A Functional Analysis of Mouse Models of Cardiac Disease through Metabolic Profiling

Since the completion of the human and mouse genomes, the focus in mammalian biology has been on assessing gene function. Tools are needed for assessing the phenotypes of the many mouse models that are now being generated, where genes have been “knocked out,” “knocked in,” or mutated, so that gene expression can be understood in its biological context. Metabolic profiling of cardiac tissue through high resolution NMR spectroscopy in conjunction with multivariate statistics has been used to classify mouse models of cardiac disease. The data sets included metabolic profiles from mouse models of Duchenne muscular dystrophy, two models of cardiac arrhythmia, and one of cardiac hypertrophy. The metabolic profiles demonstrate that the strain background is an important component of the global metabolic phenotype of a mouse, providing insight into how a given gene deletion may result in very different responses in diverse populations. Despite these differences associated with strain, multivariate statistics were capable of separating each mouse model from its control strain, demonstrating that metabolic profiles could be generated for each disease. Thus, this approach is a rapid method of phenotyping mouse models of disease.

Following the sequencing of the mouse (1) and human genomes (2), attention has focused on “omic” technologies for understanding gene function. In conjunction with the increased availability of techniques for manipulating the genome, this has led to a proliferation of mouse models of human pathology. However, many mouse models do not express the desired phenotype associated with the gene or protein in human sufferers. For example, disruption of the gene for dystrophin results in severe muscle wasting culminating in death within the 2nd decade in humans (3, 4) but produces a mild pathology in the mouse, with only a small reduction in longevity in this animal (5). Thus, tools are needed for assessing the phenotype of a model so that gene expression and protein activity can be understood in their biological context.

A similar phenomenon has also been described in yeast (Saccharomyces cerevisiae) where genetic manipulations may produce “silent phenotypes” in terms of the growth rate of yeast strains, the main characteristic for phenotyping this organism. To measure a phenotype in such “silent” strains, Raamsdonk and co-workers (6, 7) suggested an approach described as Functional ANalysis by Co-responses in Yeast (FANCY), which uses global analytical tools such as 1H NMR spectroscopy or mass spectrometry (8) to study the metabolic changes induced in different yeast mutants. These profiles were then used to classify samples, clustering mutants that arise from similar deletions together. For example, yeast mutants involving the deletion of one of two genes encoding the same enzyme, 6-phosphofructo-2-kinase, produced the same metabolic phenotype, and deletions involving oxidative phosphorylation also clustered together (7). Thus, such a process of defining a phenotype through the global changes induced in metabolism may be used to predict the function of genes deleted or up-regulated in a given system through comparative metabolomics.

The definition of a metabolic phenotype, or metabotype, by large scale analysis of metabolites using either 1H NMR spectroscopy or mass spectrometry has found a number of applications in genetic engineering, toxicology, and disease diagnosis in plants, animals, and microbes (9–12). In this study we demonstrate that the FANCY approach described for yeast can be extended to mammalian systems by using high resolution NMR spectroscopy to classify mouse models of cardiac disease. By using multivariate analysis, the metabolic profiles of a mouse model of Duchenne muscular dystrophy (mdx) (13), two models of cardiac arrhythmia (a heterozygous gene deletion of the cardiac sodium channel (Scnβ±) as well as a heterozygous gain in function for the previous gene deletion (Scnβ−/−) (14)) and one of cardiac hypertrophy (muscle LIM protein knock out (MLPKO) (15)), were used to classify and co-cluster cardiac tissues. Table I summarizes the reported phenotypical characteristics of these mouse models in terms of visual observations and gross pathology. In each model the primary genetic lesion is not associated with an enzyme or metabolic pathway, indicating that such approaches of deriving metabolic phenotypes can be applied generally to functional genomic studies. We have termed this approach “phenotype information by metabolic profiles.” Furthermore, we demonstrate that the strain background can have a profound impact on the resultant metabolism of the mouse, which may provide insights into how a
given gene deletion results in very different responses across genetically diverse populations.

**MATERIALS AND METHODS**

**Animal Handling**—All mice were maintained according to the UK Home Office guidelines. Male 4–5-month-old mice were removed from stable colonies of C57BL/10 control mice (n = 15), mdx mice (n = 16), MLPKO mice (n = 6), B6129S2Svcs control mice to MLPKO (n = 6), Scn16 (n = 8), Scn16 (n = 8), and 129Sv control mice (n = 16). Animals were sacrificed by cervical dislocation, and cardiac tissue was removed rapidly. Tissue was immediately frozen in liquid nitrogen and stored at −80 °C prior to NMR analysis. Genetic integrity of the colonies was monitored throughout by standard genotyping techniques.

**NMR Spectroscopy**—Frozen cardiac tissue was pulverized using a pestle and mortar, and metabolites were extracted using perchloric acid (100 mg wet weight tissue in 1 ml of 6% perchloric acid). To further aid extraction, the perchloric acid/tissue powder mixture was further mixed using a Polytron (3 bursts for 30 s). The solution was neutralized with KOH and the precipitate discarded. The resultant solution was lyophilized, and the extracts were redissolved in D$_2$O containing 1 mM TSP. The solution was neutralized with NaOH and metabolites were extracted using perchloric acid monitored throughout by standard genotyping techniques.

All solution state spectra were acquired using a 9.6-tesla superconducting magnet interfaced to an INOVA spectrometer (Varian, CA). Solvent suppressed spectra were acquired into 16,384 data points, summed over 256 scans.

**HRMAS Spectroscopy**—Spectra were phased, baseline corrected, and referenced to the singlet of TSP at 1.3 ppm. The output vector representing each spectrum was normalized across the integral regions, excluding the water resonance, effectively standardizing all the individual integrals to the total integral of all the low molecular weight metabolites (16, 17). Data sets were imported into the SIMCA package (Umetrics, Umeå, Sweden) and then preprocessed using Pareto scaling by multiplying each variable by (1/sk$^2$) where sk is the variance of the variable. This scaling effectively increases the importance of low concentration metabolites in the resultant models but not to an extent where the noise significantly contributes to the model.

Data were analyzed by using principal components analysis (PCA) and the supervised regression extension, partial least squares discriminant analysis (PLS-DA), within the package SIMCA (Umetrics, Umeå, Sweden). PCA is a quantitatively rigorous method for replacing a group of variables with a smaller number of new variables, called principal components (PC), which are linear combinations of the original variables. All the principal components are orthogonal to each other so there is no redundant information. Projecting the observations on one of these axes generates another new variable designed to maximize the description of the variance in the data set. PLS-DA is a generalization of PCA where a projection model is developed predicting class membership from the variables (X matrix) via scores of these variables through a generalized multiple regression method that can cope with a number of variables being correlated with class membership.

Cross-validation of PLS-DA was carried out by leaving out every 6th observation and predicting the observation’s class membership on a new model as part of a jack-knifing routine. The prediction error sum of squares (PRESS) is the squared differences between observed and predicted values for the data. For every component, the overall PRESSSS was calculated, where SS is the residual sum of squares of the previous dimension. The final PRESS score then has contributions from all data. The goodness of fit algorithm was used to determine whether a correlation was significant ($Q^2 > 0.05$), and is defined in Equation 1.

$$Q^2_{cum} = 1 - \sum \left( \text{PRESS/SS} \right)$$

The major metabolic perturbations between cardiac tissues from different mouse models were determined from loadings scores and the variable importance parameters for each pattern recognition model. Loading plots display the correlation between the X variables, in the first dimension, or the residuals of the X variables in subsequent dimensions, and class membership. X variables with large weights (positive or negative) are highly correlated with class. Variable importance parameter is the influence on class membership (Y) of every term in the model, summed over all model dimensions, and is equal to the squared PLS weight of that term, multiplied by the explained SS of that PLS dimension (18). To confirm the importance of these metabolites, the integral regions were excluded from the analysis to examine their leverage on the models produced. New models were produced in an analogous manner to the jack-knifing routine described previously, and the goodness of fit algorithm was used to determine whether a metabolite was responsible for a statistically significant classification.
the age range examined, with all mice showing similar levels of activity, and no increase in mortality rate was detected for any of the mouse mutants at this age. Consistent with previous literature reports there was no significant difference in cardiac mass for the mdx and sodium channel modification mouse models (data not shown). However, as previously reported, the MLPKO mouse heart demonstrated mild hypertrophy at this age (mass of MLPKO hearts, $0.255 \pm 0.051$ g; B6129S2Svs control strain, $0.185 \pm 0.023$ g; unpaired $t$ test, $p = 0.0119$). No pathological changes were detected post-mortem by visual inspection of the hearts from mdx, SCN$^{+/+}$, and class $PLS$-DA model was built with the data set (Fig. 2B). The C57BL/10 strain background mice all had increased concentrations of creatine (chemical shift of resonances identified in the loadings scores $\delta$; 3.04 and 3.92), lactate (3.14), phosphocholine (3.24), and choline (3.20) compared with the other strains, whereas the C57BL/10 strain background was characterized by increased concentrations of glucose (3.48, 3.52, and 3.56) and $\beta$-hydroxybutyrate (1.12 and 1.16). Aqueous extracts of cardiac tissue from the B6129S2Svs strain background exhibited a higher concentration of taurine (3.28 and 3.44).

To examine whether these differences in relative metabolite concentrations in tissue extracts could be used to predict the mouse strain from which the tissue was derived, a supervised PLS-DA model was built with the data set (Fig. 2C), and class membership was predicted for each strain. This model correctly predicted the strain for both mice on C57BL/10 and 129Sv backgrounds with 100% accuracy, but only produced a model that was 53% accurate for the B6129S2Svs background strain. To define further the difference between strains measured in this data set, a Cooman’s plot was formed using PCA models of the C57BL/10 and the 129Sv backgrounds (Fig. 2D). For this a PCA model was built to describe the variation in just the subset of data relating to a particular strain. These two models were then used to predict whether each observation belonged to the C57BL/10 background, the 129Sv background, and could not be separated (i.e. the model predicted the sample to be both C57BL/10 and 129Sv), or belonged to neither model. Only one observation from the 129Sv background subset was outside the 95% confidence limit for this strain, whereas all the C57BL/10 background mice were within the 95% confidence limit for this strain (i.e. the strain of only one mouse was not correctly
predicted for the 129Sv and C57BL/10 mice). By using this Cooman’s plot, two spectra from the B6129S2Svs background were misclassified, and the rest of the data set were determined to lie outside the two PCA models (i.e. were correctly predicted as being neither 129Sv nor C57BL/10 strain mice).

To examine whether the different strain backgrounds also had different proportions of NMR-detectable lipids, a similar analysis was performed on the HRMAS 1H NMR spectra data set. Again, strain differences dominated the PCA of the complete HRMAS data set. This separation was further improved by building a PLS-DA model with this model predicting class membership for all 48 spectra in terms of the strain (Fig. 2E).

The 10 most important resonance regions responsible for this classification as identified by the variable importance parameter were from the metabolites taurine (δ 3.28 and 3.44), CH$_2$CH$_2$CH$_2$ lipids (boldface type indicates the resonance observed; δ 1.24–1.36), lactate (δ 1.32), choline (δ 3.2), CH$_3$CH$_2$ lipid (δ 0.92), phosphocholine (δ 3.24), and creatine (δ 3.04) (Fig. 2F).

**Despite Strain Differences, Distinct Metabolic Profiles Are Still Detectable for Each Mouse Model**—As strain differences dominated the first components of the PCA model, and PLS-DA models were used to distinguish the four animal models of disease. We used three different routines to identify metabolic changes with the disease process (Fig. 3).

For the first set of models the data were correlated to distinguish one disease model (one group) from the total remaining data set (the other group representing all other disease models and all control mice). The most significant model in terms of the goodness of fit algorithm ($Q^2$) was produced for Scn$^{-/-}$ mice ($Q^2_{cum} = 0.46$), then Scn$^{+/-}$ mice ($Q^2_{cum} = 0.38$), then MLPKO mice ($Q^2_{cum} = 0.25$), and the least for the model of DMD ($Q^2_{cum} = 0.17$) (models not shown). These models were used to predict disease status across the complete group (Table II).

However, because of the large differences associated with strain, the data from the individual disease models and their respective control strain were processed alone using PLS-DA. Highly predictive models were produced for each disease Fig. 4, A–C. Each disease state had a distinctive metabolic profile and could be distinguished by the variable importance parameter.
scores of key metabolites (Fig. 4, D–F). A PLS-DA analysis of the aqueous cardiac spectra from the sodium channel mouse models and the respective control was able to predict the class of 26 out of 28 samples correctly using a jackknifing procedure (Table III). The most discriminatory metabolites for this model were taurine (3.28 and 3.40–3.44), lactate (1.36 and 1.32), phosphocholine (3.24), choline (3.20), creatine (3.04 and 3.92), and glucose (3.48). By applying PLS-DA to the data set containing the MLPKO transgenic mouse and its control strain, the model was 93% correct in predicting disease presence, with the most perturbed metabolites being citrate (2.76 and 3.40–3.44), lactate (3.28, 3.40, and 3.44), phosphocholine (3.24), lysine (3.12), alanine (1.40 and 1.44), creatine (3.92), taurine (3.44 and 3.28), and glutamate (2.16). The least predictive model was produced for the mdx mouse and its control strain. Whereas the model predicted the control strain correctly, only 57% of the mdx mice were predicted correctly. The most discriminatory metabolites in this model were lactate (2.76 and 2.72), phosphocholine (3.24), lysine (3.12), alanine (1.40 and 1.44), creatine (3.92), taurine (3.44 and 3.28), and glucose (3.48, 3.68, and 3.76).

To examine whether disease had an impact on global metabolism by increasing or decreasing the variation in the complete set of metabolites, PCA, an unsupervised approach, was applied to measure the variation across the data set. To ensure similar sampling for the PCA, a randomly selected equal number of spectra (n = 6) were chosen for each mouse type. PCA models were built for spectra from disease models and control strains. For each disease model/strain control comparison, the diseased animal demonstrated greater variation according to the sum of squares of the maximum eigenvalues of the first two components. Of the disease models, the largest variation was detected in the mdx mouse. By comparing each individual

### Table II

| Mouse models | NMR experiment | Components | $R^2_{cum}$ | $Q^2_{cum}$ | Correct predictions for mouse model | Correct predictions for other models |
|-------------|----------------|------------|------------|-------------|-----------------------------------|-----------------------------------|
| Scn$^{+/+}$ | Solution       | 2          | 0.38       | 0.39        | 5/8                               | 64/64                             |
| Scn$^{-/-}$ | HRMAS          | 2          | 0.31       | 0.14        | 4/8                               | 40/40                             |
| MLPKO       | Solution       | 4          | 0.51       | 0.46        | 7/8                               | 65/65                             |
| MLPKO       | HRMAS          | 2          | 0.31       | 0.29        | 6/7                               | 37/41                             |
| Mdx         | Solution       | 2          | 0.36       | 0.25        | 8/8                               | 63/64                             |
| Mdx         | HRMAS          | 2          | 0.38       | 0.29        | 8/8                               | 40/40                             |

**Fig. 4. Pattern recognition models of metabolic changes associated with genetic modification in solution state spectra.** In each model, only mice on the same strain background were considered to investigate the impact genetic modification had on the metabolic phenotype of the heart. In each case a PLS-DA model was created correlating metabolic changes with group membership (i.e. disease status). A, sodium channel modifications. B, muscle LIM protein modification. C, Duchenne muscular dystrophy. D–F, show the corresponding variable importance parameter plots for A–C, respectively.
control strain the greatest variation was measured for the C57BL/10 background strain compared with the other two strains (data not shown).

For the intact tissue data set, as with the spectra from tissue extracts, individual PLS-DA models distinguished a particular disease state from the rest of the data set (data not shown) or when the mouse model was compared with its control strain only (Fig. 5, A–C). However, unlike the aqueous extracts, the MLPKO mouse was most readily distinguished from the other models (Q^2cum/0.29), then Scn^−/− mice (Q^2cum/0.28), then Scn^+/− mice (Q^2cum/0.14), and mdx the least (Q^2cum/0.07). These models were used to predict disease status (Table III) and to identify the metabolites most important for these classifications (Fig. 5, B and C). Both the Scn^+/− and Scn^+/− mice had decreased lactate and CH2CH2CH2 lipids (δ 1.32, δ 1.28) compared with their control strain, whereas the Scn^+/− mice had an increased concentration of taurine (δ 3.28) compared with both the control strain and the Scn^+/− mice. The mdx mice had increased concentrations of taurine (δ 3.28 and δ 3.44), lactate (δ 1.32), CH2CH2CH2 lipids (δ 1.32 and δ 1.28), CH3CH2 lipids (δ 0.80, δ 0.84, δ 0.88, δ 0.90, and δ 0.94), glutamate (δ 2.04), and decreased glucose/glycogen (δ 3.32, δ 3.46, δ 3.52, and δ 3.56), and creatine (δ 3.96) compared with its control strain. The MLPKO and control mice were distinguished by increased taurine (δ 3.28 and 3.44), creatine (δ 3.04), lactate (δ 1.32), CH3CH2CH2 lipids (δ 1.32 and δ 1.28), and CH=CH lipids (δ 5.32), and by decreased phosphocholine/phosphatidylcholine (δ 3.24), glutamate (δ 2.36), and CH3CH2 lipids (δ 0.88).

| Mouse models | NMR experiment | Components | R^2cum | Q^2cum | Correct predictions for disease model | Correct predictions for control strain |
|--------------|----------------|------------|--------|--------|--------------------------------------|--------------------------------------|
| Scn^+/−      | Solution       | 4          | 0.81   | 0.14   | 7/8                                  | 12/13                                |
| Scn^−/−      | HRMAS          | 6          | 0.89   | 0.28   | 7/7                                  | 7/8                                  |
| Scn^+/−      | HRMAS          | 2          | 0.45   | 0.29   | 8/8                                  | 8/8                                  |
| MLPKO        | Solution       | 2          | 0.53   | 0.18   | 8/8                                  | 6/7                                  |
|             | HRMAS          | 2          | 0.46   | 0.07   | 8/14                                 | 13/15                                |

**TABLE III** Comparing a given disease group against its control strain

PLS-DA models were built that distinguished a given mouse model of a disease from its control strain. For the two mouse models where the cardiac sodium channel was modified, a single PLS-DA model was built to distinguish Scn^+/−, Scn^+/−, and control strain. Models were built by both solution state and HRMAS NMR spectroscopy. The predictions are separated into two groups as follows: correct prediction of the disease and correct prediction of control strain status.
Orthogonal Signal Correction Spectral Filtering Can Remove Strain Differences and Highlight Disease Differences Simultaneously in All Models—The spectral filter orthogonal signal correction removes variation from a data set (the X variables) that is not correlated with a Y variable (in this case a variable signifying disease status) by using PLS regression. Applying this filter to the data set to identify the variation correlated with disease status removed two components representing 36% of the variation in the original data set. By applying PLS-DA to the resultant data set, all five disease models were separated across three PLS-DA components (Fig. 6), and the three control strains were clustered together. The metabolites responsible for this separation were lactate (δ 1.32 and 1.36), taurine (δ 3.28, δ 3.40, and δ 3.44), creatine (δ 3.04), citrate (δ 2.72 and δ 2.80), phosphocholine (δ 3.24), and succinate (δ 2.40).

**DISCUSSION**

For both technical and financial reasons, the mouse is currently the most commonly used tool for studying mammalian genomics through transgenes and gene targeting strategies. This necessitates rapid tools for phenotyping animals so that a given mouse model can be placed in context with existing models and the human disease that is being modeled. For cardiac diseases, a range of physiological measures can be used to assess the heart shape and function (echocardiography, conductance volumetry, sonomicrometry, and magnetic resonance imaging), pressure, flow (Doppler echocardiography), and electrophysiology (19). However, a comprehensive phenotyping of mice using these physical approaches would be both costly and time consuming. In addition to these physical techniques, biochemical assays have been applied as a blood biochemistry screen to assess mutants produced as part of a large scale mutagenesis program (20). Such biochemical phenotyping is appropriate to high throughput screening and is also relatively cheap on a per animal basis. However, the diseases that can be screened could be limited by the assays chosen.

High resolution 1H NMR spectroscopy of biofluids in conjunction with pattern recognition has been used to screen drug effects in toxicology studies (12, 17, 21, 22), providing a rapid method of identifying biochemical changes following drug-induced lesions. Biofluid 1H NMR spectroscopy has also been used to diagnose coronary artery disease and high blood pressure through metabolic profiles of blood sera in patients (23, 24). In this study we have applied a similar approach to phenotype four mouse models of cardiac disease. We chose a range of mouse models of cardiac disease to investigate the utility of this approach as a phenotype screening tool for genetically

**Fig. 6.** An orthogonal signal corrected (OSC) PLS-DA analysis of all the mouse models of disease using the solution state NMR data set. A, three-dimensional plot of the PLS-DA model of all the disease models compared with one control group (containing mice from the three strains). B, VIP scores for the most discriminatory NMR regions for this model. ○, control; ↑, mdx; ♦, MLPKO; ■, Scn-/-; ▲, Scn+/

modified mice. Abnormalities in cardiac function were present in each mouse model, but these abnormalities did not have a significant impact on mortality and morbidity rates at this age. However, although the MLPKO mice have left ventricular dilatation (25) and the mdx mouse has a pronounced hunch and lower muscle regenerative capacity (26, 27), neither mouse model has a mortality rate significantly different from their wild type control strains. Although our approach was sensitive enough to distinguish a given disease model from its control strain, the largest influence on the total data sets was that of the background strain of the mouse.

Influence of Strain Background—The mdx, MLPKO, and the scid channel mouse models were all produced on separate background strains, and thus, three separate control strain backgrounds were included in this study. These background strains were C57BL/10 for the mdx mouse, a mixed cross of 129SV and C57BL/6 for the MLPKO mice (referred to as B6129S2Sv), and 129SV for the scid channel sodium channel modifications. Applying both PCA and PLS-DA to the data, the largest correlated variation across both the solution state and HRMAS spectra data sets were associated with the background strain, with this variation dominating any differences associated with disease. Gavaghan et al. (11) have previously separated C57BL10J and Alpk:Apx1CD mouse strains of healthy mice using NMR spectroscopy of the urinary metabolites. This largely resulted from Krebs cycle intermediates and methyamine pathway products, demonstrating that some of the key metabolic intermediates were highly variable across different strains.

In the present study the metabolites that were most discriminatory in classifying the different strains included creatine, lactate, phosphocholine, choline, glucose, β-hydroxybutyrate, taurine, and saturated lipids, accounting for many of the high concentration metabolites detected using 1H NMR spectroscopy. These metabolites represent some of the key metabolic processes in the heart, including energy transfer (creatinine), osmoregulation (taurine, creatine), ATP generation (lactate, glucose, β-hydroxybutyrate, and lipids), Ca2+ homeostasis (taurine), and cell membrane constituents (phosphocholine and lipids). The relatively low sensitivity of NMR spectroscopy means that our approach detects only a fairly small number of metabolites, but because metabolic networks are dominated by a few highly connected nodes, changes detected in the metabolome in seemingly unrelated pathways can have a profound effect. Furthermore, these networks can operate as amplification mechanisms, producing large changes in concentration for key nodes (28–31). A corollary of this is that for the pattern recognition models, it is as much the ratio of metabolic changes that are important in the classifications as the actual metabolite concentrations. Rather than identify a unique biomarker for a strain or disease process, classifications have been produced according to changes in the relative proportions of key node metabolites. Although this complicates cross-correlations between the cardiac metabolome and the genome, this property suggests that 1H NMR spectroscopy, even of only the high concentration metabolites, may still have wide applicability to high throughput screening of diverse phenotypes.

The Impact of Disease on High Concentration Metabolites—A number of studies have already demonstrated that mdx mice are categorized by both increased concentration of taurine and decreased creatine in the heart and muscle tissue in general (26, 27, 32). The concentration of taurine has been correlated with the ability of muscle tissue to regenerate (26). We have also previously used 1H NMR spectroscopy and pattern recognition techniques to compare mdx mice with utrophin transgene (Tgfull length/Dmdmdx) transgenic mice lacking dystrophin, but with up-regulated utrophin expression in skeletal muscle but not cardiac tissue. The dystrophic phenotype normally observed in mdx mice is absent when muscles overexpress utrophin (33), and utrophin-based gene therapy has been suggested as one potential treatment for Duchenne muscular dystrophy (34). The concentration of taurine in skeletal muscle of utrophin Tgfull length/Dmdmdx transgenic mice was intermediate between mdx and control mice, suggesting that the metabolic profiles determined by 1H NMR spectroscopy could potentially be used to follow gene therapy. One role for the increased taurine in dystrophic tissue could be to compensate for increased intracellular Ca2+ in the tissue (35).

To our knowledge this is the first description of metabolic deficits associated with both the MLPKO and scid channel modified mouse models. Both animal models represent important human diseases (cardiac hypertrophy and cardiac arrhythmia, respectively). The approach was also sensitive to distinguish whether the cardiac sodium channel was deleted (SCN−) or a gain of function was produced (SCN+/−), with both genetic modifications resulting in cardiac arrhythmia. In all cases high concentration metabolites distinguished the disease model from the control strain. This suggests that in vivo MRS of the heart may be capable of distinguishing a number of common cardiac disorders in the future in a similar manner to the capability of in vivo MRS of the brain by distinguishing a number of disorders based on the concentration of N-acetyl aspartate, creatine, choline and lactate, all found in high concentrations in the brain. A corollary of this is that certain mouse models of disease may be more appropriate on a given background, and this may be particularly important for multifactorial diseases such as diabetes and dyslipidemia where there is significant interaction between several genes. Indeed, Linder (37) has suggested that all the control strains available should be used as the wild type con-
trols for a transgenic or knock-out mouse model. Furthermore, the background for many disease models may be dynamic in nature. Many transgenic and knock-out mice are generated on a mixed background, and with subsequent generations any number of alleles, all potentially interacting with the gene under investigation, could be fixed into the final genome (38). Thus, rapid and high through-put processes for phenotyping mouse models are set to become increasingly important to address such issues.

Conclusions—The combined metabolic approach of $^1$H NMR spectroscopy and pattern recognition techniques provides a rapid method of phenotyping mouse models of disease. Despite the relatively small number of metabolites detected in tissue extracts of heart muscle, the ratios of these metabolites were highly discriminatory, reflecting the central nodes that many of these metabolites have in different metabolic pathways.

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