Remodeling the cardiac transcriptional landscape with diet

Elizabeth D. Luczak,1* Kristen K. B. Barthel,1* Brian L. Stauffer,1,2 John P. Konhilas,1 Tom H. Cheung,3 and Leslie A. Leinwand1,2

1Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder; 2Department of Medicine, Division of Cardiology, University of Colorado Health Sciences Center, Denver; and 3Department of Chemistry and Biochemistry, University of Colorado, Boulder, Colorado

Submitted 1 December 2010; accepted in final form 12 April 2011

Luczak ED, Barthel KK, Stauffer BL, Konhilas JP, Cheung TH, Leinwand LA. Remodeling the cardiac transcriptional landscape with diet. Physiol Genomics 43: 772–780, 2011. First published April 12, 2011; doi:10.1152/physiolgenomics.00237.2010.—The perception that soy food products and dietary supplements will have beneficial effects on cardiovascular health has led to a massive consumer market. However, we have previously noted that diet profoundly affects disease progression in a genetic model of hypertrophic cardiomyopathy (HCM). In this model, a soy-based diet negatively impacts cardiac function in male mice. Given the frequent connection between functional changes and transcriptional changes, we investigated the effect of diet (soy- vs. milk-based) on cardiac gene expression and how it is affected by the additional factors of sex and disease. We found that gene expression in the heart is altered more by diet than by sex or an inherited disease. We also found that the healthy male heart may be sensitized to dietary perturbations of gene expression in that it displays a gene expression profile more similar to diseased male and female hearts than to healthy female hearts. These observations may in part account for documented divergence in HCM phenotypes between males and females and between diets.

hypertrophy; nutrigenomics

For example, consumption of polyunsaturated fatty acids or carboxydrates has been shown to alter gene expression both in rodent models and in humans (18). However, relatively little attention has been paid to the fact that diet can contain many bioactive compounds. One food source that is rich in bioactive compounds is soy. American consumers will spend over $8 billion this year on soy foods, beverages, and dietary supplements, in part because of the perception that these products will lead to cardiovascular health benefits including improvement of blood lipid profiles and decreased blood pressure (15). However, the proposed health benefits of soy-containing products are still under debate, especially in light of the high concentrations of phytoestrogens found in soy (16). Phytoestrogens, such as genistein and daidzein, are isoflavones that are produced essentially only by legumes. They are able to bind estrogen receptors, activating estrogen response elements (4, 8, 16, 21), as well as act as broad spectrum receptor tyrosine kinase inhibitors (1, 9, 13, 24, 25), suggesting that they may have diverse and profound biological effects (19). In fact, the American Heart Association now recommends against the use of phytoestrogen supplements (15).

In practice, laboratory mice are almost always fed a soy-based, phytoestrogen-rich diet. Although these mice are being used to model human diseases, this diet does not recapitulate a typical human diet, even those that incorporate a lot of soy-based foods. In light of this concern, we were interested in the contribution of diet to disease development, in particular familial hypertrophic cardiomyopathy (HCM). HCM can arise from many disease-causing mutations in genes encoding sarcomeric proteins, including myosin heavy chain (MyHC). HCM patients initially experience cardiac hypertrophy to compensate for the mutation and preserve cardiac function. In the worst cases, the hypertrophic compensation is eventually not sufficient, and the heart enters a decompensatory phase that is characterized by ventricular dilation and wall thinning. This leads to contractile dysfunction and ultimately to heart failure.

We previously discovered that feeding a milk protein-based, soy-free (casein) diet to both wild-type mice and mice that model an inherited form of HCM resulted in significantly larger hearts and accumulation of more body fat with age than feeding mice a standard soy-based diet. These effects may seem pathologic, but casein-fed HCM mice have significantly improved cardiac phenotypes, including contractile function, compared with those fed a soy-based diet (20).

To investigate the molecular basis for these dietary effects on heart structure and function, we employed microarrays and pathway-oriented bioinformatics analyses to examine how gene expression in the young rodent heart is affected by diet. HCM is a progressive disease (3, 23), thus we hypothesized that studying gene expression in animals before most pathologic features of the disease are present could also give insight into how HCM develops at the molecular level.

MATERIALS AND METHODS

Animals

Wild-type or HCM C57BL/6 mice were euthanized at 2–4 mo of age, and left ventricle tissue was rapidly excised and frozen. HCM mice used for this work are described in (23). In brief, C57BL/6 mice express a transgene for rat α-MyHC cDNA harboring the point mutation R403Q and a 59 amino acid deletion in the actin binding domain substituted with nine dissimilar amino acids. Expression is driven by the rat α-MyHC promoter, ensuring an expression pattern restricted to cardiomyocytes. Mice were housed under standard conditions. All animal protocols were approved by the Institutional Animal Care and Use Committee at the University of Colorado at Boulder.

Diets

Mice were fed ad libitum either a standard soy diet (8640; Harlan Teklad, Indianapolis, IN) or a casein-based diet (D10001; Research
Diets, New Brunswick, NJ). To control for in utero and neonatal effects of diet, breeding females were maintained on the appropriate casein- or soy-based diet. Analysis of the soy-based rodent diet revealed isoflavone concentrations of 206 mg daidzein/kg and 229.5 mg genistein/kg dry food weight. The casein and soy diets are composed of approximately the same percentage of protein (20–24%), fat (4–5%), carbohydrate (65%), and fiber (5%) with similar amounts of vitamins and minerals.

Gene Expression Arrays

Total RNA was isolated from frozen left ventricles using TRI Reagent (MRC, Cincinnati, OH) and further purified using RNeasy Mini kits (Qiagen, Valencia, CA) according to the manufacturer’s instructions. Gene expression data were collected at The Genomics and Microarray Core of the University of Colorado Denver. Briefly, biotin-labeled amplified RNA was fragmented and hybridized onto the microarrays (U74Av2; Affymetrix, Fremont, CA) according to the Affymetrix protocol. Each experiment was done with biological triplicates or quadruplicates, and each replicate comprised equal amounts of pooled RNA from two animals. Scanned chip images were analyzed and normalized using the robust multiarray averaging method. Significant genes were identified using a statistical limit of \( P \leq 0.05 \) and fold enrichment \( \geq 1.5 \) using Genespring 7.2 (Agilent Technologies, Santa Clara, CA). Gene expression data can be viewed and retrieved at the National Center for Biotechnology Information Gene Expression Omnibus database with accession number GSE25700.

Bioinformatic Analyses

Gene pathway analysis was performed with Ingenuity Pathway Analysis (IPA) version 8.7 (Ingenuity Systems, Redwood City, CA).

![Fig. 1. Differential cardiac gene expression in diet, sex, and disease. Number of genes up- and downregulated (≥1.5-fold, \( P \) value ≤ 0.05) in comparisons of diet (A), sex (B), and disease (C). Unique genes annotated with an Entrez Gene ID were considered for this analysis. WT, wild type; HCM, hypertrophic cardiomyopathy; M, male; F, female.](http://physiolgenomics.physiology.org/)

Physiol Genomics • VOL 43 • www.physiolgenomics.org
CA). Affymetrix probe set IDs conforming to the fold enrichment and significance threshold criteria described above for the four diet comparisons (casein vs. soy in wild-type male, wild-type female, HCM male, and HCM female) were uploaded to IPA. IPA extracted those transcripts that were annotated as known genes; those that were not annotated were not included in further gene pathway analysis. The total numbers of genes included in the analysis for each comparison were: 544 for wild-type male, 355 for wild-type female, 1,815 for HCM male, and 1,393 for HCM female. The probability that a given gene pathway or disease category was significantly represented in the dataset was determined by a \( P \) value \( \leq 0.05 \), calculated with a right-tailed Fisher’s exact test [sometimes represented by \(-\log(P\text{ value}) \geq 1.3\)]. The reference set for these analyses was all genes represented on the MG U74Av2 microarray.

The IPA comparison analysis tool was used to assess common and distinct pathways between the comparisons. The significance of a given pathway is determined by calculating the ratio of the number of genes from the dataset that map to the total possible number of genes within the canonical pathway and by assigning a \( P \) value determined by Fisher’s exact test, which indicates how likely the association of the dataset genes with the pathway is to occur by random chance alone.

Gene ontology (GO) analysis of molecular functions of regulated genes was performed with the Expression Analysis Systematic Explorer (EASE) version 2.0 (http://david.abcc.ncifcrf.gov/content.jsp?file=/ease/ease1.htm&type=1) (6). Differentially expressed probe set IDs (separated into casein-enriched and soy-enriched gene lists) were analyzed for overrepresented gene categories; EASE extracted the annotated genes with associated GO terms and eliminated redundancies. The probability that a given molecular function category was significantly represented in the dataset was determined by a \( P \) score, where a \( P \) score is the upper bound of the jackknife Fisher exact probabilities for overrepresented gene categories; EASE extracted the annotated genes with associated GO terms and eliminated redundancies. The probability that a given molecular function category was significantly represented in the dataset was determined by calculating the ratio of the number of genes from the dataset that map to the total possible number of genes within the canonical pathway and by assigning a \( P \) value determined by Fisher’s exact test, which indicates how likely the association of the dataset genes with the pathway is to occur by random chance alone.

Gene ontology (GO) analysis of molecular functions of regulated genes was performed with the Expression Analysis Systematic Explorer (EASE) version 2.0 (http://david.abcc.ncifcrf.gov/content.jsp?file=/ease/ease1.htm&type=1) (6). Differentially expressed probe set IDs (separated into casein-enriched and soy-enriched gene lists) were analyzed for overrepresented gene categories; EASE extracted the annotated genes with associated GO terms and eliminated redundancies. The probability that a given molecular function category was significantly represented in the dataset was determined by a \( P \) score, where a \( P \) score is the upper bound of the jackknife Fisher exact probabilities for overrepresented gene categories; EASE extracted the annotated genes with associated GO terms and eliminated redundancies.

The IPA comparison analysis tool was used to assess common and distinct pathways between the comparisons. The significance of a given pathway is determined by calculating the ratio of the number of genes from the dataset that map to the total possible number of genes within the canonical pathway and by assigning a \( P \) value determined by Fisher’s exact test, which indicates how likely the association of the dataset genes with the pathway is to occur by random chance alone.

Table 1. Genes differentially expressed between wild-type males and females fed either soy diet or casein diet

<table>
<thead>
<tr>
<th>Soy Enriched</th>
<th>Casein Enriched</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fin14 (Ddx3x)</td>
<td>Dbp</td>
</tr>
<tr>
<td>Utx</td>
<td>MGL:2384747 (Erdr1)</td>
</tr>
<tr>
<td>Eif2s3x</td>
<td>Nrl1d2</td>
</tr>
<tr>
<td></td>
<td>Cebbp</td>
</tr>
<tr>
<td></td>
<td>Bhlhb2</td>
</tr>
<tr>
<td></td>
<td>Lmx2</td>
</tr>
<tr>
<td></td>
<td>Usp2</td>
</tr>
<tr>
<td></td>
<td>Mt2</td>
</tr>
<tr>
<td></td>
<td>Tspan4</td>
</tr>
<tr>
<td></td>
<td>Per1</td>
</tr>
<tr>
<td></td>
<td>Mlap5</td>
</tr>
<tr>
<td>Eif2s3y*</td>
<td>Eif2s3y*</td>
</tr>
<tr>
<td>Ddx3y</td>
<td>Cpxm2</td>
</tr>
<tr>
<td></td>
<td>Nppb</td>
</tr>
<tr>
<td></td>
<td>Arml</td>
</tr>
<tr>
<td></td>
<td>Nihl3</td>
</tr>
</tbody>
</table>

Soy diet, left column; casein diet, right column. Eif2s3y* is highlighted with an asterisk because it is enriched in males fed either diet. WT, wild type.

RESULTS

Cardiac Gene Expression is More Strongly Affected by Diet Than by Sex or an Inherited Cardiomyopathy

We have previously observed a striking, diet-dependent difference in phenotype between males and females carrying a mutation in α-MyHC leading to HCM (20). We noted that, while soy-fed male HCM mice experience a dramatic decrease in cardiac function by 8 mo of age, this can be largely prevented by feeding them the casein-based diet. Females are generally more resistant to the effects of the HCM mutation; thus we did not see much functional response of the heart to the diet change, including the parameters of percent fractional shortening and wall thickness. While these two diets are calorically similar (20), they also differ in a number of ways. For instance, the protein in the casein-based diet is derived exclusively from casein, while the soy-based diet derives protein from a wider variety of sources, including soybeans, corn, wheat, whey, and yeast. In addition, isoflavones are absent from the casein diet but are abundant in the soy diet.

Diet effects. To quantitatively assess the effects of diet on gene expression, we used microarrays to measure mRNA levels in both HCM and WT male and female hearts from mice consuming the soy or the casein diet. There are 498 genes differentially expressed between diets in the hearts of WT male animals and 329 in females. Even more impressively, in diseased (HCM) hearts the number of genes differentially expressed between the two diets increases over threefold to 1,639 in males and 1,265 in females (Fig. 1A).

Sex effects. In contrast to the observed effects of diet, there are <20 genes differentially expressed between males and females consuming either diet and from either genetic background (Fig. 1B). Despite this relatively weak effect, diet still influences sex-dependent gene expression differences. As presented in Table 1, the five genes differentially regulated in wild-type males and females (corresponding to the first bar in Fig. 1B) can be segregated into male-specific and female-specific. The three female-specific genes are all X-linked, while the two male-specific genes are Y-linked. Moreover, two of the three female genes (Ddx3x and Eif2s3x) are homologs of the male-specific genes (Ddx3y and Eif2s3y). However, in casein-fed wild-type mice, the number of genes differentially expressed between males and females is not only greater (16 vs. 5), the gene lists are nearly unique (Table 1, right column, corresponding to the third and fourth bars in Fig. 1B). In the sex-based comparison, only one gene is common between the soy and casein diets: Eif2s3y, enriched in males. None of the other genes differentially expressed between males and females on the casein diet are encoded by the sex chromosomes.

Disease effects. We also examined the extent to which gene expression in both sexes responds to the HCM-causing mutant α-MyHC transgene. This analysis was again performed within the context of the different diets. In animals consuming soy, 96 genes are differentially expressed in HCM vs. WT hearts in...
males and 99 in females. Curiously, for males and females consuming casein, these numbers increase approximately six-fold to 602 and 597, respectively (Fig. 1C). This indicates that cardiac gene expression is much more plastic in mice eating a casein-based diet, perhaps accounting for the increased adaptability of casein-fed males to the HCM transgene. These results show that 1) diet has the most profound effect on gene expression in hearts and 2) that gene expression in the diseased heart is far more affected by diet than in a healthy heart.

The Cellular Pathways Differentially Regulated by Diet in Wild-type Males are Very Similar to HCM Animals of Either Sex

Since such a large number of genes are differentially regulated between diets, we further analyzed the data using bioinformatics tools to ask more process-oriented questions. In particular, what distinguishes these sets of differentially expressed genes, and how might these processes account for the
observed phenotypic differences? To address these questions, we first employed IPA to analyze the differentially regulated genes in each of the four diet comparisons (HCM male, HCM female, wild-type male, and wild-type female).

We utilized the canonical pathways analysis tool within IPA to predict which pathways are responsive to diet. In comparing the top five predicted pathways for the four comparisons (Fig. 2A), we noted that both male and female HCM comparisons have identical pathways (white and light gray bars, respectively). Three of these five are related to energy metabolism and, importantly, to dysfunction in energy metabolism, which has been shown to contribute to cardiac dysfunction and heart failure (11, 14). Intriguingly, these three pathways are also predicted for the wild-type male comparison (charcoal gray bars), including mitochondrial dysfunction. However, while analysis of the wild-type female comparison (black bars) categorizes oxidative phosphorylation as significant, mitochondrial dysfunction is not predicted.

Since we expected similar genes to populate the oxidative phosphorylation and mitochondrial dysfunction categories, we investigated which genes distinguish the mitochondrial dysfunction category from the oxidative phosphorylation category (Fig. 2B). Several interesting points emerge from this analysis. First, while the majority of the genes shared between the two categories are subunits of Complexes I–IV in the electron transport chain, the majority of the genes unique to the mitochondrial dysfunction pathway are involved in reactive oxygen species (ROS) generation or detoxification. Given their relation to ROS handling, these unique genes can be classified as either pro- or antiapoptotic. It is important to note that most of the antiapoptotic genes (which are dedicated to ROS reduction) are enriched in casein hearts (black text in Fig. 2B). Additionally, most of the soy-enriched genes are in the proapoptotic class (gray text). This suggests that ROS detoxification may be a mechanism by which the casein diet exerts cardioprotective effects in the context of HCM.

In the case of wild-type females, it is noteworthy that four of the top five canonical pathways are not shared with any of the other comparisons, revealing that they have a unique molecular response to the two diets. Of these pathways, both insulin receptor signaling and IGF-1 signaling are predicted to respond to diet. Both pathways are related to cell growth and prolifer-
ation as well as metabolism. IGF-1 in particular is linked to survival (10). Our expression data reveal that IGF-1 is more highly expressed in the hearts of wild-type female mice fed soy, and this is not seen in wild-type males.

**GO Analysis of Molecular Functions**

To determine which functional classes of genes change with diet, we performed GO analysis of casein-enriched and soy-enriched genes. Figures 3 and 4 (casein- and soy-enriched, respectively) display the statistically significant, nonredundant categories affiliated with each experimental group. It is immediately apparent that more similarities exist between comparisons amongst the casein-enriched functional categories than soy. In the casein analyses (Fig. 3), the most statistically significant and heavily populated categories in the wild-type male and both HCM groups are related to energy metabolism and protein synthesis. However, wild-type females are once again distinct, just as in the IPA pathway analysis. There are only two statistically significant molecular function categories for this group: cation transporter activity and vitamin binding.

When the same GO analysis was performed on the soy-enriched genes, we saw fewer total associated GO terms and yet there is a broader diversity of terms among the comparisons, particularly when comparing the HCM males to females (Fig. 4). Whereas the casein-enriched molecular function categories are very similar between the HCM males and females, the terms assigned to the soy-enriched genes in either sex are much more distinct.

**Genes Differentially Expressed in Response to Diet are More Similar Within Genotype Than Within a Sex**

To understand which genes distinguish each comparison, we further refined our lists of differentially expressed genes by applying more stringent filters. We asked which genes were differentially expressed between casein and soy by more than twofold with a $P$ value of $<0.0001$. We then extracted those genes (either casein- or soy-enriched, corresponding to the nonoverlapping regions of the Venn diagram in Fig. 5) that were unique to wild-type male, wild-type female, HCM male, and HCM female (genes listed in Supplemental Table S1). When we considered genes that were shared between groups in either the casein- or soy-enriched comparisons, we observed that the similarities are within genotype and not within a sex (Fig. 5, red circled regions). For instance, out of 95 genes enriched in casein HCM male hearts, 43 overlap with casein HCM female hearts, whereas there is no overlap with genes from the wild-type casein males. Correspondingly, 15 of the 28 total casein wild-type male genes overlap with casein wild-type females. Unsurprisingly, many of these genes are related to protein translation and metabolism.

**A Small Set of Genes are Always Regulated by Diet Regardless of Sex or Genotype**

Finally, we wanted to know which genes are always influenced by diet in the rodent heart, regardless of sex or genetic

---

1 The online version of this article contains supplemental material.
background. Using our original stringency filters, we extracted these common genes and present them in Table 2. There are 23 genes that are always casein-enriched and 21 genes that are always soy-enriched. While these lists are too small for a large-scale analysis of molecular functions or pathways, we do observe that there are several ribosomal proteins enriched in the casein signature (Rps28, Rps4y2, and Rps18). This could lead to increased translation and perhaps account in part for the increased ventricle weight/tibia length seen on the casein diet in all experimental groups except wild-type males (20). The soy-enriched genes do not show any obvious connections in molecular function or pathways.

**DISCUSSION**

In summary, this study aimed to understand how sex, diet, and a genetic form of HCM impact gene expression patterns in the mouse heart. Figure 6 summarizes the cumulative effect of these three variables. Surprisingly, diet was by far the strongest modifier of gene expression among these variables. Moreover, although the number of genes differentially regulated by diet in wild-type animals was high (Fig. 1A: 498 male, 329 female), this number increased to 1,639 genes in males and 1,265 genes in females in the context of HCM, indicating that the diseased heart is much more sensitive to the environmental perturbations of diet. Interestingly, the genes regulated by diet in each sex only partially overlap, which points to a potential transcriptional basis for the sexually dimorphic phenotype observed in HCM (20). By comparison, we see <20 genes differentially regulated between the sexes on either diet (Fig. 1B). The finding that diet has the greatest impact on global gene expression illustrates the dramatic effect that environmental factors can have on health and disease.

Pathway-oriented analysis of the gene expression data led us to speculate that one reason HCM males respond so negatively to the soy diet is because even wild-type male animals show gene expression patterns consistent with mitochondrial dysfunction. As a result, they may be more sensitive to the

---

**Table 2. Genes always enriched in hearts of mice fed either casein or soy diet, regardless of sex or genotype**

<table>
<thead>
<tr>
<th>Casein Enriched</th>
<th>Soy Enriched</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cnbp1</td>
<td>Fnbp4</td>
</tr>
<tr>
<td>Tnp1</td>
<td>P4ha2</td>
</tr>
<tr>
<td>Gnas</td>
<td>Mcp6</td>
</tr>
<tr>
<td>Rps28</td>
<td>Scn11</td>
</tr>
<tr>
<td>B230364F10 (Mobb11a)</td>
<td>Akap8</td>
</tr>
<tr>
<td>Ah282936 (Nesp4)</td>
<td>P13xa3</td>
</tr>
<tr>
<td>Commd1</td>
<td>Sck394</td>
</tr>
<tr>
<td>Pcp41</td>
<td>Ptk3</td>
</tr>
<tr>
<td>Dmnt1</td>
<td>Inpp1</td>
</tr>
<tr>
<td>Anapc13</td>
<td>Ad5</td>
</tr>
<tr>
<td>Myb9</td>
<td>Atp5g1</td>
</tr>
<tr>
<td>Rgs19ip1</td>
<td>Stom</td>
</tr>
<tr>
<td>1500006009Rik (Thoc7)</td>
<td>Prss25</td>
</tr>
<tr>
<td>111003319Rik (Rps4y2)</td>
<td>Sipi</td>
</tr>
<tr>
<td>Lig3</td>
<td>Enpep</td>
</tr>
<tr>
<td>Shd1g1</td>
<td>Pcdha12</td>
</tr>
<tr>
<td>Nduv2</td>
<td>Got2</td>
</tr>
<tr>
<td>2900010M23Rik</td>
<td>Cyp2c39</td>
</tr>
<tr>
<td>Rps18</td>
<td>C1q9</td>
</tr>
<tr>
<td>Ptp4a3</td>
<td>S100a14</td>
</tr>
<tr>
<td>11100010J03Rik (Fmcl)</td>
<td>Rab6</td>
</tr>
<tr>
<td>C77604 (Bud31)</td>
<td></td>
</tr>
<tr>
<td>Tceaa</td>
<td></td>
</tr>
</tbody>
</table>

---

Fig. 6. Diet is the strongest modifier of gene expression as judged by total number of statistically significant, differentially expressed genes. The numbers represent the combined sum of unique genes from all comparisons corresponding to the modifier in question: sex, disease, or diet. For instance, the 2,251 genes in the diet category collectively represent casein vs. soy for wild-type male, wild-type female, HCM male, and HCM female. This is not equal to the sum of genes presented in Fig. 1A because there is redundance between comparisons.
transgene effect than casein-fed mice. We have previously shown that male HCM hearts display decreased state 3 respiration at 4 mo and ultrastructural abnormalities as well as decreased Complex I and IV activity by 8 mo old (11). Results presented here indicate that gene changes associated with these processes are already occurring by 2 mo of age. The particular genes regulated in the mitochondrial dysfunction category suggest that hearts from male soy-fed mice are more sensitized to an apoptotic response; therefore, they may be less able to adapt to the HCM-causing transgene. In fact, we have previously shown that, while caspase-3 activity is induced in male HCM mice fed either diet, it is over twice as active in soy-fed mice compared with casein-fed mice (20).

Conversely, females do not respond to the transgene or to the different diets in the same way as males. To begin with, female hearts do hypertrophy, but they do not typically proceed to dilation, even when the mice are fed the standard soy-based diet. In addition, cardiac function is better preserved and fibrosis is less extensive (12). These measures of cardiac health are similar in casein-fed females (20). As we were interested in why soy-fed transgenic females do not develop as severe a form of HCM as soy-fed transgenic males, we inspected the pathways that respond to diet in females. If the gene expression profile of soy-fed wild-type male hearts suggests that they are poised to adapt poorly to the transgene, we hypothesized that the opposite may be true for females. We anticipated a gene expression profile congruent with cardioprotection and cell survival. We observed that genes in the IGF-1 pathway change with diet in wild-type female hearts. IGF-1 is known to exert prosurvival effects in cardiomyocytes through the activation of Akt (5); this may explain why females are more able to adapt to the transgene. Interestingly, premenopausal women display significantly higher levels of nuclear phospho-Akt in cardiomyocytes compared with men or postmenopausal women (2).

If we consider this analysis as a whole, it appears that wild-type males fed a soy diet may experience a cardiac environment that is less able to adapt to stresses on the heart, such as the HCM-causing transgene. However, casein-fed HCM males experience a cardiac environment where antioxidant genes, including peroxiredoxin 5 (Prdx5), thioredoxin reductase 2 (Txnrdr2), and glutathione peroxidases 4 and 7 (Gpx4, Gpx7), are more abundant, thus allowing the heart to adapt to stress. In contrast, hearts from female WT mice are not predisposed to mitochondrial dysfunction. Instead, they may benefit from the cardioprotective effects of IGF-1 signaling. This may in part explain why HCM female mice manifest a less severe phenotype than males.

Most prior experimentation in laboratory rodents has been conducted without great attention to diet. However, it is clear from our studies that diet is a potent modifier of disease and gene expression and should be taken into account when designing animal studies. Also, if laboratory rodents are intended to serve as models of human disease, the soy-based diet used almost exclusively by investigators certainly does not recapitulate either a human diet or the diet ingested by mice in the wild. Finally, laboratory rodent studies on sex differences in disease could be confounded by the presence of phytoestrogens, which are known to bind estrogen receptors and inhibit tyrosine kinases (19). Several cautionary articles have recently appeared about the impact of consuming large amounts of such potent, bioactive compounds in soy food and dietary supple-

ACKNOWLEDGMENTS

We thank Laura Edgerly for technical assistance. We also thank Pamela Harvey and Ben Barthel for critical reading of the manuscript.

Current addresses: E. D. Luczak, Dept. of Internal Medicine, Univ. of Iowa, Iowa City, IA 52242; J. P. Konhilas, Molecular Cardiovascular Research Program, Univ. of Arizona, Tucson, AZ 85724; T. H. Cheung, Dept. of Neurology and Neurological Sciences, Stanford Univ. School of Medicine, Stanford, CA 94305.

REFERENCES


