Published in final edited form as:

Mech Ageing Dev. 2008 March ; 129(3): 138–153. doi:10.1016/j.mad.2007.11.003.

COMPARATIVE ANALYSIS OF MICROARRAY DATA IDENTIFIES COMMON RESPONSES TO CALORIC RESTRICTION AMONG MOUSE TISSUES

William R. Swindell

University of Michigan, Department of Pathology, Ann Arbor MI, 48103

Abstract

Caloric restriction has been extensively investigated as an intervention that both extends lifespan and delays age-related disease in mammals. In mice, much interest has centered on evaluating gene expression changes induced by caloric restriction (CR) in particular tissue types, but the overall systemic effect of CR among multiple tissues has been examined less extensively. This study presents a comparative analysis of microarray datasets that have collectively examined the effects of CR in ten different tissue types (liver, heart, muscle, hypothalamus, hippocampus, white adipose tissue, colon, kidney, lung, cochlea). Using novel methods for comparative analysis of microarray data, detailed comparisons of the effects of CR among tissues are provided, and 28 genes for which expression response to CR is most shared among tissues are identified. These genes characterize common responses to CR, which consist of both activation and inhibition of stress-response pathways. With respect to liver tissue, transcriptional effects of CR exhibited surprisingly little overlap with those of aging, and a variable degree of overlap with the potential CR-mimetic drug resveratrol. These analyses shed light on the systemic transcriptional activity associated with CR diets, and also illustrate new approaches for comparative analysis of microarray datasets in the context of aging biology.

Keywords

aging; diet restriction; gene expression; longevity; microarray

1. Introduction

Caloric restriction is an effective intervention for delaying age-related disease and extending good health into later ages of the mammalian lifespan. Much interest in caloric restriction (CR) has centered on its unique life-extending effects, but increasingly, its impact on age-related disease and biomarkers of the aging process have received attention. In mammals, CR has been found to combat inflammation (Kalani et al., 2006), reduce endogenous levels of oxidative stress (Yu, 2006), increase insulin sensitivity (Anderson and Weindruch, 2006), decrease core body temperature (Mattison et al., 2003) and promote elevated resistance to stress treatments (Bruce-Keller et al., 1999; Apte et al., 2003). Taken together, these effects appear to combat many diseases of aging. Mammals that consume 30–40% fewer calories exhibit a lower incidence of Type 2 diabetes (Astrup, 2001), neurodegenerative disorders (Logroscino et al., 1996; Patel et al., 2005), hearing loss (Someya et al., 2006) and cardiovascular disease (Fontana et al., 2004). An especially important effect of CR is tumor suppression and reduced mortality

due to cancer (Kritchesky, 2001; Hursting et al., 2003; Klebanov, 2007), which in mice, may be the key factor underlying lifespan extension (Spindler, 2005; Spindler and Dhabi, 2007). These results have been supported by ongoing experimental studies with rhesus monkeys (Ingram et al., 2006), and several observational analyses have revealed health advantages of CR diets in humans as well (Holloszy and Fontana, 2007).

Gene expression patterns are a fundamental level of investigation that may reveal key mechanisms underlying the health benefits of CR diets (Spindler, 2006; Spindler and Mote, 2007). DNA microarrays are especially valuable in this regard, since microarrays allow the genome-wide transcriptional effects of CR to be quantified. This is useful for evaluating the expression patterns of genes already known to mediate the effects of CR, but more importantly, can identify new candidate genes that were previously unlinked to CR mechanisms. In mice, previous studies have used microarrays to investigate the effects of CR in a wide range of tissue types (Lee et al., 2002; Higami et al., 2003; Massaro et al., 2004; Tsuchiya et al., 2004; Dhabi et al., 2005; Dhabi et al., 2006; Fu et al., 2006; Selman et al., 2006; Someya et al., 2006; Edwards et al., 2007; Wu et al., 2007). Results from these studies have identified genes that may be important mediators of health benefits that stem from CR diets. Genes for which expression is induced by CR in one study, however, often differ from those identified in other studies, even when the same tissue has been examined in both cases. Such discrepancy may result from Type I errors, alternative statistical analysis methods or experimental differences between two studies (Rosati et al., 2004). Results obtained from a single microarray study may therefore be of limited generality. Additionally, since previous studies have examined the effects of CR in one or only a small number of tissue types, only limited conclusions regarding expression patterns among tissues have been obtained. Few studies, for example, have been sufficiently comprehensive to identify genes induced by CR in the same direction across multiple tissue types (Fu et al., 2006; Selman et al., 2006; Spindler and Dhabi, 2007).

The comparative analysis of DNA microarray datasets can identify highly robust gene expression patterns that occur most consistently among multiple experimental investigations (Larsson et al., 2006). In the context of studies investigating genes activated by CR, comparative analysis yields two specific points of insight. First, with respect to any one tissue type, identifying genes correspondingly induced across multiple studies provides a way of filtering out false positive identifications, as well as genes whose identification may be dependent upon the strain of mouse used, nutritional composition of diet, tissue processing prior to array hybridization and other methodological practices that may vary among laboratories. This yields a list of highly supported candidate genes, for which the effects of CR on expression patterns is most likely repeatable in other contexts. Secondly, with respect to multiple tissue types, comparative analysis can identify genes for which the effect of CR is most common among tissues (Spindler and Dhabi, 2007). Given that some mechanisms of CR may be conserved between invertebrates and mammals (Motta et al., 2004), and that some mechanisms could be mediated by a common set of genes independently of tissue type (e.g., combating oxidative stress), it is plausible that such shared CR response genes exist. If so, their identification would shed light on systemic processes underlying the health benefits of CR, and also provide targets whose expression patterns can be mimicked through interventions not localized to individual tissues.

This study presents a comparative analysis of microarray datasets that have examined the effects of CR in a wide range of mouse tissues. The analysis is based on data generated from studies that have collectively examined the effect of CR in ten different tissue types (liver, heart, muscle, hypothalamus, hippocampus, white adipose tissue, colon, kidney, lung, cochlea). A detailed comparison of the effects of CR among tissues is provided, and genes for which response to CR is most shared among tissue types are identified. Further analyses were aimed at evaluating the effects of both CR and aging on gene expression in liver, since this tissue type

has been a focus in many studies (Amador-Noguez et al., 2004; Tsuchiya et al., 2004; Dhahi et al., 2005; Boyleston et al., 2006; Niedernhofer et al., 2006; Someya et al., 2006; Fu et al., 2006; Edwards et al., 2007; Wu et al., 2007). With regard to liver, it was possible to identify candidate CR genes based on consistency among CR studies, as well as expression changes due to aging. The effects of CR in liver were also compared to those of resveratrol, a promising CR mimetic compound that has received much interest (Baur et al., 2006). Results from these analyses characterize robust gene expression patterns associated with CR, and also demonstrate new analytical approaches for the comparative analysis of microarray datasets.

2. Materials and Methods

Microarray datasets were obtained from Gene Expression Omnibus (Barrett et al., 2007), ArrayExpress (Parkinson et al., 2007) or directly from the contact author of published studies. All datasets were generated from experiments that evaluated the effects of CR on gene expression patterns in one tissue, or in some cases, multiple types of tissue. While most datasets were generated using Affymetrix microarray platforms, data generated from commercial non-Affymetrix platforms were also incorporated into the analysis. Since the Affymetrix 430 2.0 array is the most recent Affymetrix microarray platform and provides the most comprehensive coverage of the murine genome, data from all experiments were joined through linkage with this platform. Mappings between the 430 2.0 array and other platforms were not strictly one-to-one, since probesets from other arrays sometimes matched more than one probeset on the more comprehensive 430 2.0 array. Probesets from different generations of Affymetrix microarrays were linked using best match tables published by Affymetrix. Probesets from non-Affymetrix commercial arrays were linked to the 430 2.0 array using maps generated by the Resourcerer online database (Tsai et al., 2001).

All experiments featured treatments with mice provided a CR diet, along with a corresponding control treatment in which mice were provided a normal diet (usually about 90 kcal per week). Each CR and control treatment pair was used to formulate a statistical *contrast* evaluating the effect of CR within a particular tissue type and under a particular set of conditions. A total of 23 such contrasts were included in the analysis (see Table 1). Six contrasts evaluated the effects of CR in liver, while five contrasts evaluated the effects of CR in heart tissue. For other tissues, the effects of CR were evaluated by either two (muscle, hypothalamus, white adipose tissue) or one contrast (hippocampus, colon, kidney, lung, cochlea). Each contrast corresponded to a series of differential expression tests, in which probesets significantly upregulated were assigned a score of 1, probesets significantly downregulated were assigned a score of -1, and probesets exhibiting no significant differential expression were assigned a score of 0. The pattern of 1, -1 and 0 values among all probesets defined the *differential expression signature* associated with a given contrast (Swindell, 2007a). Nearly all differential expression signatures analyzed in this study were generated from raw data using the same statistical methodology. This methodology consisted of normalization by Robust Multichip Average (Irizarry et al., 2003), and differential expression analysis using the Limma linear modeling package (Smyth, 2004), with P-value adjustment using the Benjamini-Hochberg method (Benjamini and Hochberg, 1995). In two cases where raw data was not available, differential expression signatures were generated using results provided as supplemental data in original research reports (Corton et al., 2004; Fu et al., 2006). For all signatures, functional analysis of differentially expressed genes was based on gene ontology terms, and overrepresentation analysis of differentially expressed genes was carried out using GOSTats (Falcon and Gentleman, 2007). Overrepresentation analysis was carried out by first identifying ontology terms significantly overrepresented with respect to at least one signature for each of the ten tissues examined. Terms overrepresented with respect to five or more tissues were then identified. Following p-value adjustments for multiple testing, a significance level of 0.05 was

used to identify differentially expressed genes as well as significantly overrepresented gene ontology terms.

Differential expression signatures generated by each statistical contrast were clustered based upon their overlap (Swindell, 2007a). Equation (1) describes the similarity measure used to carry out an average linkage hierarchical clustering of differential expression signatures. Notations associated with Equation (1) are provided in Table 2.

$$s = \frac{n_{+,+} + n_{-,-}}{n_{+,+} + n_{-,-} + n_{+,-} + n_{-,+} + \text{Min}[(n_{+,0} + n_{-,0}), (n_{0,+} + n_{0,-})]} \quad (1)$$

The value of s ranges between 0 and 1, such that the distance between two differential expression signatures is defined as $d = 1 - s$. For a given pair of signatures, the value of s is a proportion representing the number of genes with corresponding differential expression patterns ($n_{+,+} + n_{-,-}$), divided by the number of genes differentially expressed within the signature associated with fewer differentially expressed genes. This similarity measure is configured to ensure that signatures with many genes differentially expressed can be similar to signatures with few genes differentially expressed, provided that most genes differentially expressed in the latter signature have the same pattern in the former signature. This criterion is sensible because the total number of genes differentially expressed within any one signature is a function of statistical power. It should not be concluded that signatures with many differentially expressed genes are dissimilar from those with fewer, since the difference may be attributable to statistical power and sample size. The similarity measure represented by Equation (1) circumvents this potential difficulty. It should also be noted that Equation (1) does not assign any similarity based upon the $n_{0,0}$ genes that are not differentially expressed with respect to either of two signatures being compared. This is appropriate, since $n_{0,0}$ is typically large and would otherwise mask similarity based upon genes that are differentially expressed.

Statistical significance of the overlap between two differential expression signatures was evaluated by simulation analysis. For any one pair of differential expression signatures, the test statistic is T as defined by Equation (2) (see Table 2 notations).

$$T = n_{+,+} + n_{-,-} \quad (2)$$

The null hypothesis of interest is that the probability of a gene being differentially expressed within any one signature is independent of its probability of differential expression within another signature. Under this condition, the null distribution of T can be described analytically based upon approximations to the binomial distribution (Smid et al., 2003; Swindell, 2007a). In the present study, however, probeset maps between the Affymetrix 430 2.0 array and other platforms were not strictly one-to-one. In some cases, the same probeset from one platform was assigned to multiple probesets on the Affymetrix 430 2.0 array. It is therefore unrealistic to assume that the probability of up or downregulation is independent among probesets within the same signature. The Bernoulli trial condition is therefore untenable and the distribution of T cannot be modeled using a Binomial model. It was possible, however, to generate a null distribution of T for each pair of differential expression signatures by simulation. With marginal totals in Table 2 fixed for a given pair of signatures being compared, values of 0, 1 and -1 were randomly permuted among probesets within signatures, and the value of T was calculated after each of 10,000 permutations. For each pair of signatures being compared, this procedure generated a null distribution for T against which an observed value of T was compared. The analysis was carried out for all $23(23-1)/2 = 253$ pairwise combinations of signatures included

in the analysis, and resulting P-values were adjusted for multiple comparisons using the Benjamini-Hochberg method (Benjamini and Hochberg, 1995).

3. Results

3.1 Overview

Fewer than 23% of examined genes were significantly induced by CR in any one experiment, and in most cases, CR had a significant effect on less than 5% of examined genes (Table 3). The overall effects of CR varied among studies that examined the same type of tissue. With regard to liver, for instance, 22.1% of genes were differentially expressed with respect to the lvr1 contrast, while less than 1% of genes were differentially expressed with respect to the lvr4b and lvr10 contrasts. Among all tissue types examined, CR most commonly led to upregulation of genes involved in lipid metabolism and metal ion response, and downregulation of genes associated with immunity and protein folding. For each contrast, gene ontology terms significantly overrepresented among significantly up and downregulated genes were identified, pooled within tissue types, and then compared across tissue types. For CR-upregulated genes, this analysis revealed that some individual biological process terms were overrepresented with respect to as many as five tissue types, including metabolic process (GO:0008152), response to metal ion (GO:0010038), detoxification of copper ion (GO:0010273) and cellular lipid metabolic process (GO: 0044255). For CR-downregulated genes, the terms protein folding (GO: 0006457), phosphate transport (GO:0006817), humoral immune response (GO: 0006959), immunoglobulin mediated immune response (GO:0016064) and antigen processing and presentation of exogenous peptide antigen via MHC class II (GO:0019886) were overrepresented with respect to seven tissue types.

3.2. Effects of CR among tissues

Tissue type was the strongest factor determining gene expression changes induced by a CR diet. This was demonstrated by cluster analysis of differential expression signatures (see Figs. 1 and 2). Signatures associated with the same type of tissue clustered together in nearly every case, even when data were generated by separate laboratories in different studies. In Figure 2, for example, the seven signatures associated with liver clustered together in the same branch, which was also the case for signatures associated with the heart and hypothalamus. Signatures associated with muscle (msl1 and msl3), in contrast, did not cluster together, which was probably due to a large difference between msl1 and msl3 in terms of the duration of CR (Table 1).

Despite the strong effect of tissue type, the laboratory or experimental study in which data were generated also had strong influence on clustering patterns. Signatures joined at very low levels of dissimilarity ($d < 0.70$), for instance, were usually generated by the same laboratory as part of the same study (Figure 2). Within tissue-specific branches (see Figure 2), signatures clustered according to the study from which data were obtained, rather than duration of CR or age at which CR was initiated. Additionally, there were several cases in which signatures generated as part of the same study clustered together, even though each signature was associated with a different type of tissue (e.g., see cln1 and msl1 in Figure 2). These results indicate that the laboratory in which experiments are performed, which encompasses numerous technical and experimental factors, has strong influence on results obtained from microarray investigations of CR.

Genes differentially expressed in any one experiment were often not differentially expressed in other comparable experiments. An illustrative case is the comparison between hrt7 and hrt8b (Figure 3). Both signatures were obtained from studies that examined the effects of long-term CR in heart tissue (Lee et al., 2002; Dhahbi et al., 2006). While the overlap between hrt7 and

hrt8b signatures is statistically significant ($P < 0.001$, see Figure 3), fewer than 20% of differentially expressed genes were common to both studies. This level of overlap (or less) was typical of the signatures examined in this analysis. In Figure 2, for example, most branches are joined at high levels of dissimilarity ($d > 0.70$), indicating that intersections between differentially expressed gene sets are on the order of 30% or less. At the same time, however, among the many possible signature comparisons, signature overlap was commonly greater than expected on the basis of chance. Supplemental Data File 1 provides an informative similarity matrix that displays the similarity level and statistical significance of all 253 possible pairwise comparisons among the 23 signatures analyzed in this study.

3.3. Common CR Responses among Tissues

A small number of genes exhibited shared expression responses to CR across five or more tissues (Figs. 4 and 5). Such genes were a rare extreme with respect to the murine genome, since most genes did not exhibit expression response to CR in any tissue, or only responded to CR within one tissue type (Table 4). Of the ten tissues examined, 16 genes were upregulated with respect to five or more tissues (Figure 4), while 12 genes were downregulated with respect to five or more tissues (Figure 5). The biological significance of identified genes is supported by the observation that gene pairs with similar functional properties are included in both gene sets. For example, the 16 upregulated genes include two metallothioneins (Mt1 and Mt2) as well as two period homologues (Per1 and Per2) (Figure 4). Likewise, among the 12 downregulated genes, two procollagen alpha 1 genes (Col1a1 and Col3a1) and two heat shock proteins were included (Hsp110 and Serpinh1/Hsp47) (Figure 5). Among the 16 upregulated genes, the three most overrepresented biological process gene ontology terms were nitric oxide mediated signal transduction (GO:0007263), zinc ion homeostasis (GO:0006882) and circadian rhythm (GO:0007623) ($P < 0.05$). Among the 12 downregulated genes, overrepresented gene ontologies included those associated with response to heat (GO:0009408), unfolded protein (GO:0006986), biotic stimuli (GO:0009607), chemical stimuli (GO:00042221) and response to pest, pathogen and parasite (GO:0009613) ($P < 0.05$).

Per2 (period homologue 2) and Serpinh1 (serine peptidase inhibitor/Hsp47) exhibited the most shared CR response patterns among tissues. Per2 was upregulated by CR in seven tissues (liver, heart, muscle, hypothalamus, hippocampus, colon, cochlea), while Serpinh1 was downregulated by CR in seven tissues (liver, heart, muscle, white adipose tissue, hypothalamus, colon, lung) (Figs. 4 and 5). It is noteworthy that the effects of CR on Per2 and Serpinh1 expression, as well as other genes listed in Figures 4 and 5, is much more common among tissues than genes commonly cited as playing key roles in the mammalian CR response, such as Sirt1, Igf-I and mTOR (Dilova et al., 2007; Yamaza et al., 2007). For instance, Igf-I was significantly downregulated with respect to only liver and muscle (lvr4b, msl1, msl3), while mTOR was upregulated in just the liver and heart (lvr1, lvr4a, hrt7). Surprisingly, the effect of CR on Sirt1 expression was non-significant in every tissue for which data was available, and in each case, the effect of CR on Per2 expression was larger than the effects of CR on Sirt1 expression (Figure 6).

3.4. Effects of CR and Aging in Liver

Five differential expression signatures associated with aging in liver were generated using data downloaded from the Gene Expression Omnibus and ArrayExpress databases (GSE3129, GSE3150, EMEXP153, EMEXP839) (Amador-Noguez et al., 2004; Boyleston et al., 2006; Niedernhofer et al., 2006). The signatures are identified as age1 through age5, where each quantified gene expression differences between young (< 6 months) versus old mice (> 22 months). Each signature was generated using data collected from standard laboratory strains (C57B1/6J), or from mice with normal phenotypes that are heterozygous for recessive endocrine mutations (*Prop1^{df/+}*, *Pit1^{dw/+}*, *Ghrhr^{lit/+}*). There was only slight evidence to

indicate that, in liver, the effects of CR strongly overlapped with those of aging. The signatures associated with CR and aging in liver generally clustered in two separate branches (see Figure 7 and Supplemental Data File 2). The one exception was age5, which clustered in the same branch as signatures associated with CR, although it should be noted that age5 was joined to the CR cluster at a high level of dissimilarity ($d \approx 0.90$). A more striking observation was that signatures associated with CR rarely overlapped significantly with those associated with aging. Supplemental data file 2 provides a similarity matrix of the CR and aging signatures shown in Figure 7, and reveals that of the 35 pairwise comparisons between CR and aging signatures, significant overlap was present in just 6 cases.

To investigate specific anti-aging effects of CR, genes with opposite induction patterns under CR and aging in liver were identified. Ten genes were identified as upregulated by CR in three or more lvr signatures, as well as downregulated by aging in at least one age signature (Figure 8). Likewise, ten genes were downregulated by CR in three or more lvr signatures and upregulated by aging in at least one age signature (Figure 9). These genes correspond to cases in which gene expression changes induced by CR are in opposition to those that result from aging. Genes listed in Figure 8 were disproportionately associated with electron transport (GO:0006118) and cellular metabolism (GO:0044237) ($P < 0.05$). Likewise, genes listed in Figure 9 were associated with positive regulation of organismal physiological process and metabolism (GO:0057240, GO:0009893), response to stimulus (GO:0050896) and defense response (GO:0006952) ($P < 0.05$). Williams et al. (2006) has recently evaluated baseline hepatic gene expression levels of BxD mouse strains, for which lifespan data had previously been collected by Gelman et al. (1988). Combining these two data sources revealed that hepatic expression levels of Serpina12 (serine peptidase inhibitor; see Figure 9) was negatively related to lifespan among BxD mouse strains ($r_s = -0.366$, $P = 0.034$), although this relationship was non-significant after adjusting for multiple testing of all genes included in Figures 8 and 9 ($P = 0.238$).

The transcriptional effects of CR in liver were compared to those of resveratrol, a postulated CR mimetic compound that has recently received much interest (Baur et al., 2006). The similarity between the effects of CR and resveratrol on gene expression depends on the experiment to which a comparison is made (Figure 10). In three cases, the effects of resveratrol on gene expression in liver significantly overlapped with those of CR (lvr1, lvr5a and lvr5b), while in four cases no significant overlap was found (lvr4a, lvr4b, lvr10 and lvr13).

4. Discussion

Microarrays are powerful tools for screening large numbers of genes and making unbiased determinations regarding which genes are best candidates for detailed experimental study. The comparative analysis of microarray data integrates results from many independent investigations, filters out experimental noise and false positive identifications, and thus maximizes the insight generated from whole-genome expression analysis. The present study has implemented new approaches for the comparative analysis of microarray data, and has shed light on mechanisms by which caloric restriction (CR) acts among mammalian tissues and how these mechanisms relate to the aging process. Independent experiments involving CR generally identified disparate sets of genes with limited overlap ($< 30\%$). Nevertheless, a total of 28 genes that exhibit shared transcriptional responses to CR across five or more mammalian tissues were identified. Based on gene expression patterns alone, the systemic involvement of these 28 genes in the mammalian CR response is better supported than the involvement of Sirt1, Igf-I and mTOR (Dilova et al., 2007; Yamaza et al., 2007). With regard to liver tissue, surprisingly little overlap between the genome-wide effects of CR and aging were found, suggesting that the effects of CR on gene expression are not necessarily a “reversal” of the gene expression changes that occur during aging. Interestingly, the correspondence between

gene expression changes induced by CR and resveratrol in liver depended upon which experimental study comparison was made to. This result indicates that additional data is necessary to determine whether, in liver tissue, the genome-wide transcriptional effects of resveratrol serve to mimic those associated with a CR diet.

Common CR responses among tissues were characterized by upregulation of tumor suppression genes as well as genes that combat oxidative stress. It is especially striking that two period homologues (Per1) and two metallothionein (Mt1 and Mt2) genes were included among the 16 genes upregulated across five or more tissue types under CR. Period homologues are widely recognized for their role in manipulating the biological clock, but also exhibit tumor suppression activity (Lee, 2006). Per1 and Per2 null mice are more susceptible to tumor development, and also exhibit features of premature aging (Fu et al., 2002; Lee, 2005; Lee, 2006). Since decreased tumor incidence is perhaps the major factor underlying extended lifespan in CR mice (Spindler and Dhahi 2007), upregulation of Per1 and Per2 in many tissue types may be a key factor reducing mortality rates under CR. Elevated expression of Cdkn1a (cyclin-dependent kinase inhibitor 1A) and Sult1a1 (sulfotransferase family member 1A) across several tissues could also inhibit tumorigenesis in CR mice. Increased Cdkn1a (also p21) expression inhibits cellular proliferation and induces growth arrest (Xiong et al., 1993; Franklin et al., 2000; Gartel and Tyner 2002) and mice lacking this gene have severely decreased lifespan due to spontaneous tumor development (Martin-Caballero et al., 2001). Sult1a1 polymorphisms have often been linked with cancer risk (Peng et al., 2003; Wang et al., 2002; Wu et al., 2003; Zheng et al., 2003; Bardakci et al., 2007), which may be due to the role of sult1a1 in the bioactivation and inactivation of carcinogens (Miller, 1994; Fan et al., 2007). The metallothionein genes Mt1 and Mt2, also upregulated as a shared CR response, may have beneficial health effects through different mechanisms. Metallothioneins are stress-response genes with potent cytoprotective effects, most notably against oxidative stress damage (Thirumoorthy et al., 2007). Metallothioneins scavenge reactive oxygen species and have thus been found to prevent ROS-induced apoptosis in a wide range of contexts (Haidara et al., 1999; Kawai et al., 2000; Giralt et al., 2002; Penkowa, 2006; Kang, 2007). Since the accumulation of oxidative stress over the lifespan is thought to play a key role in the physiological decline associated with aging (Sohal and Weindruch, 1996), the upregulation of Mt1 and Mt2 by CR may contribute to the anti-aging effects of CR. Taken together, these findings support the hypothesis that, by increasing the expression of tumor suppressor (Per1, Per2 and Cdkn1a) and stress-response genes (Mt1 and Mt2) in multiple tissue types, CR increases lifespan in mice by preventing the onset of cancer and by limiting the accrual of oxidative stress damage during aging.

Common responses to CR include both activation and inhibition of stress-response pathways. Stress-response genes upregulated by CR include metallothioneins (Mt1 and Mt2), cyclin-dependent kinase inhibitor 1A (cdkn1a) and RNA binding motif protein 3 (Rbm3), while stress-response genes downregulated by CR include two heat shock proteins (Serpinh1/Hsp47, Hsp110) and guanine nucleotide binding protein (Gng11). At a systemic level, therefore, response to CR may be viewed as a partial hormesis effect, involving activation of certain stress-response pathways and inhibition of others. The downregulation of heat shock proteins under CR seems contrary to findings from invertebrate model systems, which have suggested that heat shock protein upregulation has positive impacts on longevity (Hsu et al., 2003; Walker and Lithgow, 2003). Evidence regarding similar effects in mammals, however, is currently lacking, and to the contrary, elevated heat shock protein expression has been associated with carcinogenesis (Kim et al., 2007). The expression of Hsp110, in particular, is elevated in tumors and appears to promote resistance of cancer cells to apoptosis (Hosaka et al., 2006). Serpinh1/Hsp47 downregulation may also influence tumorigenesis (Shirakami et al., 1995; Morino et al., 1997), although a more important effect may be to prevent accumulation of excess fibrous tissue during aging. Hsp47 is a collagen-specific chaperone that is essential for construction

and maintenance of the extracellular matrix (Nagai et al., 2000), and its overexpression has been found to promote fibrosis and glomerulosclerosis in the aging rat kidney (Razzaque et al., 1998), which is attenuated by a CR diet through Hsp47 downregulation (Razzaque et al., 1999). The inhibition of collagen-building pathways appears to be an important systemic effect of CR, since two procollagen genes (Col1a1 and Col3a1) were also downregulated as shared CR responses.

It was recently found that mice lacking 5 adenylyl cyclase (AC5-KO mice) exhibit a 30% lifespan increase (Yan et al., 2007). AC5 is a downstream signaling component of both β_1 -adrenergic receptor (Adrb1) and β_3 -adrenergic receptor (Adrb3), and mediates the synthesis of cAMP from ATP (Susulic et al., 1995). This study found that hepatic expression of β_3 -adrenergic receptor (Adrb3) increases with age and decreases due to CR (Figure 9), which suggests that Adrb3 signaling through AC5 might be inhibited by CR. If so, this would represent a shared mechanism underlying lifespan extension due to CR and in AC5-KO mice. A complicating factor, however, is that since AC5-KO mice also exhibit significantly reduced growth hormone (GH) levels (Yan et al., 2007), such that inhibition of the GH/IGF-I axis might also explain the observed lifespan increase (Brown-Borg, 2007), perhaps independently of adrenergic receptor signaling. Interestingly, inhibition of the GH/IGF-I axis in the long-lived Ames mouse leads to upregulation of Adrb3 in liver at early ages but not at late ages (Swindell, 2007b), which may reflect either increased Adrb3/AC5 signaling capacity or a compensatory mechanism to account for inhibited Adrb3/AC5 signaling.

The downregulation of immune-response genes is a common CR response among tissues that should not be overlooked. Positive effects of CR on immune response indicators have been documented in a number of studies (Grossmann et al., 1990; Effros et al., 1991; Pahlavani, 2004; Messaoudi et al., 2006), but survival rates of CR and control-fed mice in response to pathogen infection have been examined less extensively. When survival of CR and control-fed mice has been compared, discouraging results have been obtained (Gardner, 2005; Kristan et al., 2007). For instance, mortality of old mice treated with influenza virus was significantly increased by CR, resulting in complete mortality of CR mice compared to just 40–60% mortality of control mice (Gardner, 2005). Furthermore, as pointed out by Spindler and Mote (2007), a large scale study of humans aged 50–75 found that low body mass index is associated with increased mortality, which was largely attributable to elevated rates of infection (Sunder, 2005). In most tissues, genes downregulated by CR were disproportionately associated with humoral and immunoglobulin immune response, as well as antigen processing and presentation. The 12 genes downregulated across five or more tissue types included antigens (Cd74 and H2-Ea), T-Cell specific GTPase (Tgtp), complement component 1 (C1qb) and an interferon alpha stimulated gene (Ifi27). It is unclear how the downregulation of these genes by CR could affect health and survival of mammals within natural environments where pathogen exposure is unregulated. Downregulation of interferon alpha inducible protein 27 (Ifi27) may decrease the ability of mice to cope with viral infection, especially in aged animals (Labrada et al., 2002). Likewise, downregulation of T-Cell specific GTPase (Tgtp) by CR may increase susceptibility to infection, since Tgtp transfection was found to increase resistance of L cells to an RNA virus (Carlow et al., 1998). Additionally, upregulation of the metallothioneins Mt1 and Mt2, while protective against oxidative stress, may be yet another factor inhibiting immune response under CR, since elevated Mt1 and Mt2 lowers zinc bioavailability and thus weakens immunity (Cipriano et al., 2003; Mocchegiani et al., 2004).

Guanine nucleotide binding protein (Gng11) was downregulated in five tissue types under CR (liver, muscle, hypothalamus, colon and lung) and represents a possible link between cellular and organismal senescence. Hossain et al. (2006) found that downregulation of Gng11 in human fibroblasts led to an extension of replicative lifespan, while its overexpression rapidly induced cellular senescence. Gng11 expression is also induced by oxidative stress (Hossain et

al., 2006), suggesting that CR may inhibit cellular senescence by preventing the accumulation of oxidative stress and consequently inhibiting Gng11 expression.

CR is commonly viewed as a treatment that not only improves health and lifespan, but actually slows the process of aging. In support of this notion, previous microarray studies have reported that gene expression changes resulting from CR are largely a reversal of expression changes that result from aging. In a recent study, for example, Edwards et al. (2007) reported that 87% of age-related gene expression changes in skeletal muscle were countered by CR. This result was in qualitative agreement with findings from an earlier study indicating that CR reversed 19% of age-related gene expression changes in the heart (Lee et al., 2002). Results from the present analysis, however, reveal that in liver tissue, gene expression changes due to CR seldom have a statistically significant relationship with those that result from aging. This result is based on 35 comparisons among seven contrasts evaluating the effects of CR and five contrasts evaluating the effects of aging in liver, in which significant overlap between differential expression signatures was present in just 6 of 35 comparisons (see Supplemental Data File 3). A possible explanation of this result is that, in earlier studies, the effects of CR and aging were not *a priori* independent due to experimental design. Both Edwards et al. (2007) and Lee et al. (2002) examined three treatment comparisons among young mice, old mice, and old-CR mice. Given this experimental setup, the aging effect on gene expression (young vs. old mice) cannot be independent of the CR effect on gene expression (old mice vs. old-CR mice), since association between the two effects will be generated via the common comparator (the old mice treatment). This design could inflate associations between the effects of CR and aging, which may explain the difference between results of the present analysis and results from Edwards et al. (2007) and Lee et al. (2002). The ideal approach for evaluating the effects of CR and aging is to employ a two-factor factorial design with age and diet as fixed effects. Using this design, Dhabi et al. (2006) compared the effects of aging and CR on gene expression in the heart and found, in agreement with the present analysis, weak association between the two effects, with only 79 of 1075 (7.3%) age-responsive genes affected by CR.

Common responses to CR provide benchmarks for evaluating potential CR mimetic compounds. The degree to which the beneficial effects of CR arise from tissue-specific versus common mechanisms is not fully established (Spindler and Dhabi, 2007). It is unlikely, however, that CR-mediated lifespan extension depends overwhelmingly on gene expression changes within any one tissue type alone. The extended lifespan phenotype more likely depends on continued systemic functioning during the course of aging, involving all major organ systems and tissues. Thus, while DNA microarrays have been valuable for identifying important gene expression changes associated with CR (Spindler and Mote, 2007), this technology may be less critical beyond the current discovery phase. A more cost-effective and biologically meaningful strategy for screening potential CR mimetics may be to evaluate expression patterns of a small number of genes among a variety of tissues. The present study provides some support for the notion that the beneficial effects of CR diets are at least partly mediated by a set of shared gene expression changes that lead to tumor suppression and reduction of oxidative stress in multiple tissues. Such shared gene expression changes warrant special consideration when evaluating candidate CR mimetic compounds. A useful direction for future studies, therefore, is to evaluate whether the 28 common gene expression patterns identified in this study are also associated with CR mimetic compounds currently under investigation (e.g., Baur et al., 2006), or alternative dietary treatments that may also be viewed as CR mimetic strategies (e.g., Orentreich et al., 1993; Miller et al., 2005). This approach may facilitate the development of effective CR mimetic strategies, which may prove to be of considerable value in the realm of preventative medicine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIA training grant AG000114 and the University of Michigan Department of Pathology. Helpful comments and suggestions were provided by two anonymous reviewers. The author thanks laboratories for providing microarray data to the Gene Expression Omnibus and ArrayExpress databases, as well as researchers who responded to requests for experimental data (Yoshikazu Higami, Yinghe Hu, Patricia L. Mote, Thomas A. Prolla, Steven R. Spindler, James M. Vann, Richard Weindruch, Pu Wu).

References

- Amador-Noguez D, Yagi K, Venable S, Darlington G. Gene expression profile of long-lived Ames dwarf mice and Little mice. *Aging Cell* 2004;3:423–441. [PubMed: 15569359]
- Anderson RM, Weindruch R. Calorie restriction: progress during mid-2005 mid-2006. *Exp Gerontol* 2006;41:1247–1249. [PubMed: 17125949]
- Apte UM, Limaye PB, Desai D, Bucci TJ, Warbritton A, Mehendale HM. Mechanisms of increased liver tissue repair and survival in diet-restricted rats treated with equitoxic doses of thioacetamide. *Toxicol Sci* 2003;72:272–282. [PubMed: 12655029]
- Astrup A. Healthy lifestyles in Europe: prevention of obesity and type II diabetes by diet and physical activity. *Public Health Nutr* 2001;4:499–515. [PubMed: 11683545]
- Bardakci F, Arslan S, Bardakci S, Binatli AO, Budak M. Sulfotransferase 1A1 (Sult1A1) polymorphism and susceptibility to primary brain tumors. *J Cancer Res Clin Oncol*. 2007in press
- Barrett T, Troup DB, Wilhite SE, Ledoux P, Rudnev D, Evangelista C, Kim IF, Soboleva A, Tomashevsky M, Edgar R. NCBI GEO: mining tens of millions of expression profiles database and tools update. *Nucleic Acids Research* 2007;35:D760–D765. [PubMed: 17099226]
- Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, Pistell PJ, Poosala S, Becker KG, Boss O, Gwinn D, Wang M, Ramaswamy S, Fishbein KW, Spencer RG, Lakatta EG, Le Couteur D, Shaw RJ, Navas P, Puigserver P, Ingram DK, de Cabo R, Sinclair DA. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* 2006;444:337–342. [PubMed: 17086191]
- Benjamini Y, Hochberg Y. Controlling the false discovery rate: a powerful and practical approach to multiple testing. *J Roy Stat Soc B* 1995;57:289–300.
- Boylston WH, DeFord JH, Papaconstantinou J. Identification of longevity- associated genes in long-lived Snell and Ames dwarf mice. *Age* 2006;28:125–144.
- Brown-Borg HM. Hormonal regulation of longevity in mammals. *Age Res Rev* 2007;6:28–45.
- Bruce-Keller AJ, Umberger G, McFall R, Mattson MP. Food restriction reduces brain damage and improves behavioral outcome following excitotoxic and metabolic insults. *Ann Neurol* 1999;45:8–15. [PubMed: 9894871]
- Carlow DA, Teh SJ, Teh HS. Specific antiviral activity demonstrated by TGTP, a member of a new family of interferon-induced GTPases. *J Immunology* 1998;161:2348–2355. [PubMed: 9725230]
- Cipriano C, Giacconi R, Muzzioli M, Gasparini N, Orlando F, Corradi A, Cabassi E, Mocchegiani E. Metallothionein (I + II) confers, via c-myc, immune plasticity in oldest mice: model of partial hepatectomy/liver regeneration. *Mech Age Develop* 2003;124:877–886.
- Corton JC, Apte U, Anderson SP, Limaye P, Yoon L, Latendresse J, Dunn C, Everitt JJ, Voss KA, Swanson C, Kimbrough C, Wong JS, Gill SS, Chandraratna RAS, Kwak MK, Kensler TW, Stulnig TM, Steffensen KR, Gustafsson JA, Mehendale HM. Mimetics of caloric restriction include agonists of lipid-activated nuclear receptors. *J Biol Chem* 2004;279:46204–46212. [PubMed: 15302862]
- Dhahbi JM, Mote PL, Fahy GM, Spindler SR. Identification of potential caloric restriction mimetics by microarray profiling. *Physiol Genomics* 2005;23:343–350. [PubMed: 16189280]
- Dhahbi JM, Tsuchiya T, Kim HJ, Mote PL, Spindler SR. Gene expression and physiologic responses of the heart to the initiation and withdrawal of caloric restriction. *J Gerontol Biol Sci* 2006;61A:218–231.

- Dilova I, Easlon E, Lin SJ. Calorie restriction and the nutrient sensing signaling pathways. *Cell Mol Life Sci* 2007;64:752–767. [PubMed: 17260088]
- Edwards MG, Anderson RM, Yuan M, Kendzierski CM, Weindruch R, Prolla TA. Gene expression profiling of aging reveals activation of a p53-mediated transcriptional program. *BMC Genomics* 2007;8:article 80
- Effros RB, Walford RL, Weindruch R, Mitcheltree C. Influences of dietary restriction on immunity to influenza in aged mice. *J Gerontol* 1991;46:B142–B147. [PubMed: 2071828]
- Falcon S, Gentleman R. Using GOstats to test gene lists for GO term association. *Bioinformatics* 2007;23:257–258. [PubMed: 17098774]
- Fan C, Jin M, Chen K, Zhang Y, Zhang S, Liu B. Case-only study of interactions between metabolic enzymes and smoking in colorectal cancer. *BMC Cancer* 2007;7:115. [PubMed: 17603900]
- Fontana L, Meyer TE, Klein S, Holloszy JO. Long-term calorie restriction is highly effective in reducing the risk for atherosclerosis in humans. *Proc Natl Acad Sci USA* 2004;101:6659–6663. [PubMed: 15096581]
- Franklin DS, Godfrey VL, O'Brien DA, Deng C, Xiong Y. Functional collaboration between different cyclin-dependent kinase inhibitors suppresses tumor growth with distinct tissue specificity. *Mol Cell Biol* 2000;20:6147–6158. [PubMed: 10913196]
- Fu C, Hickey M, Morrison M, McCarter R, Han ES. Tissue specific and non-specific changes in gene expression by aging and by early stage CR. *Mech Age Develop* 2006;127:905–916.
- Fu L, Pelicano H, Liu J, Huang P, Lee CC. The circadian gene period2 plays an important role in tumor suppression and DNA damage response *in vivo*. *Cell* 2002;111:41–50. [PubMed: 12372299]
- Gardner EM. Caloric restriction decreases survival of aged mice in response to primary influenza infection. *J Gerontol Biol Sci* 2005;60A:688–694.
- Gartel AL, Tyner AL. The role of the cyclin-dependent kinase inhibitor p21 in apoptosis. *Molecular Cancer Therapeutics* 2002;1:639–649. [PubMed: 12479224]
- Gelman R, Watson A, Bronson R, Yunis E. Murine chromosomal regions correlated with longevity. *Genetics* 1988;118:693–704. [PubMed: 3163317]
- Giralt M, Penkowa M, Lago N, Molinero A, Hidalgo J. Metallothionein-1+2 protect the CNS after a focal brain injury. *Experimental Neurology* 2002;173:114–128. [PubMed: 11771944]
- Grossmann A, Maggio-Price L, Jinneman JC, Wolf NS, Rabinovitch PS. The effect of long-term caloric restriction on function of T-cell subsets in old mice. *Cell Immunol* 1990;131:191–204. [PubMed: 2121372]
- Haidara K, Moffatt P, Denizeau F. Metallothionein induction attenuates the effects of glutathione depletors in rat hepatocytes. *Toxicological Sciences* 1999;49:297–305. [PubMed: 10416275]
- Higami Y, Pugh TD, Page GP, Allison DB, Prolla TA, Weindruch R. Adipose tissue energy metabolism: altered gene expression profile of mice subjected to long-term caloric restriction. *FASEB J* 2003;18:415–417. [PubMed: 14688200]
- Holloszy JO, Fontana L. Caloric restriction in humans. *Exp Gerontol* 2007;42:709–712. [PubMed: 17482403]
- Hosaka S, Nakatsura T, Tsukamoto H, Hatayama T, Baba H, Nishimura Y. Synthetic small interfering RNA targeting heat shock protein 105 induces apoptosis of various cancer cells both *in vitro* and *in vivo*. *Cancer Sci* 2006;97:623–632. [PubMed: 16827803]
- Hossain MN, Sakemura R, Fujii M, Ayusawa D. G-protein γ subunit GNG11 strongly regulates cellular senescence. *Biochem Biophys Res Comm* 2006;351:645–650. [PubMed: 17092487]
- Hsu AL, Murphy CT, Kenyon C. Regulation of aging and age-related disease by DAF-16 and heat-shock factor. *Science* 2003;300:1142–1145. [PubMed: 12750521]
- Hursting SD, Lavigne JA, Berrigan D, Perkins SN, Barrett JC. Calorie restriction, aging, and cancer prevention: mechanisms of action and applicability in humans. *Ann Rev Med* 2003;54:131–152. [PubMed: 12525670]
- Ingram DK, Roth GS, Lane MA, Ottinger MA, Zou S, de Cabo R, Mattison JA. The potential for dietary restriction to increase longevity in humans: extrapolation from monkey studies. *Biogerontology* 2006;7:143–148. [PubMed: 16732404]

- Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* 2003;4:249–264. [PubMed: 12925520]
- Kalani R, Judge S, Carter C, Pahor M, Leewenburgh C. Effects of caloric restriction and exercise on age-related chronic inflammation assessed by C-reactive protein and interleukin-6. *J Gerontol A Biol Sci Med* 2006;61:211–217.
- Kang YJ. Antioxidant defense against anthracycline cardiotoxicity by metallothionein. *Cardiovasc Toxicol* 2007;7:95–100. [PubMed: 17652812]
- Kawai K, Liu SX, Tyurin VA, Tyurina YY, Borisenko GG, Jiang JF, St Croix CM, Fabisiak JP, Pitt BR, Kagan VE. Antioxidant and antiapoptotic function of metallothioneins in HL-60 cells challenged with copper nitrilotriacetate. *Chem Res Toxicol* 2000;13:1275–1286. [PubMed: 11123969]
- Kim HJ, Hwang NA, Lee KJ. Heat shock responses for understanding diseases of protein denaturation. *Mol Cells* 2007;23:123–131. [PubMed: 17464187]
- Klebanov S. Can short-term dietary restriction and fasting have a long-term anti- carcinogenic effect? *Interdiscip Top Gerontol* 2007;35:176–192. [PubMed: 17063039]
- Kristan D. Chronic calorie restriction increases susceptibility of laboratory mice (*Mus musculus*) to a primary intestinal parasite infection. *Aging Cell*. 2007In Press
- Kristan, Deborah M. Department of Biological Sciences. California State University San Marcos; San Marcos, CA 920961:
- Kritchesky D. Caloric restriction and cancer. *J Nutr Sci Vitaminol* 2001;47:13–19. [PubMed: 11349885]
- Labrada L, Liang XH, Zheng W, Johnston C, Levine B. Age-dependent resistance to lethal alphavirus encephalitis in mice: analysis of gene expression in the central nervous system and identification of a novel interferon-inducible protective gene, mouse ISG12. *J Virol* 2002;76:11688–11703. [PubMed: 12388728]
- Larsson O, Wennmalm K, Sandberg R. Comparative microarray analysis. *OMICS* 2006;10:381–397. [PubMed: 17069515]
- Lee CC. The circadian clock and tumor suppression by mammalian period genes. *Methods in Enzymology* 2005;393:852–861. [PubMed: 15817328]
- Lee CC. Tumor suppression by the mammalian period genes. *Cancer Causes Control* 2006;17:525–530. [PubMed: 16596306]
- Lee CK, Allison DB, Brand J, Weindruch R, Prolla TA. Transcriptional profiles associated with aging and middle age-onset caloric restriction in mouse hearts. *Proc Natl Acad Sci* 2002;99:14988–14993. [PubMed: 12419851]
- Logroscino G, Marder K, Cote L, Tang MX, Shea S, Mayeux R. Dietary lipids and antioxidants in Parkinson's disease: a population-based, case-control study. *Ann Neurol* 1996;39:89–94. [PubMed: 8572672]
- Martin-Caballero J, Flores JM, Garcia-Palencia P, Serrano M. Tumor susceptibility of p21(Waf1/Cip1)-deficient mice. *Cancer Res* 2001;61:6234–6238. [PubMed: 11507077]
- Massaro D, DeCarlo Massaro G, Baras A, Hoffman EP, Biadasz Clerch L. Calorie-related rapid onset of alveolar loss, regeneration, and changes in mouse lung gene expression. *Am J Physiol Lung Cell Mol Physiol* 2004;286:L896–L906. [PubMed: 14594731]
- Mattison JA, Lane MA, Roth GS, Ingram DK. Calorie restriction in rhesus monkeys. *Exp Gerontol* 2003;38:35–46. [PubMed: 12543259]
- Messaoudi I, Warner J, Fischer M, Park B, Hill B, Mattison J, Lane MA, Roth GS, Ingram DK, Picker LJ, Douek DC, Mori M, Nikolich-Zugich J. Delay of T cell senescence by caloric restriction in aged long-lived nonhuman primates. *Proc Natl Acad Sci* 2006;103:19448–19453. [PubMed: 17159149]
- Miller JA. Sulfonation in chemical carcinogenesis history and preset status. *Chem Biol Interact* 1994;92:329–341. [PubMed: 8033266]
- Miller RA, Buehner G, Chang Y, Harper JM, Sigler R, Smith-Wheelock M. Methionine-deficient diet extends mouse lifespan, slows immune and lens aging, alters glucose, T4, IGF-I and insulin levels, and increases hepatocyte MIF levels and stress resistance. *Aging Cell* 2005;4:119–125. [PubMed: 15924568]

- Mocchegiani E, Giacconi R, Muti E, Rogo C, Bracci M, Muzzioli M, Cipriano C, Malavolta M. Zinc, immune plasticity, aging, and successful aging. *Ann N Y Acad Sci* 2004;1019:127–134. [PubMed: 15247005]
- Morino M, Tsuzuki T, Ishikawa Y, Shirakami T, Yoshimura M, Kiyosuke Y, Matsunaga K, Yoshikumi C, Saijo N. Specific expression of Hsp47 in human tumor cell lines in vitro. *In Vivo* 1997;11:17–21. [PubMed: 9067768]
- Motta MC, Divecha N, Lemieux M, Kamel C, Chen D, Gu W, Bultsma Y, McBurney M, Guarente L. Mammalian SIRT1 represses forkhead transcription factors. *Cell* 2004;116:551–563. [PubMed: 14980222]
- Nagai N, Hosokawa M, Itoharu S, Adachi E, Matsushita T, Hosokawa N, Nagata K. Embryonic lethality of molecular chaperone Hsp47 knockout mice is associated with defects in collagen biosynthesis. *J Cell Biol* 2000;150:1499–1506. [PubMed: 10995453]
- Niedernhofer LJ, Garinis GA, Raams A, Lalai AS, Robinson AR, Appeldoorn E, Odijk H, Oostendorp R, Ahmad A, van Leeuwen W, Theil AF, Vermeulen W, van der Horst GT, Meinecke P, Kleijer WJ, Vijg J, Jaspers NG, Hoeijmakers JH. A new progeroid syndrome reveals that genotoxic stress suppresses the somatotroph axis. *Nature* 2006;444:1038–1043. [PubMed: 17183314]
- Orentreich N, Matias JR, DeFelice A, Zimmerman JA. Low methionine ingestion by rats extends life span. *J Nutr* 1993;123:269–274. [PubMed: 8429371]
- Pahlavani MA. Influence of caloric restriction on aging immune system. *J Nutr Health Aging* 2004;8:38–47. [PubMed: 14730366]
- Parkinson H, Kapushesky M, Shojatalab M, Abeygunawardena N, Coulson R, Farne A, Holloway E, Kolesnykov N, Lilja P, Lukk M, Mani R, Rayner T, Sharma A, William E, Sarkans U, Brazma A. ArrayExpress a public database of microarray experiments and gene expression profiles. *Nucleic Acids Research* 2007;35:D747–D750. [PubMed: 17132828]
- Patel NV, Gordon MN, Connor KE, Good RA, Engelman RW, Mason J, Morgan DG, Morgan TE, Finch CE. Caloric restriction attenuates Abeta-deposition in Alzheimer transgenic models. *Neurobiol Aging* 2005;26:995–1000. [PubMed: 15748777]
- Peng CT, Chen JC, Yeh KT, Wang YF, Hou MF, Lee TP, Shih MC, Chang JY, Chang JG. The relationship among the polymorphisms of SULT1A1, 1A2 and different types of cancers in Taiwanese. *Int J Mol Med* 2003;11:85–89. [PubMed: 12469224]
- Penkowa M. Metallothioneins are multipurpose neuroprotectants during brain pathology. *FEBS J* 2006;273:1857–1870. [PubMed: 16640552]
- Razzaque MS, Shimokawa I, Nazneen A, Higami Y, Taguchi T. Age-related nephropathy in the Fischer 344 rat is associated with overexpression of collagens and collagen-binding heat shock protein 47. *Cell Tissue Res* 1998;293:471–478. [PubMed: 9716737]
- Razzaque MS, Shimokawa I, Nazneen A, Liu D, Naito T, Higami Y, Taguchi T. Life-long dietary restriction modulates the expression of collagens and collagen-binding heat shock protein 47 aged Fischer 344 rat kidney. *Histochem J* 1999;31:123–132. [PubMed: 10416684]
- Rosati B, Grau F, Kuehler A, Rodriguez S, McKinnon D. Comparison of different probe-level analysis techniques for oligonucleotide microarrays. *Biotechniques* 2004;36:316–322. [PubMed: 14989097]
- Selman C, Kerrison ND, Cooray A, Piper MDW, Lingard SJ, Barton RH, Schuster EF, Blanc E, Gems D, Nicholson JK, Thornton JM, Partridge L, Withers DJ. Coordinated multitissue transcriptional and plasma metabolomic profiles following acute caloric restriction in mice. *Physiol Genomics* 2006;127:187–200. [PubMed: 16882887]
- Shirakami T, Tsuzuki T, Morino M, Kiyosuke YI, Yoshimura M, Yoshikumi C, Okada F, Hosokawa M. Decreased expression of HSP47 in highly malignant mouse fibrosarcoma. *In Vivo* 1995;9:513–518. [PubMed: 8900932]
- Smid M, Dorssers LCJ, Jenster G. Venn Mapping: clustering of heterologous microarray data based on the number of co-occurring differentially expressed genes. *Bioinformatics* 2003;19:2065–2071. [PubMed: 14594711]
- Smyth GK. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol* 2004;3:Article 3
- Sohal RS, Weindruch R. Oxidative stress, caloric restriction, and aging. *Science* 1996;273:59–63. [PubMed: 8658196]

- Someya S, Yamasoba T, Weindruch R, Prolla TA, Tanokura M. Caloric restriction suppresses apoptotic cell death in the mammalian cochlea and leads to prevention of presbycusis. *Neurobiology of Aging*. 2006in press
- Spindler SR. Rapid and reversible induction of the longevity, anticancer and genomic effects of caloric restriction. *Mech Ageing Dev* 2005;126:960–966. [PubMed: 15927235]
- Spindler SR. Use of microarray biomarkers to identify longevity therapeutics. *Aging Cell* 2006;5:39–50. [PubMed: 16441842]
- Spindler SR, Dhahbi JM. Conserved and tissue-specific genic and physiologic responses to caloric restriction and altered IGFI signaling in mitotic and postmitotic tissues. *Annu Rev Nutr* 2007;27:193–217. [PubMed: 17428180]
- Spindler SR, Mote PL. Screening candidate longevity therapeutics using gene-expression arrays. *Gerontology* 2007;53:306–321. [PubMed: 17570924]
- Sunder M. Toward generation XL: anthropometrics of longevity in late 20th-century United States. *Econ Hum Biol* 2005;3:271–295. [PubMed: 15921967]
- Susulic VS, Frederick RC, Lawitts J, Tozzo E, Kahn BB, Harper ME, Himms-Hagen J, Flier JS, Lowell BB. Targeted disruption of the β_3 -Adrenergic receptor gene. *J Biol Chem* 1995;270:29483–29492. [PubMed: 7493988]
- Swindell WR. Gene expression profiling of long-lived dwarf mice: longevity-associated genes and relationships with diet, gender and aging. *BMC Genomics* 2007a;8:353. [PubMed: 17915019]
- Swindell WR. Genotype-by-age interaction and identification of longevity-associated genes from microarray data. *Age* 2007b;29:97–102. [PubMed: 19424835]
- Thirumoorthy N, Kumar KTM, Sundar AS, Panayappan L, Chatterjee M. Metallothionein: an overview. *World Journal of Gastroenterology* 2007;13:993–996. [PubMed: 17373731]
- Tsai J, Sultana R, Lee Y, Perteu G, Karamycheva S, Antonescu V, Cho J, Parvizi B, Cheung F, Quackenbush J. Resourcerer: a database for annotating and linking microarray resources within and across species. *Genome Biol* 2001;2software0002
- Tsuchiya T, Dhahbi JM, Cui X, Mote PL, Bartke A, Spindler SR. Additive regulation of hepatic gene expression by dwarfism and caloric restriction. *Physiol Genomics* 2004;17:307–315. [PubMed: 15039484]
- Wang YF, Spitz MR, Tsou AM, Zhang K, Maken N, Wu X. Sulfotransferase (SULT)1A1 polymorphism as a predisposition factor for lung cancer: a case-control analysis. *Lung Cancer* 2002;35:137–142. [PubMed: 11804685]
- Walker GA, Lithgow GJ. Lifespan extension in *C. elegans* by a molecular chaperone dependent upon insulin-like signals. *Aging Cell* 2003;2:131–139. [PubMed: 12882326]
- Williams RBH, Cotsapas CJ, Cowley MJ, Chan E, Nott DJ, Little PF. Normalization procedures and detection of linkage signal in genetical-genomics experiments. *Nat Genetics* 2006;38:855–856. [PubMed: 16874319]
- Wu MT, Wang YT, Ho CK, Wu DC, Lee YC, Hsu HK, Kao EL, Lee JM. SULT1A1 polymorphism and esophageal cancer in males. *Int J Cancer* 2003;103:101–104. [PubMed: 12455060]
- Wu P, Shen Q, Dong S, Xu Z, Tsien JZ, Hu Y. Calorie restriction ameliorates neurodegenerative phenotypes in forebrain-specific presenilin-1 and presenilin-2 double knockout mice. *Neurobiology of Aging*. 2007in press
- Xiong Y, Hannon GJ, Zhang H, Casso D, Kobayashi R, Beach D. p21 is a universal inhibitor of cyclin kinases. *Nature* 1993;366:701–704. [PubMed: 8259214]
- Yamaza H, Komatsu T, To K, Toyama H, Chiba T, Higami Y, Shimokawa J. Involvement of insulin-like growth factor-1 in the effect of caloric restriction: regulation of plasma adiponectin and leptin. *J Gerontol A Biol Sci Med Sci* 2007;62:27–33. [PubMed: 17301034]
- Yan L, Vatner DE, O'Connor JP, Ivessa A, Ge H, Chen W, Hirotsu S, Ishikawa Y, Sadoshima J, Vatner SF. Type 5 adenylyl cyclase disruption increases longevity and protects against stress. *Cell* 2007;130:247–258. [PubMed: 17662940]
- Yu BP. Why calorie restriction would work for human longevity. *Biogerontology* 2006;7:179–182. [PubMed: 16676136]
- Zheng L, Wang Y, Schabath MB, Grossman HB, Wu X. Sulfotransferase 1A1(SULT1A1) polymorphism and bladder cancer risk: a case-control study. *Cancer Lett* 2003;202:61–69. [PubMed: 14643027]

Figure 1.

Differential expression signatures. Each row corresponds to a contrast that evaluates the effects of CR on the expression of a large number of genes (see Table 1). Information listed in Table 1 has been incorporated into the labels shown in the figure. Labels indicate the contrast ID, followed by the duration of CR and the age at which CR was initiated. Within a given row, genes upregulated by CR are shown in red, while genes downregulated by CR are shown in green. Light yellow regions correspond to genes for which expression is not significantly affected by CR and blank white regions indicate where data was not available. The ordering of signatures corresponds to that generated by hierarchical clustering analysis (see Figure 2). Cluster analysis was based on all genes for which data was available. Signatures displayed in

the figure, however, are based on a subset of 1347 genes, which were obtained by filtering to eliminate genes not differentially expressed with respect to more than two signatures.

Figure 2.

Differential expression signature cluster analysis. The dendrogram provides results of a cluster analysis performed on differential expression signatures using the similarity metric described by Equation (1). Each leaf corresponds to a contrast involving the effects of caloric restriction (see Table 1). Information listed in Table 1 has been incorporated into the leaf labels shown in the figure. Labels indicate the contrast ID, followed by the duration of CR and the age at which CR was initiated. The horizontal axis indicates the average level of dissimilarity at which two clusters were joined, where dissimilarity is defined as $1 - s$ (see Equation 1).

Figure 3. Overlap of differential expression signatures. (A) A total of 719 probesets were induced by CR with respect to contrast hrt7, while 120 probesets were induced by CR with respect to contrast hrt8b (see Table 1). The Venn diagram shows the overlap between these two groups, where up and down triangles indicate the number of probesets up and downregulated, respectively. (B) The overlap between differential expression signatures associated with hrt7 and hrt8b consists of $T = 8 + 14 = 22$ probesets (see Equation 2). Part (B) shows the null distribution of T generated by simulation under the null hypothesis that the probability of differential expression with respect to contrast hrt7 is independent of the probability of differential expression with respect to hrt8b. The dotted vertical line indicates the observed value of $T = 22$, as indicated in the overlap region of the Venn Diagrams shown in part (A).

	lvr1	lvr4a	lvr4b	lvr5a	lvr5b	lvr10	lvr13	hrt7	hrt8a	hrt8b	hrt8c	hrt13	msl1	msl3	wat9a	wat9b	hyp1	hyp13	hip12	cln1	lng6	kid11	coc2	
	Δ	-	-	-	-	-	Δ	Δ	Δ	-	-	Δ	Δ	-	-	-	-	Δ	Δ	Δ	-	×	Δ	Per2
	Δ	-	-	-	-	-	Δ	Δ	-	-	-	-	Δ	-	-	-	Δ	-	-	Δ	Δ	×	-	Pnpla2
	Δ	-	-	-	▽	-	-	Δ	-	-	-	-	Δ	-	-	-	Δ	-	-	Δ	Δ	×	-	Mt1
	Δ	-	-	-	-	-	-	Δ	-	-	-	-	Δ	-	-	-	Δ	-	-	-	Δ	-	-	Cdkn1a
	Δ	-	-	-	-	-	Δ	-	-	-	-	Δ	Δ	-	-	-	-	Δ	-	Δ	-	×	-	Tspan4
	Δ	-	-	-	-	-	Δ	Δ	-	-	-	-	Δ	-	-	-	-	-	-	Δ	Δ	×	-	Pim3
	Δ	Δ	Δ	-	-	Δ	Δ	Δ	Δ	-	Δ	Δ	Δ	-	-	-	Δ	Δ	×	Δ	-	-	-	Rbm3
	Δ	-	-	-	-	-	-	Δ	-	-	-	Δ	Δ	-	-	-	-	-	-	Δ	Δ	×	-	Peci
	Δ	-	-	Δ	Δ	-	Δ	Δ	-	-	-	-	Δ	-	-	-	-	-	-	Δ	Δ	-	-	Fkbp5
	Δ	-	-	-	-	-	Δ	Δ	-	-	-	-	Δ	-	-	-	-	-	-	Δ	Δ	-	-	Per1
	Δ	-	-	-	-	-	Δ	Δ	-	-	-	-	Δ	-	-	-	Δ	-	-	-	Δ	×	-	Nfkbia
	Δ	-	-	-	-	-	-	Δ	-	-	-	-	Δ	-	-	-	Δ	-	-	-	Δ	-	-	Ddit4
	Δ	-	-	-	-	-	-	-	-	-	-	-	Δ	-	-	-	Δ	-	×	Δ	Δ	×	-	MGI:1924575
	Δ	-	-	-	-	-	Δ	Δ	-	-	-	Δ	Δ	-	-	-	Δ	-	×	-	Δ	-	-	Sult1a1
	Δ	-	-	-	-	-	-	-	Δ	Δ	-	-	Δ	-	-	-	Δ	-	-	Δ	-	-	-	Ppara
	Δ	▽	-	-	▽	-	-	Δ	-	-	-	-	Δ	-	-	▽	-	-	▽	Δ	Δ	-	-	Mt2

Figure 4.

Genes upregulated by CR in five or more tissue types. A total of 16 genes met this criterion and are included in the figure. Each row corresponds to an individual gene, while each column corresponds to a contrast (see Table 1). Within the grid, up-triangles indicate a gene was significantly upregulated by CR, and down-triangles indicate a gene was significantly downregulated by CR. A dash indicates where a gene was not significantly induced by CR, and “×” indicates where data was not available.

	lvr1	lvr4a	lvr4b	lvr5a	lvr5b	lvr10	lvr13	ht7	ht8a	ht8b	ht8c	ht13	ms1	ms3	wat9a	wat9b	hyp1	hyp13	hip12	cin1	lng6	kid11	coc2	
	▽	-	-	-	-	-	-	▽	-	-	-	▽	▽	-	-	▽	-	▽	×	▽	▽	-	-	Serpinh1
	▽	-	-	-	-	-	-	▽	-	-	-	-	-	▽	-	▽	-	-	△	▽	▽	-	-	Ifi27
	▽	-	-	-	-	-	-	▽	-	▽	▽	▽	▽	-	-	-	▽	-	△	-	▽	-	-	Col3a1
	-	-	-	-	△	-	▽	▽	-	-	-	▽	-	-	-	▽	-	▽	-	▽	-	×	-	Hsp110
	-	-	-	-	-	-	-	▽	-	-	-	▽	-	-	-	▽	-	▽	▽	-	-	▽	-	C1qb
	▽	-	-	-	-	-	-	-	-	-	-	-	▽	-	-	-	▽	-	-	▽	▽	×	-	Gng11
	▽	-	-	-	-	-	-	-	-	▽	▽	▽	▽	-	-	▽	-	-	-	-	▽	-	-	Col1a1
	-	-	-	-	▽	-	-	▽	▽	▽	-	▽	-	-	-	▽	-	-	-	-	▽	▽	-	H2-Ea
	-	-	-	▽	▽	-	-	×	-	▽	-	-	-	-	-	-	▽	-	-	▽	▽	×	-	Ifitm3
	-	-	-	-	▽	-	-	-	-	-	-	▽	-	-	-	▽	-	-	▽	-	▽	×	-	Ccl9
	▽	-	-	-	-	-	-	-	-	-	-	-	▽	-	-	-	▽	-	-	▽	▽	-	-	Tgtp
	-	-	-	-	-	-	-	-	▽	▽	-	▽	-	▽	-	▽	-	-	-	-	▽	▽	-	Cd74

Figure 5.

Genes downregulated by CR in five or more tissue types. A total of 12 genes met this criterion and are included in the figure. Each row corresponds to an individual gene, while each column corresponds to a contrast (see Table 1). Grid symbols are described in the Figure 4 legend.

Figure 6.

Effects of CR on Per2 versus Sirt1 expression. Solid circles connected by solid lines represent log-transformed fold-changes associated with Per2, and open circles connected by dotted lines represent log-transformed fold-changes associated with Sirt1. The horizontal axis indicates the contrast to which each symbol corresponds. Since Sirt1 was not represented on all microarray platforms considered, only seven contrasts for which data was available are listed. In some cases, Per2 and/or Sirt1 genes were represented by multiple probesets. In these instances, log-transformed fold-change was calculated for each probeset individually, and the maximum value associated with each gene is plotted in the figure.

Figure 7.

Effects of CR and aging in liver: Differential expression signature cluster analysis. Differential expression signatures associated with the effects of CR in liver tissue were clustered together with those corresponding to the effects of aging in liver. The similarity metric described by Equation (1) was used to cluster signatures, and the horizontal axis indicates the average level of dissimilarity at which two clusters were joined (dissimilarity = $1 - s$). Aging effects were defined as Young – Old, such that age signatures would be comparable to the effect of CR, defined as CR – control. Supplemental Data File 2 includes a heatmap image of differential expression signatures shown in the figure. Supplemental Data File 3 provides a similarity matrix of differential expression signatures and provides indication of which pairwise signature comparisons were associated with significant levels of overlap.

	lvr1	lvr4a	lvr4b	lvr5a	lvr5b	lvr10	lvr13	age1	age2	age3	age4	age5	
	△	△	△	-	△	-	△	-	△	-	-	-	Glrx
	△	△	-	-	-	△	△	-	-	-	-	△	Cyp4a14
	△	-	-	△	△	-	△	-	△	-	-	-	Cyp2c39
	△	△	△	-	-	-	-	-	△	-	-	-	Slc20a1
	-	△	△	-	-	-	△	-	△	-	-	-	Grem2
	△	-	-	-	△	-	△	▽	△	-	-	-	Klhl21
	△	△	-	-	-	-	△	-	△	-	-	-	Gstt2
	△	△	-	-	-	-	△	-	△	-	-	-	Pcsk4
	△	-	-	-	△	-	△	-	△	-	-	-	Mknk2
	△	△	-	-	-	-	△	-	△	-	-	-	Usp2

Figure 8.

Effects of CR and aging in liver: Genes upregulated by CR and downregulated by aging. A total of 10 genes were upregulated by CR with respect to three or more liver contrasts, and downregulated with respect to at least one aging contrast. Each row corresponds to an individual gene, while each column corresponds to a CR or age contrast associated with liver tissue. For lvr contrasts, triangles indicate whether a gene was significantly up or downregulated by CR. For age contrasts, up-triangles indicate where expression was significantly higher in young mice versus old mice, and down-triangles indicate where expression was significantly lower in young mice versus old mice. For all contrasts, the dash indicates where either CR or aging had no significant effect on gene expression. It should be noted that genes Slc20a1 and Grem2 do not meet selection criteria if CR data generated from a long-lived mutant mouse strain are excluded from the analysis (contrast lvr4b).

	lvr1	lvr4a	lvr4b	lvr5a	lvr5b	lvr10	lvr13	age1	age2	age3	age4	age5	
	▽	-	-	-	▽	▽	-	-	▽	-	-	▽	Ly6a
	▽	▽	-	-	-	-	▽	-	▽	-	▽	-	Igh-6
	▽	-	-	▽	△	-	▽	-	▽	-	-	-	Chka
	▽	-	-	▽	△	-	▽	-	▽	-	-	-	Nedd4l
	▽	-	-	-	▽	-	▽	-	▽	-	-	-	Ifi47
	▽	-	-	-	▽	-	▽	-	-	▽	-	-	Serpina12
	▽	-	-	-	▽	-	▽	▽	-	-	-	-	Adrb3
	▽	-	-	-	-	▽	▽	-	▽	-	-	-	Tubb2a
	-	-	-	▽	▽	-	▽	-	▽	-	-	-	Lgals3bp
	▽	-	-	▽	▽	-	-	-	▽	-	-	-	Lgals1

Figure 9.

Effects of CR and aging in liver: Genes downregulated by CR and upregulated by aging. A total of 10 genes were downregulated by CR with respect to three or more lvr contrasts, and upregulated with respect to at least one age contrast. Each row corresponds to an individual gene, while each column corresponds to a CR or age contrast associated with liver tissue. Symbols used within the grid a defined in the Figure 8 legend.

Figure 10.

Effects of CR and resveratrol in liver. The effects of resveratrol in liver were evaluated by Baur et al. (2006) (Gene Expression Omnibus series GSE6089). Differential expression signatures from data generated by Baur et al. (2006) were compared to those associated with the effects of CR in liver (lvr1, lvr4a, lvr4b, lvr5a, lvr5b, lvr10, lvr13). Each panel shows the null distribution of T associated with each comparison (see Methods), along with the observed value of T (dotted vertical line). The panels correspond to the comparison of the resveratrol differential expression signature to (A) lvr1, (B) lvr4a, (C) lvr5a, (D) lvr5b, (E) lvr10 and (F) lvr13 (lvr4b is not shown).

Contrasts evaluating the effects of CR on gene expression. The contrast ID indicates the type of tissue examined and ends with an identification number that differentiates multiple contrasts associated with the same tissue type. The % CR column indicates the reduction of calories in the CR treatment relative to a corresponding control treatment. Duration indicates the length of time that CR was enforced, while Age indicates the ages over which CR was applied (in months). The gender and strain of CR-treated and control-fed mice are indicated by the final two columns of the table.

Table 1

Contrast ID	% CR	Duration	Age	Gender	Strain
lvr1 ^a	10–30%	0.50	3.5–4	m	C57BL/6
lvr4a ^b	30%	4	2–6	f	<i>Prop1</i> ^{dl/+}
lvr4b ^b	30%	4	2–6	f	<i>Prop1</i> ^{dl/dl}
lvr5a ^c	44%	2	20–22	m	B6C3F1
lvr5b ^c	44%	17+	5–22+	m	B6C3F1
lvr10 ^d	35%	1	N/A	m	SV129
lvr13 ^e	40%	2.5–4.5	1.5–6	m	C57BL/6
hrt7 ^f	41%	16	14–30	m	B6C3F1
hrt8a ^g	44%	6	1–7	m	B6C3F1
hrt8b ^g	44%	24	7–31	m	B6C3F1
hrt8c ^g	44%	2	29–31	m	B6C3F1
hrt13 ^e	40%	2.5–4.5	1.5–6	m	C57BL/6
msl1 ^a	10–30%	0.5	3.5–4	m	C57BL/6
msl3 ^h	26%	23.5	1.5–25	m	C57BL/6NHsd
wat9a ⁱ	41%	0.75	9.75–10.5	m	C57BL/6
wat9b ⁱ	41%	9	1.5–10.5	m	C57BL/6
hyp1 ^a	10–30%	0.5	3.5–4	m	C57BL/6
hyp13 ^e	40%	2.5–4.5	1.5–6	m	C57BL/6
hip12 ^j	30%	4	8–12	m	B6CBA
cln1 ^a	10–30%	0.5	3.5–4	m	C57BL/6
lng6 ^k	66%	2 days	N/A	m	C57BL/6J
kid11 ^l	N/A	N/A	?–30	N/A	C57BL/6
coc2 ^m	26%	13	2–15	m	C57BL/6

^aEMEXP748, Selman et al. (2006)

- ^b GSE1093, Tsuchiya et al. (2004)
- ^c GSE2431, Dhahi et al. (2005)
- ^d Corton et al. (2004)
- ^e Fu et al. (2006)
- ^f Lee et al. (2002)
- ^g Dhahbi et al. (2006)
- ^h GSE6323, Edwards et al. (2007)
- ⁱ Higami et al. (2003)
- ^j Wu et al. (2007)
- ^k GSE478, Massaro et al. (2004)
- ^l GSE459
- ^m GSE4786, Someya et al. (2006)

Table 2

Notations associated with Equation (2). Consider two contrasts A and B . Contrast A evaluates whether gene expression levels differ significantly between CR mice and mice provided a normal diet. Contrast B evaluates this same hypothesis for another experiment involving CR and normal-fed mice. Considering contrasts A and B jointly, there are nine outcome categories to which a given probeset may be assigned. These categories depend on whether, with respect to both A and B , a probeset is upregulated by CR, downregulated by CR or CR has no significant effect on expression. Among all N probesets evaluated in a given comparison, the number of probesets assigned to each category is indicated by the values of n given in the table.

			Contrast A	
		Upregulated	Downregulated	No CR Effect
Contrast B	Upregulated	$n_{+,+}$	$n_{-,+}$	$n_{0,+}$
	Downregulated	$n_{+,-}$	$n_{-,-}$	$n_{0,-}$
	No CR Effect	$n_{+,0}$	$n_{-,0}$	$n_{0,0}$

Table 3

Overview of differential expression results. For each signature, the Total column indicates the number of unique genes for which expression data was available. The Upregulated column indicates the number of unique genes for which expression was upregulated significantly by CR ($P < 0.05$). Likewise, the Downregulated column indicates the number of unique genes for which expression was significantly downregulated by CR ($P < 0.05$).

Contrast ID	Total	Upregulated	Downregulated
lvr1	20958	1767	2862
lvr4a	7862	91	65
lvr4b	7862	22	22
lvr5a	7862	58	91
lvr5b	7862	273	524
lvr10	7862	12	54
lvr13	7862	439	215
hrt7	7862	324	376
hrt8a	7862	101	48
hrt8b	7862	48	79
hrt8c	7862	16	22
hrt13	7862	59	91
msl1	20958	1778	1064
msl3	13415	554	456
wat9a	7862	14	21
wat9b	7862	120	122
hyp1	20958	852	655
hyp13	7862	21	66
hip12	14459	115	18
cln1	20958	622	355
lng6	7862	254	190
kid11	5481	1	8
coc2	20958	138	18

Table 4

Common responses to CR among tissues. Results from experimental studies were linked to probesets associated with the Affymetrix 430 2.0 microarray. A total of 45,037 probesets (approximately 20,000 unique genes) were considered in the analysis and the effect of CR on expression was examined for each probeset with respect to as many as ten tissue types. Most probesets were not induced by CR with respect to any tissue type and only two probesets (two unique genes) were induced by CR with respect to seven tissue types (Per2 and Serpinh1, see Figures 4 and 5) ($P < 0.05$). The table lists the number of unique genes significantly upregulated and downregulated by CR with respect to 0, 1, ..., 6 and 7 different tissue types.

Number of Tissues	Upregulated	Downregulated
7	1	1
6	2	1
5	13	10
4	48	30
3	200	128
2	917	805
1	4491	4664
0	19139	19079