Cardiac hypertrophy in mice with long-chain acyl-CoA dehydrogenase or very long-chain acyl-CoA dehydrogenase deficiency

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Cardiac hypertrophy is a common finding in human patients with inborn errors of long-chain fatty acid oxidation. Mice with either very long-chain acyl-coenzyme A dehydrogenase deficiency (VLCAD–/–) or long-chain acyl-coenzyme A dehydrogenase deficiency (LCAD–/–) develop cardiac hypertrophy. Cardiac hypertrophy, initially measured using heart/body weight ratios, was manifested most severely in LCAD–/– male mice. VLCAD–/– mice, as a group, showed a mild increase in normalized cardiac mass (8.8% hypertrophy compared with all wild-type (WT) mice). In contrast, LCAD–/– mice as a group showed more severe cardiac hypertrophy (32.2% increase compared with all WT mice). On the basis of a clear male predilection, we analyzed the role of dietary plant estrogenic compounds commonly found in mouse diets because of soy or alfalfa components providing natural phytoestrogens or isoflavones in cardioprotection of LCAD–/– mice. Male LCAD–/– mice fed an isoflavone-free test diet had more severe cardiac hypertrophy (58.1% hypertrophy compared with WT mice fed the same diet). There were no significant differences in the female groups fed any of the diets. Echocardiography measurement performed on male LCAD-deficient mice fed a standard diet at the age of ∼3 months confirmed the substantial cardiac hypertrophy in these mice compared with WT controls. Left ventricular (LV) wall thickness of the interventricular septum and posterior wall was remarkably increased in LCAD–/– mice compared with that of WT controls. Accordingly, the calculated LV mass after normalization to body weight was increased by about 40% in the LCAD–/– mice compared with WT mice. In summary, we found that metabolic cardiomyopathy, expressed as hypertrophy, developed in mice because of either VLCAD deficiency or LCAD deficiency; however, LCAD deficiency was the most profound and seemed to be attenuated either by endogenous estrogen (in females) or by phytoestrogens present in the diet as isoflavones (in males).

Inborn errors of long-chain fatty acid metabolism are often characterized by cardiac hypertrophy, arrhythmia and sudden death in the disease phenotype[1] of affected children. These metabolic defects involve disruption of long-chain fatty acid catabolism, either in the transport of long-chain acylcarnitine esters into the mitochondria (as in carnitine palmitoyl-transferase-2 or carnitine/acylcarnitine translocase deficiencies) or in long-chain fatty acid β-oxidation (as in very long-chain acyl-CoA dehydrogenase or long-chain 3-hydroxy-acyl-CoA dehydrogenase deficiencies). In addition, in some of these cases, attenuation of disease severity has been correlated with the presence of residual enzyme activity.[2–4] The molecular basis of metabolic cardiomyopathy, expressed as hypertrophy, that results from primary defects in metabolism is poorly understood. Because the heart relies heavily on fatty acid oxidation (FAO) for energy production after birth, it has been considered that energy deprivation contributes to the development and progression of metabolic cardiomyopathy.[5] In addition, certain inborn errors of long-chain fatty acid metabolism, as well as ischemia and reperfusion injury, can result in accumulation of toxic long-chain acylcarnitines. These amphipathic molecules are thought to

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be arrhythmogenic because they alter membrane properties and integral proteins such as the sarcolemmal Na^+/K^+-ATPase.[6] Long-chain acyl-coenzyme A dehydrogenase (LCAD)[7] and very long-chain acyl-coenzyme A dehydrogenase (VLCAD)[8] deficient mouse models have been developed.

It has long been established that hormone status as determined by sex and age has an important role in the development and progression of cardiovascular disease.[9] More recently, plant-derived estrogen-like compounds, such as the isoflavones, have been cited as agents that can influence the development and course of cardiovascular disease in people who eat a diet rich in certain plant proteins such as those derived from soy.[10–15] There are no known human patients with LCAD deficiency; however, the LCAD-deficient mouse model, which has a more severe phenotype[7,8] than the VLCAD-deficient model, has many shared features of human patients with VLCAD deficiency. We hypothesized that the mouse models for VLCAD and LCAD deficiencies would develop cardiac hypertrophy as seen in the corresponding human VLCAD deficiency disease. We initially analyzed cardiac mass in both male and female LCAD-deficient, VLCAD-deficient and wild-type (WT) control mice. We then determined the effects of dietary isoflavones (phytoestrogens) on cardiac mass changes, and finally evaluated heart mass and cardiac function in the C57BL/6 congenic LCAD-deficient mice by echocardiography.

MATERIALS AND METHODS
Animals
All mutant mice in the study were homozygous at either the long-chain acyl-coenzyme A dehydrogenase locus (*Acadl*) (B6,129–*Acadl*[tm1Uab] LCAD–/–) (LCAD = enzyme; *Acadl*-mouse, *ACADL*-human = genes, respectively)[7] or the very long-chain acyl-coenzyme A dehydrogenase locus (*Acadvl*) (B6,129–*Acadvl*[tm1Uab] VLCAD–/–) (VLCAD = enzyme; *Acadvl*-mouse, *ACADVL*-human = genes, respectively),[8] or had B6,129–*Acadl*^{+/+} and B6,129–*Acadvl*^{+/+} WT alleles. An equivalent B6, 129 background was maintained by using intercrosses within each of the three genotypes. LCAD–/–, VLCAD–/– and WT mice in the initial studies were derived from mice of 129/SvEvTacBR (129S6) and C57BL/6NTacfBR (B6) (Taconic) genetic backgrounds. Mice used for the echocardiography studies were LCAD–/– B6 congenic mice, all males, ∼12 weeks old, and fed a standard diet (Harlan-Teklad 7913—NIH-31). Mice were negative for murine pathogens based on serological assays for 10 different viruses, aerobic bacterial cultures of the nasopharynx and cecum, examinations for endo- and ectoparasites, and histopathology of all major organs. All animal protocols were approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham.

Heart Mass Measurement
Before killing and sample collection, all mice except those in the diet studies were fasted for 22–28 h. Following measurement of body weight, hearts were carefully excised and gently blotted on absorbent paper to remove blood from the ventricles before wet weight measurement.

Echocardiography Measurement
M-mode echocardiography was performed using the small animal echocardiography analysis system (Vevo 770, VisualSonics, Canada). In brief, mice were anesthetized with isoflurane inhalation in oxygen. Fur was removed from the upper sternal and subxiphoid areas. Limbs were attached to electrodes for electrocardiographic monitoring and timing of cardiac cycles. A two-dimensional short-axis view and M-mode tracings of the left ventricle were obtained with a 30 MHz transducer. Using analysis software provided by the manufacturer, the following data were obtained: left ventricular dimension at systole and diastole (LVID;s and LVID;d), posterior wall thickness at systole and diastole (LVPW;s and LVPW;d), interventricular septal wall thickness at systole and diastole) (IVS;s and IVS; d:), ejection fraction (EF%), fractional shortening (FS%), left ventricle volume at systole and diastole (volume;s and volume;d), and left ventricle stroke volume, heart rate, cardiac output and left ventricular mass.

Diet Study Design
At 21 days of age LCAD–/– and LCAD+/+ mice were weaned onto either a standard laboratory diet (Teklad LM-485—the basal diet) or an isoflavone-free diet (Teklad diet TD.96155—the test diet) (Harlan-Teklad, Inc., Indianapolis, IN, USA). Mice were housed under 12-h light–dark cycles in microisolator cages and killed at 90–115 days of age for sample collection. The mice in the diet study were killed by CO_2 inhalation and the body and wet heart weight measurements were taken as above, but without prior fasting.

Measurement of Isoflavones in Diets
Individual samples (0.5 g) of each diet were analyzed in duplicate. The isoflavones were extracted by mixing with 80% aqueous methanol (5 ml) at 4°C for 2 h. Dichlorofluorescein was added as an internal standard to the extracting solvent.[16] After centrifugation of the mixture at 3000 g for 10 min, the supernatants were analyzed using reverse-phase HPLC on an Agilent Technologies HP 1100 instrument. Aliquots (10 μl) were separated on a 22 cm × 4.6 mm i.d. Aquapore C_8 column with a 3 cm × 4.6 mm i.d. C_8 guard column. The column was equilibrated in solvent A (90% double-distilled water:10% acetonitrile containing 0.1% trifluoroacetic acid). The isoflavones were eluted with a 0–30% gradient of solvent B (10% double-distilled water:90% acetonitrile containing 0.1% trifluoroacetic acid) in solvent A over a 30-min period at a flow rate of 1.5 ml/min. Eluted isoflavones were monitored with a diode array detector and assayed from their absorbance at 262 nm. Standard isoflavone mixtures also containing the internal standard were analyzed as part of the group of samples.

Quantitative data were obtained by comparing the ratios of the areas of the unknown peaks to the internal standard with those in the standard isoflavone mixtures. Results are reported in aglucone units, that is, the equivalent weight of the isoflavone aglucone regardless of the nature of glucose moiety (the isoflavones are present in soy as at least three β-glucoside conjugates, each with a different molecular weight). Within-assay variation for duplicate samples ranged from 1 to 3% and inter-assay variation from 3 to 5%.
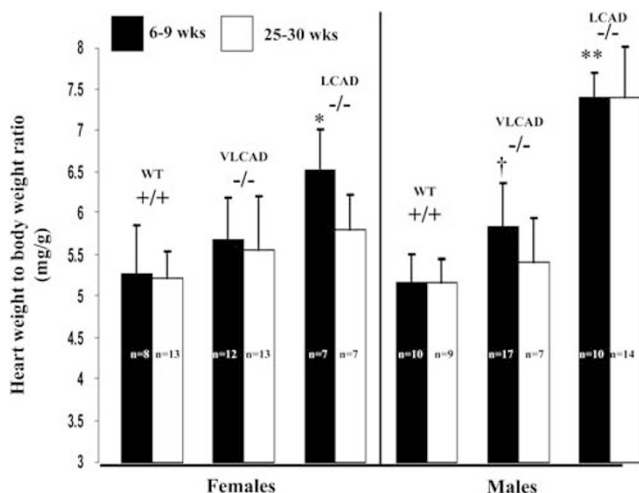
Statistics

Groups were analyzed by either one-way or two-way analysis of variance (ANOVA) using the SigmaStat 2.03 as program parameters permitted.

RESULTS

Heart Weight to Body Weight Ratio in LCAD- and VLCAD-Deficient Mice

Figure 1 shows the effects of genotype, sex and age on cardiac mass normalized to body weight. Genotype had the greatest effect on the development of cardiac hypertrophy ($P < 0.001$). The VLCAD–/– mice had a milder cardiac phenotype. VLCAD–/– mice, as a group, showed a mild increase in normalized cardiac mass (8.8% hypertrophy compared with all WT mice). In contrast, LCAD–/– mice as a group showed more severe cardiac hypertrophy (32.2% increase compared with all WT mice). A closer examination of cardiac mass in subgroups divided along genotype, age and sex lines showed

that cardiac hypertrophy was less severe in females and certain groups of older mice with either LCAD or VLCAD deficiency, as detailed below.

Sex was the next most influential modulator of cardiac mass ($P < 0.001$). Although sex had no effect on cardiac mass in WT mice, cardiac hypertrophy was more severe in both VLCAD–/– and LCAD–/– males than in females of like genotype. Sex and genotype interacted significantly ($P < 0.001$). Even in younger VLCAD–/– males, when cardiac hypertrophy was most prominent, the sex disparity in cardiac mass was greater in LCAD–/– mice (13% difference between the sexes) than in VLCAD–/– mice (3% difference).
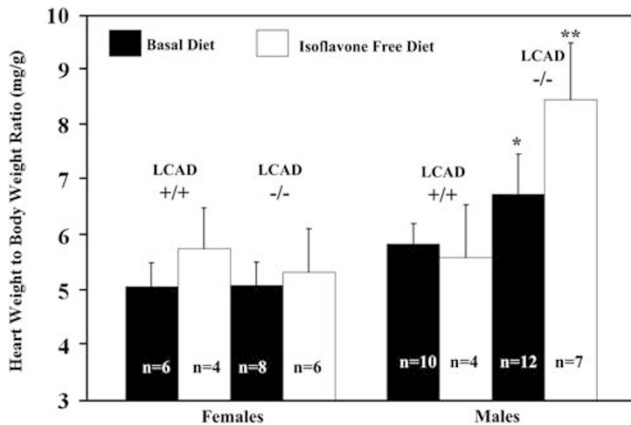
Age was significant, but least influential ($P < 0.02$). Mice were divided into young (6–10 weeks old) and adult mice (15–44 weeks old). Some groups of mice showed a modest decrease in cardiac hypertrophy in adults. The greatest attenuation in cardiac hypertrophy, toward the normal range, was seen in older, female LCAD–/– mice (a 12% decrease when compared with young LCAD–/– females). Older VLCAD–/– males also showed regression in cardiac hypertrophy back to the normal range. No age effect on hypertrophy was seen in male LCAD–/– mice. When VLCAD–/– mice were excluded from the analysis, the age effect on cardiac hypertrophy was not significant ($P = 0.06$).

Diet Effects on the Severity of Metabolic Cardiomyopathy in LCAD-Deficient Mice

Recent studies have associated high dietary intake of isoflavones, especially those found in soy proteins, with resistance to human cardiovascular disease.[10] Because soy and alfalfa are used as protein sources in the standard autoclavable rodent diet that we fed our colony, we considered that the diet might be rich in isoflavones and other phytoestrogens. We hypothesized that these compounds may have a sparing effect on the severity of the cardiac hypertrophy phenotype in LCAD–/– mice, especially males, by providing a 'feminizing' effect, much like the endogenous estrogen of LCAD–/– females.

We designed a feeding trial experiment to test this hypothesis. Most striking was the effect of the isoflavone-free test diet on cardiac hypertrophy in male LCAD–/– mice (Figure 2). No differences were detected between any of the female groups. As expected, 32.4% hypertrophy was observed in LCAD–/– males on the basal soy-based diet compared with WT males fed the same basal diet ($P < 0.001$). Cardiac hypertrophy, however, was exacerbated in LCAD–/– males fed the isoflavone-free test diet, with 58.1% hypertrophy as compared with WT males on the same test diet ($P < 0.001$). The difference between the LCAD–/– males fed the basal or test diets was also significant ($P < 0.01$). The test diet had no significant effect on cardiac mass in WT mice.

Male LCAD–/– mice fed the isoflavone-free test diet were significantly smaller than WT males on the test diet ($P < 0.01$) and male LCAD–/– mice on the basal diet ($P < 0.001$, Figure 3). The difference between LCAD–/– males

Figure 1 Heart weight to body weight ratios in younger (6- to 9-week old) and older (25- to 30-week old) male and female LCAD–/–, VLCAD–/– and WT mice. Error bars equal 1 s.d.. LCAD–/– male (**) and young LCAD–/– female (*) mice showed significant cardiac hypertrophy compared with VLCAD–/– and WT mice of like sex and LCAD–/– males (**) showed significantly greater hypertrophy than all other female groups ($P < 0.001$, one-way ANOVA). Younger VLCAD–/– males (†) showed significant hypertrophy compared with age-matched WT males ($P = 0.032$) but older age-matched VLCAD–/– and WT males did not. In contrast with young LCAD–/– females, VLCAD–/– females did not show significant hypertrophy compared with age-matched WT females.

Figure 2 Diet effects on heart weight to body weight ratio in LCAD−/−) mice. Male LCAD−/− mice weaned onto and maintained on an isoflavone-free (test diet) (**) showed significantly greater hypertrophy than LCAD−/− mice weaned onto the soy containing basal diet or LCAD + / + ($P < 0.005$, one-way ANOVA) or LCAD + / + mice on either diet ($P < 0.001$, one-way ANOVA). LCAD−/− mice weaned onto the basal diet (*) showed significant hypertrophy compared with WT mice on either diet ($P < 0.001$, one-way ANOVA). No differences in heart weight to body weight ratio were noted between WT males on different diets and among any female groups.
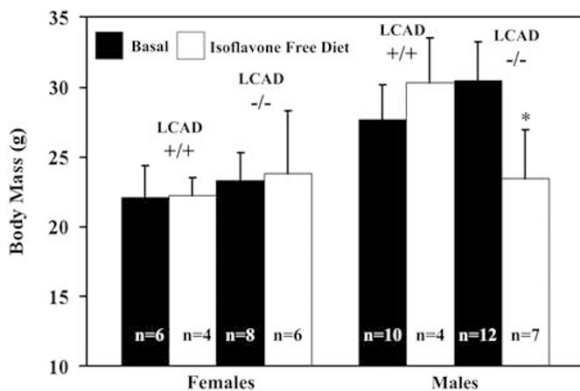
Figure 3 Body mass differences between LCAD−/− and LCAD + / + mice. Body mass was measured in LCAD−/− and WT mice fed either the basal diet or an isoflavone-free test diet. No differences were detected in body mass between any female groups. LCAD−/− male mice fed test diet were significantly smaller than LCAD−/− mice fed the basal diet and the WT males fed the test diet ($P < 0.01$).

on the test diet and WT males on the standard diet was not significant ($P = 0.065$). No detectable effects of diet on weight gain were noted between WT groups of the same sex. In addition, no differences in weight gain were noted among any of the female groups on either diet.

To define the isoflavone content of the two diets used in the diet study, the isoflavone content of each feed was measured (Table 1). The patterns of the isoflavone conjugates (no malonylglucosides and a large proportion of acetylglucosides) were consistent with the use of toasted soy protein in the diet. Total isoflavone concentration implied that the soy protein constituted about 5% by weight of the total diet.

Table 1 Isoflavone analyses of basal and test (soy-free) diets

Isoflavone	Basal diet (μg/g diet) mean ± 1 s.d.	Test diet
Daidzin	54.3 ± 2.28	0.00
Glycitin	8.50 ± 0.12	0.00
Genistin	51.5 ± 3.70	0.00
Malonyl-daidzein	0.00	0.00
Malonyl-genistein	0.00	0.00
Malonyl-glycitein	0.00	0.00
Acetyl-daidzein	24.8 ± 1.14	0.00
Acetyl-glycitein	7.08 ± 0.14	0.00
Acetyl-genistein	16.1 ± 1.07	0.00
Daidzein	0.00	0.00
Glycitein	0.00	0.00
Genistein	0.00	0.00
Total	162 ± 8.46	0.00

In order to further quantify the original observation of cardiac hypertrophy based on heart-to-body weight ratios, we performed echocardiography studies on later groups of mice. Echocardiography measurement performed on male LCAD-deficient mice at the age of ∼3 months confirmed the substantial cardiac hypertrophy in these mice compared with WT controls (Figure 4, representative M-mode image of the hearts from a LCAD−/− and a WT mice). The left ventricular wall thickness of the interventricular septum and posterior wall was remarkably increased in LCAD−/− mice compared with that of control mice (Table 2). Accordingly, the calculated LV mass after normalization to body weight was increased by about 40% in the LCAD−/− mice compared with WT mice (Table 2 and Figures 4 and 5). Interestingly, cardiac performance appeared to be well maintained with normal EF%, FS% and cardiac output (Table 2). Heart rate in the LCAD−/− mice was also comparable with that in WT mice (Table 2).

DISCUSSION

We propose the LCAD-deficient mice as a model of the metabolically induced cardiomyopathy seen in children with severe VLCAD deficiency. Children born with severe deficiencies in long-chain FAO usually present in metabolic crisis before 6 months of age and episodes of metabolic crisis pose serious threats to survival.[17] One group reported successful treatment of a VLCAD-deficient patient with medium-chain triglyceride therapy.[18] Another approach of therapy for cardiomyopathy in human VLCAD deficiency was administration of the anaplerotic, odd-chain fatty acid containing triglyceride and triheptanoin.[19] No human cases of LCAD deficiency have been documented. Earlier reported cases of human LCAD deficiency have since been reclassified as
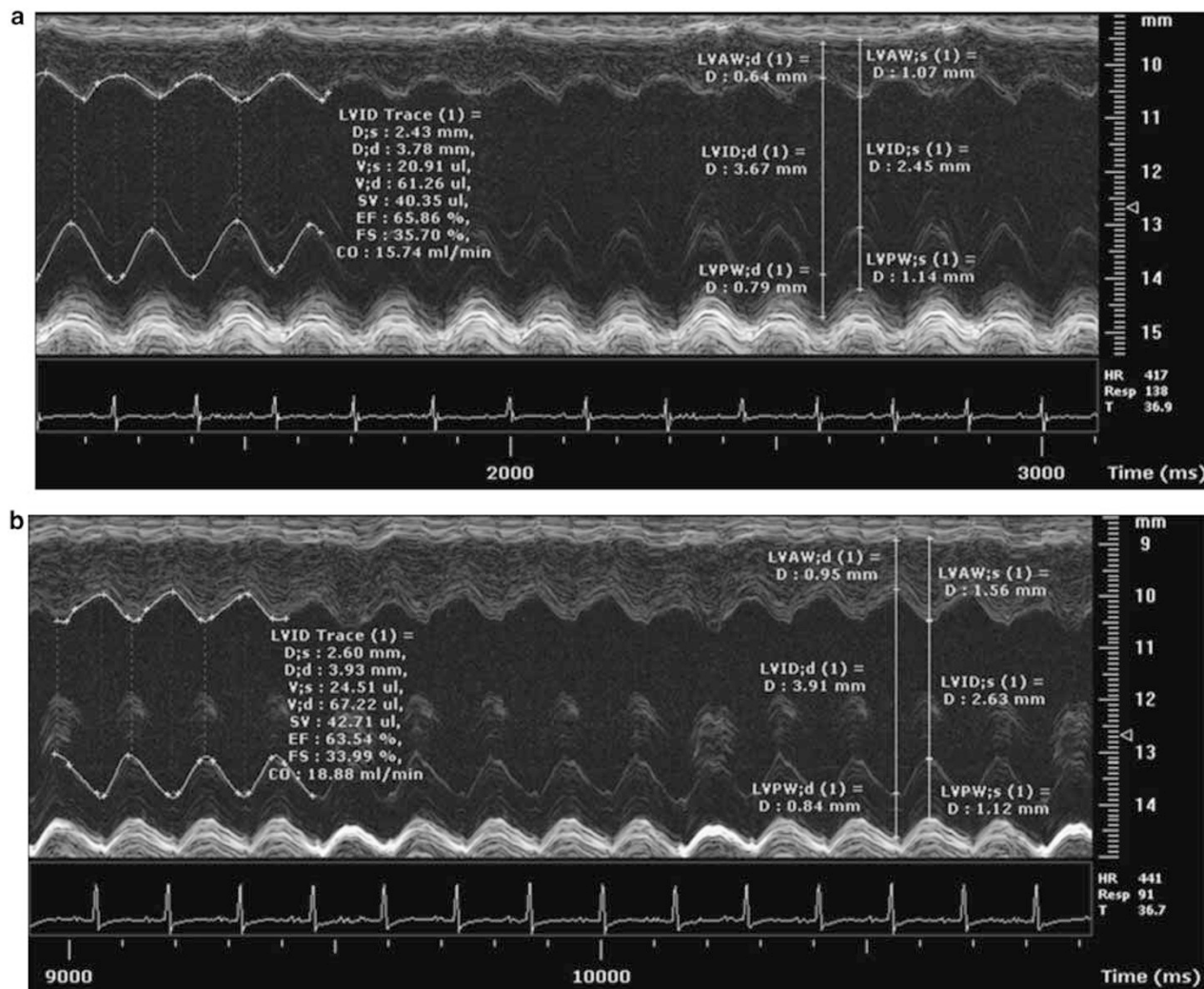
Figure 4 Representative M-mode image of the hearts from a wild-type (WT) (**a**) and an LCAD−/− (**b**) mice. Anatomic M-mode (AM-Mode) images obtained from short-axis measurements were used to evaluate left ventricular function (described in Materials and methods). The M-mode spectrum was acquired in WT or LCAD−/− mice at 3 months of age. IVS;s and IVS; d, interventricular septal wall thickness (diastole and systole); LVPW; d and LVPW; s, left ventricular posterior wall thickness (diastole and systole); LVID;d and D;d, left ventricular internal diameter (diastole); LVID;s and D;s, left ventricular internal diameter (systole); V;s and V;d, volume; systole and diastole; SV: stroke volume; EF: ejection fraction; FS: fractional shortening; CO: cardiac output. Three to five cardiac cycles were averaged for each experimental animal ($n = 9$ mice for each genotype group) for every measurement for each time point.

VLCAD deficiency.[20] We also have developed VLCAD-deficient mice and they also develop metabolic disease, such as cardiac hypertrophy, albeit milder than that seen in LCAD-deficient mice.[8] The cardiac hypertrophy seen in mice with LCAD deficiency is comparatively milder than the cardiac hypertrophy associated with VLCAD deficiency in human beings. Reasons for this disparity may arise from a diminished reliance on fatty acid β-oxidation for cardiac energy homeostasis in mice, from a greater substrate overlap between LCAD and VLCAD activities in mice, or from a greater resistance in mice to the effects of toxic metabolites.

Although cardiac hypertrophy in the LCAD−/− mice was quite remarkable compared with many mouse models with cardiac defects, it is interesting to observe that cardiac performance of the LCAD−/− mice was well maintained at least for the ages analyzed. Furthermore, left ventricular dimension was not changed despite the increasing thickness of the left ventricular wall. This is similar to a pattern of concentric cardiac hypertrophy seen in patients with left ventricular pressure overload. Therefore, the cardiac hypertrophy manifested in the LCAD−/− mice represents a typical compensatory hypertrophy. It is not clear yet whether any physiological and/or pathological stress would lead to the development of decompensatory hypertrophy or even progress to heart failure. It is interesting that at least aging, as studied here, did not exacerbate the hypertrophy progression.

As we report here, variation in more chronic features of cardiac hypertrophy can be found as well, eg, the mild cardiac

Table 2 Echocardiography measurements

| Parameter	WT	LCAD−/−	P
LVID;d (mm)	3.53 ± 0.08	3.73 ± 0.1	>0.05
LVID;s (mm)	2.24 ± 0.09	2.43 ± 0.15	>0.05
LVPW;d (mm)	0.71 ± 0.04	0.9 ± 0.06	*<0.01
LVPW;s (mm)	1.14 ± 0.05	1.34 ± 10.08	*<0.01
IVS;d (mm)	0.75 ± 0.01	0.99 ± 0.03	*<0.01
IVS;s (mm)	1.18 ± 0.04	1.36 ± 0.05	*<0.05
EF (%)	72 ± 2.1	69 ± 2.9	>0.05
FS (%)	41 ± 1.8	38 ± 2.4	>0.05
Volume;d (μl)	56 ± 2.7	63 ± 3.4	>0.05
Volume;s (μl)	15.9 ± 1.6	20.5 ± 2.7	>0.05
SV (μl)	40 ± 1.8	42.7 ± 0.9	>0.05
Heart rate (BPM)	420 ± 25	428 ± 16	>0.05
CO (ml/min)	18 ± 0.7	18.8 ± 0.57	>0.05
LV mass (mg)	68.7 ± 4.7	112.2 ± 7.3	*<0.01

Abbreviations: LVID;s and LVID;d, left ventricular dimension at systole and diastole; LVPW;s and LVPW;d, posterior wall thickness at systole and diastole; IVS;s and IVS; d, interventricular septal wall thickness (diastole and systole); EF%, Ejection fraction; FS%, fractional shortening; volume;s and volume;d, left ventricle volume at systole and diastole; SV, left ventricle stroke volume; BPM, beats per minute; CO, cardiac output.

Data were expressed as mean ± s.e.m., *$P < 0.05$, $n = 9$ in each group.

hypertrophy of VLCAD−/− male mice that resolves with age contrasts with the more severe hypertrophy of LCAD−/− males that persists well into adulthood. Furthermore, a protective effect against cardiac hypertrophy was documented in both VLCAD−/− and LCAD−/− females, with the greater effect in the latter. Whether the females benefit more from the presence of higher estrogenic hormones or from the absence of androgenic hormones remains to be proven. If the effect results from the presence of estrogens, then we would predict cardiac hypertrophy to be more severe in females after reproductive senescence.

The interaction between hormone status and the development of cardiac disease is seen not only in the different cardiac disease rates between men and premenopausal women, but also in the rapid rise in heart disease rates seen in postmenopausal women.[21] These studies show the protective effects of estrogenic hormones in the setting of metabolic disease. The plasticity of the hypertrophic response evident in LCAD−/− mice suggests that the hypertrophic response, even an insult as specific as a metabolic enzyme deficiency, is likely the result of complex processes involving signaling pathways that overlap with those downstream of the estrogen receptor. Another possibility is that the gene expression patterns in response to estrogenic compounds yield a myocardium resistant to metabolic insults.

Perhaps most surprising is the demonstrable benefits of a diet rich in soy isoflavones to male LCAD−/− mice.
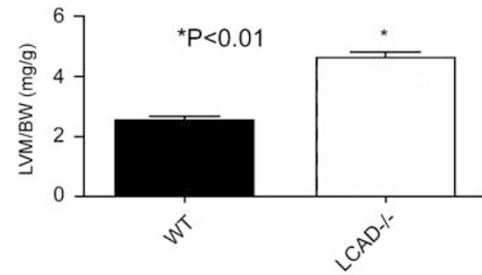
Figure 5 Left ventricular mass to body weight ratio: Left ventricular mass (LVM) of LCAD−/− and WT male mice at their ages of 3 month were derived from M-mode echocardiographic measurements. The resultant LVM was normalized to body weight. Data were expressed as mean ± s.e.m., $n = 9$, *$P < 0.01$.

In addition, we found reduced body weight (Figure 3) in the group with the most severe cardiac hypertrophy, the LCAD−/− male mice eating the phytoestrogen-free test diet. We speculate that the two effects are not linked. It is noteworthy that this degree of hypertrophy was achieved on a low-fat diet (5% by weight) and that the mice were not challenged with fasting or exercise, conditions that favor the oxidation of fatty acids in the heart. Although a simpler design might have been to add back one or more purified isoflavones to the isoflavone-free diet, a comparison of the measurable isoflavones in each of the diets illustrates the complexity of phytoestrogen composition in standard diet. Of the isoflavones present in the laboratory diet, genistein, binds to the estrogen -receptor with an affinity comparable to that of 17β-estradiol.[22] The -glucosides daidzin, genistin and glycitin and their corresponding 6′-O-acetyl esters are hydrolyzed and the resulting aglucones absorbed in the intestine both proximally and distally; they are also metabolized further to equol and other bioactive compounds that are also absorbed.[23] As a result of this variability in absorption kinetics and because several isoflavone compounds are present in the standard laboratory diet, it is difficult to ascertain which compound or combination of compounds produces attenuation of cardiac hypertrophy in LCAD−/− males. We speculate that the isoflavones are modulating cardiomyocyte cell signaling through activation of the estrogen β receptor as described for other organ systems.[22] The LCAD−/− mice are severely cold intolerant; however, we showed previously that soy-based, isoflavone-rich diets make LCAD−/− mice more cold resistant as compared with those fed a casein-isoflavone-free diet.[24] In contrast, the positive attributes of isoflavone-rich diets are not universal, as in other studies this type of diet was detrimental to mice with a mutation in the α-myosin heavy chain gene in which the male mice developed severe, dilated cardiomyopathy when fed a soy-based diet.[25]

Cardiac hypertrophy associated with primary defects in FAO has been considered, at least in part, to be a disease arising out of inadequate energy production.[19] This was supported by the observation that in adult human beings the

majority of cardiac energy production is derived from long-chain fatty acid β-oxidation under fasting conditions or during sustained exercise.[26] Furthermore, children born with 'downstream' deficiencies of medium- and short-chain fatty acid β-oxidation do not develop cardiac hypertrophy, presumably because they can partially oxidize long-chain fatty acids and derive energy production from the pathway.[5] An alternate hypothesis is that the formation of long-chain fatty esters such as long-chain acylcarnitine or long-chain acyl-CoA stimulates the development of cardiac hypertrophy. Studies using rats treated with etomoxir showed that blocking long-chain fatty acid β-oxidation by preventing the entrance of long-chain fatty acid substrates into the mitochondria is sufficient to evoke a hypertrophic response.[27] Another possibility is that the more robust hypertrophy seen in LCAD−/− mice results more from abnormal metabolite effects than from interruption of energy production by long-chain fatty acids. The similarity of response among male and female mice to etomoxir treatment suggests that inhibition of FAO at the level of CPT1 differs from that seen in LCAD−/− and VLCAD−/− mice[8] in that it is not influenced by estrogen-modulated effects.

These studies show the complexity underlying what has been characterized as the programmed, stereotypic response of cardiac hypertrophy. We expect that further investigation of these models will reveal more facets of the interplay between energy metabolism, gene regulation and the hormonal milieu that underlie metabolic cardiomyopathy.

DISCLOSURE/CONFLICT OF INTEREST
The authors declare no conflict of interest.

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