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AGING OF THE LIVER

1. INTRODUCTION

Most organs are altered morphologically and functionally in old animals. To a varying extent, these age-related changes lead to a progressive loss of differentiated functions and physiologic capacities[1]. For the liver, the data are inconsistent and conflicting regarding the effects of aging on structure and function[2]. A number of reviews dealing with the physiology, structure and function of the aging liver have been published recently[3-5]. Reported morphological and structural changes do not generally correlate with the functional alterations found in the liver with age. These disparities raise the question of whether or not the observed changes with age actually compromise liver function, which led to the idea that the liver ages well when compared to other organs.

However, as described in a number of the reviews cited above, the liver does decline functionally with age. Our recent microarray studies of mice found that aging was accompanied by changes in gene expression linked to the development of the characteristic age-related liver pathologies[6]. These include hepatocellular carcinoma, fibrosis, cirrhosis, and unhealthful apolipoprotein and fatty acid biosynthesis. Aging increased gene expression associated with inflammation, cellular stress, and fibrosis, and reduced capacity for apoptosis, negative cell-growth control, and phase I and II xenobiotic metabolism. In this study, CR (calorie restriction) from weaning [long-term CR (LT-CR)], reversed the majority of these changes. Surprisingly, in very old mice just 2 to 4 weeks of CR [short-term CR (ST-CR)] reversed approximately 70% of these age-related changes in gene expression.

LT-CR and ST-CR also produced changes in the expression of genes which did not change with age. These *CR-specific* changes involved increased gluconeogenesis and disposal of the byproducts of extrahepatic protein catabolism, reduced glycolysis, and healthful changes in apolipoprotein and fatty acid biosynthesis. In addition, LT-CR and ST-CR produced changes in gene expression associated with enhance anti-proliferative growth control, increased apoptosis and reduced chemical carcinogenesis. A number of other alterations in gene expression are associated with enhanced longevity in mice.

2. MICROARRAYS

Quantitative change in the activity of specific genes can control the rate of aging in invertebrates and mammals[7,8]. Although there have been many studies of the relationship between aging, CR and hepatic gene expression, there are serious shortcomings in this literature. There are numerous cross-sectional studies of gene expression in animals of various ages which are interpreted as showing that the major effect of CR is to *prevent* age-related changes in gene expression (e.g. Ref 9). This interpretation has become pervasive in the literature, despite the cross-sectional nature of the studies. Funding and publication bias has reinforced this notion, producing a literature replete with reports of age-related changes in gene expression which appear to be *prevented* by CR.

Genome-wide DNA-microarrays are capable of quantifying the expression of all know genes in a single experiment. A significant strength of this approach is the absence of hypothesis-based bias in the choice of genes which are studied. Instead, a comprehensive profile of the relationship between a physiological state and gene expression is generated. Application of this technology has revealed the gene expression signatures underlying the physiological effects of aging, CR, and the dwarf mutations[6,10-14]. In this way, microarrays are providing insights into aging, the development of age-related diseases, and the ameliorative actions of CR.

Our studies using this technology suggest that rather than simply preventing age-related changes in gene expression, CR instead acts rapidly to establish a new profile of gene expression which may better resist aging. Overall, ST-CR reproduced nearly 70% of the effects of LT-CR on genes that changed expression with age[6]. Thus, CR rapidly reversed, rather than prevented, many age-related changes in gene expression.

Another important effect of LT- and ST-CR was to establish the CR-specific patterns of gene expression. These CR-specific changes were in the same functional categories as the age-related changes. Further, CR in

young mice produced gene expression changes which were a subset of those produced in old CR mice. These similarities between CR in young and old mice, and between ST- and LT-CR led us to conclude that CR rapidly produces a new pattern of gene expression which better resists aging.

3. INTRA- AND INTERCELLULAR SIGNALING

Recently, a small family of single gene mutations which interfere with growth hormone (GH)/insulin-like growth factor-1 (IGF-1) signaling, resulting in dwarfism, have been shown to increase mean and maximal lifespans of mice by 40% to 70% beyond those of their heterozygous siblings[8,15,16]. The dwarf mice are homozygous for loss-of-function mutations in the Pit1 (Snell dwarf mice), Prop1 (Ames dwarf mice), or GH receptor (GHR KO mice) loci. The Pit1 and Prop1 mutations prevent differentiation of the anterior pituitary, decreasing levels of thyroid hormone, growth hormone, IGF-1 and prolactin. The GHR KO is a more focused mutation, which prevents receptor mediated GH responsiveness. The mutations appear to slow the intrinsic rate of aging. Snell dwarf mice show delays in age-dependent collagen cross-linking and in six age-sensitive indices of immune system status. These findings demonstrate that a single gene can control maximum lifespan and the timing of both cellular and extracellular senescence in mammals. The already enhanced lifespan of Ames dwarf mice can be further extended ~25% by CR[17].

Our microarray studies found that the hepatic expression of IFG-I binding protein 1 decreased with age. This protein plays an important role in the negative regulation of the IGF-1 system, a stimulator of mitogenesis[18]. Given what is now known about the apparent role of IGF-1 signaling in aging, this change may have both pro-cancer and anti-aging effects.

CR repressed expression of GH receptor and iodothyronine deiodinase type I mRNA in the liver of both young and old mice, and induced overexpression of IGF binding protein mRNA, which inhibits IGF-1 signaling. Reduction in iodothyronine deiodinase type I expression should reduce hepatic conversion of the pro-hormone form of thyroid hormone (T_4) to the active form (T_3). Down-regulation of this enzyme is likely responsible for the reduced levels of circulating T_3 found in CR rodents[19]. Short-term treatment with low-calorie diets rapidly reduces circulating T_3 levels in morbidly obese men, apparently by reducing type I deiodinase activity[20]. Thus, CR appears to reduce thyroid hormone action in CR mice and humans.

Thus, some of the changes in gene expression induced by CR in mouse liver are associated with decreased GH receptor, IGF-1, and thyroid hormone signaling. This is highly suggestive of the lifespan extending effects of the Prop-1 and Pit-1 mutations[21]. It suggests that the dwarf mutations and CR may work in part through the same signal transduction pathways.

4. AGE-RELATED INFLAMMATION

Published microarray studies of mammalian aging found that aging was associated with changes in gene expression linked to the development of the characteristic age-related pathologies of tissues such as liver, muscle and brain[6,13,22,23]. Our microarray studies of mouse liver revealed that aging was associated with other gene expression changes consistent with pathogenesis. We found age-associated induction in the expression of several genes important in inflammation including lysozyme and complement component 1, q, β[6]. Lysozyme is a myeloid cell-specific marker. Induction of this gene is normally associated with macrophage activation[24]. Complement component 1, q, β, a macrophage expressed protein, is a part of the recognition set of the complement C system, the primary humoral mediator of antigen-antibody reactions[25]. Activated macrophages, along with other inflammatory cells, are involved in a large number of liver diseases including cirrhosis, hepatitis, and sepsis- and endotoxin-induced liver injury[26].

Old mice also overexpressed the mRNA for biglycan, a proteoglycan of the hepatic extracellular matrix, serum amyloid P-component, a glycoprotein present in all amyloid deposits, and cystatin B, an inhibitor of cysteine proteinases. In areas of inflammation, fibrogenic myofibroblasts express biglycan and other proteoglycans, lead to hepatic fibrosis[27]. Serum amyloid P-component is one of the major acute phase reactants induced by inflammation in hepatocytes[28]. Cystatins and their target enzymes play a role in many pathological events, including inflammatory disease[29]. In the liver, an imbalance between cystatins and their targets can disregulate matrix degradation and accumulation, leading to hepatic fibrosis[30].

CR suppressed the age-associated increase in inflammatory and stress response genes. Consistent with decreased inflammatory response gene expression, CR delays the onset and diminishes the severity of autoimmune and inflammatory diseases in mice[31]. Decreased chaperone and stress response gene expression suggests that CR reduces the age-related physiological stress on the liver. Further, as discussed below, reduced chaperone expression is proapoptotic and anti-neoplastic. Thus, these effects may explain the delayed onset of hepatoma in CR mice[32].

5. APOPTOSIS AND TUMORIGENESIS

Hepatocytes are mitotically competent, although they have long, mostly intermitotic lifespans. These lifespans appear to lead to the incremental accumulation of damage, and the gradual impairment of physiological functions. Thus, there is an important role for apoptosis in the maintenance of hepatic function. Apoptosis was initially viewed as potentially injurious to tissues, because it destroys cells. The current view recognizes that the role of apoptosis in aging is most likely tissue-specific. In every tissue, a balance must be struck between the need to maintain cell number and function, and the need to eliminate damaged, potentially toxic or neoplastic cells. This decision is crucial in largely postmitotic tissues such as brain. Brain apoptosis can contribute to neurological disorders of aging, including Alzheimer's disease, Parkinson's disease and stroke[33].

Hepatocytes are exposed to genotoxins from the diet and from free radicals generated by xenobiotic metabolism and beta-oxidation. These can produce elevated levels of DNA damage, a potential source of neoplasia. Apoptosis acts to eliminate the damaged and preneoplastic cells, which are then replaced by cell proliferation, thus maintaining homeostatic liver function.

The predominant morphologic change in aging human liver is termed *brown atrophy*[34]. A brown color in aged liver cells results from the accumulation of lipofuscin in lysosomes. Liver atrophy results from an age-related decline in liver mass, resulting in fewer, larger hepatocytes. These observations suggest that aging is accompanied by a disregulation of apoptosis and cell division which fails to maintain youthful hepatocyte number and function during aging. Consistent with this idea, aging is associated with a decline in the rate of liver regeneration and apoptosis[35,36]. Likewise, aging is accompanied by an increase in liver tumors[37,38].

6. AGING, CR AND HEPATIC CELL DIVISION

Our microarray studies found that 23% of the genes which decreased expression with age are involved in DNA replication and regulation of the cell cycle[6]. Most of these genes have a negative effect on cell growth and division. Thus, hepatic aging may be accompanied by a general loss of negative control of cell division. Among these genes, the product of phosphatase and tensin homolog gene is a tumor suppressor which induces cell-cycle arrest through inhibition of the phosphoinositide 3-kinase pathway[39]. B-cell translocation gene 2 is a tumor suppressor which increases expression in response to DNA damage[40]. The murine gene product of the amino-terminal enhancer of split is a potent co-repressor of gene expression and cellular proliferation[41]. Calcium binding protein A11 binds to and regulates the activity of annexin II, which is involved in the transduction of calcium-related mitogenic signals[42]. As discussed above, IGF binding protein 1 negatively regulates the IGF-1 signaling[18]. Therefore, this change may be mitogenic.

Seventy-eight percent of the mice of this strain and sex fed the control diet used here die of some form of neoplasia, and the death rate from neoplasia accelerates dramatically with age[32]. Approximately 21% of these mice die of hepatoma, mostly late in life. Decreased expression of the negative growth regulators and overexpression of the chaperone genes with age are consistent with this higher incidence of hepatoma in aged mice.

LT- and ST-CR induced the expression of cyclin-dependent kinase 2-associated protein 1, a putative tumor suppressor gene[43]. Overexpression of this gene suggests that LT- and ST-CR enhance anti-proliferative growth control. Consistent with this idea, IGF binding protein 7 gene expression was induced by LT-CR. The product of this gene functions both as an IGF binding protein and independently of IGF as a growth-suppressing factor[44]. The expression of IGF binding protein 1, which has anti-growth activity through its inhibition of IGF-1 signaling, was reduced by age and restored by ST-CR. Thus, LT- and ST-CR may produce additional anti-proliferative effects on preneoplastic cells of the liver through their effects on the expression of these IGF binding protein family members.

7. AGING AND APOPTOSIS

Our microarray studies revealed that aging in mice was accompanied by elevated chaperone levels and the over expression of the other anti-apoptotic genes, myeloid cell leukemia sequence 1 and apoptosis inhibitory protein 6. These observations suggested that aging should be accompanied by a decrease in apoptotic potential of the liver. In contrast to this expectation, a number of studies reported that aging is accompanied by an increase in the intrinsic rate of apoptosis in rodent liver[45,35,46].

However, a recent study has clarified this conundrum. Suh et al. showed that the intrinsic rate of apoptosis in liver does increase slightly with age. But the increase was not significant in their study. However, they found a large and significant decrease in the apoptotic potential of the liver with age[36]. Brief exposure to a direct-acting genotoxic alkylating agent produced high rates of apoptosis in the liver of young rats, but little apoptosis in the livers of old. These results suggest that the apoptotic capacity of the liver declines with age, while the basal rate of apoptosis may

increase slightly. These data suggest that damaged and preneoplastic cells likely accumulate with age in the liver. This interpretation is consistent with the increase in brown atrophy and hepatocellular neoplasms with age in mouse and man[37,47].

8. CR AND APOPTOSIS

Our genome-wide microarray studies found that 21% of the genes which changed expression in response to LT- and ST-CR are associated with apoptosis, cell growth, or cell survival[6]. LT-CR induced the expression of the Bcl2 homologous antagonist / killer and voltage-dependent anion channel 1 (porin) genes. Bcl2 homologous antagonist / killer is a pro-apoptotic member of the Bcl2 family of apoptosis regulators. It directly interacts with porin to release the pro-apoptotic factor cytochrome c from mitochondria, initiating apoptosis[48]. The overexpression of porin found in ST-CR mice is consistent with the increase in apoptosis and reduction in chemical carcinogenesis found in fasting rodents[49,50]. LT-CR decreased the expression of the anti-apoptotic genes interferon inducible ds RNA dependent inhibitor, X-box binding protein, and lymphocyte antigen 6 complex, locus E[51-53].

ST-CR reproduced the effects of LT-CR on the expression of 50% of the cell-cycle / DNA replication and apoptosis genes. The combination of these effects on gene expression suggests that ST-CR may be capable of rapidly reproducing the anti-neoplastic effects of LT-CR in very old animals. This conclusion is consistent with studies showing that short-term fasting increases apoptosis in preneoplastic lesions, and reduces rates of chemical carcinogenesis in the liver[49].

There is compelling evidence that CR increases the rate of apoptosis in preneoplastic and normal cells. The rate of apoptosis in the liver of mice, as measured using terminal dUTP nick end labeling (TUNEL) of apoptotic bodies, was 3 times higher in CR mice[54]. Increased hepatocyte apoptosis was associated with a significantly lower incidence of spontaneous hepatomas throughout the lifespan of the CR mice. Using glutathione S-transferase-II (GST-II) as an immunohistochemical marker of preneoplastic liver cells, a progressive rise in GST-II labeling was seen with age in control mice[38]. This increase was associated with a high incidence of GST-II positive liver tumors. GST-II expression was negligible in CR mice, which had a significant decrease in tumor incidence. One week of CR induced apoptosis in the GST-II-positive hepatocytes. In another study, CR eliminated 20-30% of liver cells by apoptosis, decreasing the number of preneoplastic liver foci by 85%[55]. Apoptosis is significantly higher at all ages in hepatocytes from CR mice[35]. CR enhances apoptosis in other organs as well, including gastrointestinal tract, bladder, spleen and lymph nodes[56-58].

9. CHAPERONES, AGING, AND CR

A consistent finding of our genome-wide microarray and conventional studies was that the mRNA and protein levels of essentially every endoplasmic-reticulum chaperone increased with age and decreased with CR in the liver[6,59-61]. Similar results were obtained in several other tissues. The induction of chaperone gene expression in the livers of aged mice may be a physiological adaptation to increased oxidative or possibly other stress during aging. For a number of years the meaning of these changes was unclear. In the past few years, the relationship between chaperones and health is beginning to be understood.

Stress-inducible chaperones respond to a diverse group of stimuli including heat, oxidative and ischemic stress, inflammation, hemodynamics, and exposure to toxic chemicals[62-64]. Under such conditions, these inducible chaperones associate with abnormally folded proteins to promote their renaturation, prevent their aggregation, or promote their degradation if they cannot be properly refolded. A number of years ago, Richardson and his colleagues found that the heat inducibility of the stress-responsive chaperone, hsp70 was significantly reduced in hepatocytes isolated from old rats[65]. Similar results were found in fibroblasts from donors of various ages[66]. Richardson and colleagues also found that in old rats maintained on LT-CR, there was no decrease in the response of hsp70 to hyperthermia[65].

However, inducible chaperones like hsp70 cannot be detected in the absence of physiological stress. They play a different role than the chaperones which are present continuously in cells in the absence of physiological stress, which is by far the most common physiological state. Most proteins require interactions with constitutively expressed molecular chaperones for their biosynthesis, maturation, processing, intracellular transport, and secretion[67]. Chaperones also perform cytoprotective functions, including prevention of protein denaturation and aggregation, the repair of structurally damaged proteins[68], and promotion of the ubiquination and proteasomal degradation of malfolded, damaged proteins[69,70]. In this context, it might appear that constitutive overexpression of chaperones would be healthful, perhaps by preventing the accumulation of lipofuscin. However, another function of chaperones appears to mitigate this possible benefit.

Chaperone levels are a part of molecular decision making following genotoxic stress. Elevated chaperone levels tip the balance away from apoptosis and toward cell survival[71,72]. As described above, aging increases chaperone expression and decreases the apoptotic response to genotoxic stress[36]. The increase in chaperone expression with age may explain why hepatocellular neoplasms are the most common lesions in older mice[37,47]. In contrast, CR, which reduces endoplasmic reticulum chaperone levels in the liver and other tissues, enhances apoptosis[72-76]. Enhanced apoptosis by CR may account for its well-documented anti-cancer benefits[77].

The linkage between chaperone levels and apoptosis also extends to fasting and feeding. While feeding increases chaperone levels, fasting reduces the levels of nearly every endoplasmic reticulum and cytoplasmic chaperone we investigated[61,78]. Fasting also increases apoptosis of preneoplastic lesions and reduces the rate of chemical carcinogenesis[49,50]. This connection between caloric intake and chaperone levels may link food intake to the capacity for protein folding, assembly, and processing within cells. The level of chaperone expression in response to feeding does not depend on endoplasmic reticulum protein trafficking[78]. It appears to be regulated by the blood insulin-to-glucagon ratio.

10. MOLECULAR MECHANISMS LINKING CHAPERONES, PROTEIN SECRETION AND CARCINOGENESIS

Chaperone induction has emerged as a new anti-apoptotic mechanism[79,80]. Elevated chaperone levels during tumorigenesis allow cells to survive carcinogenesis and tumor formation[81]. Induced GRP78, GRP94 and GRP170 are essential for the survival, growth and immuno-resistance of transformed cells[82-84]. Tumorigenesis-associated chaperone induction confers drug resistance to the tumors[74,85-89]. Chaperone induction allows precancer cells to survive the DNA damage and mutations which result in transformation, proliferation and onset of carcinogenesis[73-76,90].

Chaperone induction might reduce the production or secretion of apoptogenic signals, or increase the production or secretion of apoptosis inhibitory proteins. Several studies indicate that the abundance of endoplasmic reticulum chaperones influences the secretion efficiency of many liver proteins[91-93]. The interaction between chaperones and other proteins can enhance either protein folding, maturation and processing, or enhance the degradation of proteins[94,95]. It appears that the longer a protein spends in association with chaperones, the greater the chance it will undergo degradation[95-98]. We found that CR, which decreased the level of most endoplasmic reticulum chaperones, increased the rate, efficiency and level of hepatic protein secretion[61]. It is thus possible, that the effect of CR on endoplasmic reticulum chaperone levels and secretion efficiency may change the activity of receptor mediated apoptotic pathways. It may change the display or secretion of pro- or anti-apoptotic receptors or ligands.

The increase in secretory protein output in response to CR may also enhance the turnover of serum proteins. This may decrease circulating levels of glycated serum proteins, which are associated with micro- and macrovascular damage, nephropathy, neuropathy, retinopathy and atherosclerotic disease[99,100]. Modified plasma proteins appear to be significant contributors to the development of age- and diabetes-related renal, vascular, ocular and neurological pathologies, and to aging itself[101-103]. CR reduces the age-related accumulation of glycoxidation products in blood and tissue proteins[104-106].

11. XENOBIOTIC METABOLISM

The effect of aging on hepatic drug metabolism is extremely important due to its effects on both carcinogenesis and its practical implications in determining the drug doses that are safe for older individuals. A decline in hepatic drug metabolism and clearance, and an increase in adverse drug reactions are common hallmarks of human and rodent aging. The liver's capacity to metabolize xenobiotics declines with age[4]. Pharmacokinetic evidence in humans indicates that aging is accompanied by reduced liver phase I drug metabolism. For example, cytochrome P450 content in human subjects decreases 30% after 70 years of age[107]. Altered drug metabolism has been attributed to a decline in liver volume and blood flow in humans, although these changes may only partly account for the decline in the metabolism and clearance of drugs with aging in man[107]. In rodent studies, there is compelling evidence for a decline in phase I and phase II enzyme activities and expression, although the specific enzymes which are altered may vary with strain and species[6,108-110].

In our microarray and conventional studies, aging decreased expression of xenobiotic metabolism genes[6]. This is an additional class of pro-neoplastic changes in gene expression encountered in our microarray studies. The genes for the phase I enzymes amine N-sulfotransferase and three cytochrome P450 isozymes, as well as the gene for the phase II enzyme glutathione S-transferase-like gene were negatively regulated by age. Decreased expression of Phase I enzyme genes in the liver of aged rodents has been reported in many studies[108,111,112]. Decreased expression of such genes is likely responsible in part for the age-related decline in the xenobiotic metabolizing capacity of the liver. This decline is a recognized

source of adverse drug reactions in aged mammals[2]. It may contribute to the increase in neoplasms with age in mice.

LT- and ST-CR reversed the age-related decrease in the expression of genes such as the B-cell translocation gene 2, amino-terminal enhancer of split, glutathione-S-transferase like, amine N-sulfotransferase, and cytochrome P450, 2f2 mRNAs. This CR effect is consistent with the delayed onset of hepatoma in CR mice. Partial restoration of the hepatic drug metabolizing and detoxifying functions of the liver may be a source of the anti-aging and anti-cancer effects of CR. These results suggest that ST-CR may rapidly restore some differentiated functions in tissues of older animals.

12. INTERMEDIARY METABOLISM

Energy metabolism in the liver is altered by aging. For example, at least two studies have shown decreased mitochondrial respiratory rates in the liver with age[113,114]. Perhaps the major effects of age are on homeostatic glucose regulation. The liver plays a critical role in maintaining glucose homeostasis. This homeostasis is controlled by hormones such as insulin, glucagon, growth hormone, and IGF-1. High levels of glucose and insulin are implicated in many age-associated pathologies[115]. Likewise, loss of homeostatic glucose regulation is a hallmark of mammalian aging[116]. CR reduces blood glucose and insulin concentrations in rodents, primates and humans[117-119]. Disorders associated with elevated glucose levels are reduced or mitigated entirely by CR. These facts indicate that the anti-aging effects of CR may be mediated by alteration of the normal sequence of age-related metabolic changes in the liver.

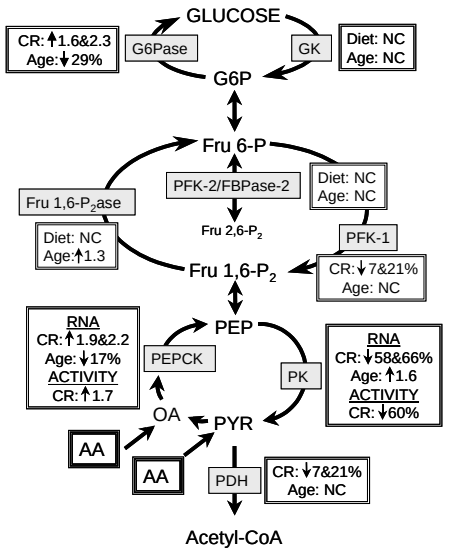

Figure. 1. Summary of the effects of age and CR on the glycolytic and gluconeogenic pathways of the liver. Glycolytic metabolism in the liver involves three irreversible, regulated steps. Glucokinase (GK) initiates glucose metabolism by phosphorylation of C6 yielding glucose 6-phosphate (G6P). The committed step in glycolysis, and the second irreversible and regulated step, is the phosphorylation of Fru 6-P by phosphofructokinase (PFK-1) to produce fructose 1,6-bisphosphate (Fru 1,6-P_2). The third irreversible step controls the outflow of the pathway. Phosphoenolpyruvate (PEP) and ADP are utilized by pyruvate kinase (PK) to produce pyruvate (PYR) and ATP. Pyruvate dehydrogenase (PDH) oxidatively decarboxylates pyruvate to form acetyl-CoA, which is a bridge between glycolysis and the tricarboxylic acid cycle. Phosphoenolpyruvate carboxykinase (PEPCK) catalyzes the first committed step in gluconeogenesis. The main non-carbohydrate precursors for gluconeogenesis are amino acids from the diet, and from muscle protein breakdown. Other organs also contribute amino acids, but muscle is the major source. Most of these amino acids are converted to oxaloacetate (OA), which is metabolized to PEP by PEPCK. In the second regulated and essentially irreversible step in gluconeogenesis, fructose 1,6-bisphosphatase (Fru 1,6-P_2ase) catalyzes the formation of fructose 6-phosphate (Fru 6-P) from fructose 1,6-bisphosphate (Fru 1,6-P_2). Finally, in the third essentially irreversible reaction of gluconeogenesis, glucose is formed by the hydrolysis of G6P in a reaction catalyzed by glucose 6-phosphatase (G6Pase). Substrates are not boxed, enzyme names are in shaded boxes, summaries of experimental results are in double bordered boxes, and amino acids are indicated by "AA" in triple bordered boxes. When two values are given following "CR", they represent the fold change in the young and old mice, respectively. The value after "Age" is the main effect of age. A down arrow indicates the percent decrease, an up arrow indicates the fold increase. The value given for age is a combination of both dietary groups. NC is no change

13. AGING AND HEPATIC ENERGY METABOLISM

In general terms, our studies of the effects of aging on key hepatic and muscle enzymes of glucose homeostasis indicated that aging is accompanied by a decline in the enzymatic capacity for the turnover and utilization of peripheral protein for the production of glucose by the liver (Figs. 1 and 2). We found an age-related decrease in the expression of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6pase) mRNA in the liver of mice (Fig. 1)[120,121]. An age-related decrease in PEPCK mRNA also was reported in hepatocytes isolated from rats of various ages[122]. This enzyme catalyzes the committing step in gluconeogenesis, the conversion of oxaloacetate to phosphoenolpyruvate (Fig. 1). Once carbon is converted to phosphoenolpyruvate it will be converted to glucose in the liver. PEPCK controls the flow of carbon for hepatic glucose production. This carbon is derived from amino acid intermediates (principally glutamine) derived from the turnover of protein in the periphery for energy generation. There are no known allosteric modifiers of the activity of any PEPCK isoform[123]. PEPCK mRNA and activity are excellent indicators of the enzymatic capacity for gluconeogenesis in the liver. Thus, aging appears to reduce the gluconeogenic capacity of the liver (Fig. 1).

Liver gluconeogenesis derives its substrates mainly from protein turnover in the peripheral tissues, suggesting that aging is accompanied by a decrease in the turnover of peripheral protein. During the postabsorptive state, muscle and other tissues utilize amino acids derived from protein turnover to generate energy via the TCA cycle. This amino acid catabolism is initiated in the muscle by two enzymatic steps, collectively called the transdeamination reaction (Fig. 2). Transdeamination leads to the liberation of the amino nitrogen as ammonia. Because of its extreme toxicity, this ammonia is transferred to glutamate by glutamine synthetase, producing glutamine. Glutamine serves to transfer both carbon and nitrogen to the liver. Aging leads to a decrease in the activity of muscle glutamine synthetase. This is consistent with an age-related decrease in the turnover of peripheral protein for energy production. It is also consistent with decreased expression of hepatic carbamylphosphate synthase-1, glutamine synthetase, and tyrosine aminotransferase (TAT; Fig. 2).

Glutamine produced in the muscle is metabolized in the liver by glutaminase into glutamate and ammonia. The ammonia derived from this reaction can be returned to the glutamine pool by liver glutamine synthetase (Fig. 2). An age-related decrease in glutamine synthetase activity would channel glutamine into gluconeogenesis. The nitrogen from this glutamine would be channeled by carbamylphosphate synthase-1 into the urea cycle for detoxification and disposal. These effects are likely responsible for a part of the decrease in muscle protein synthesis and turnover known to occur with age[124].

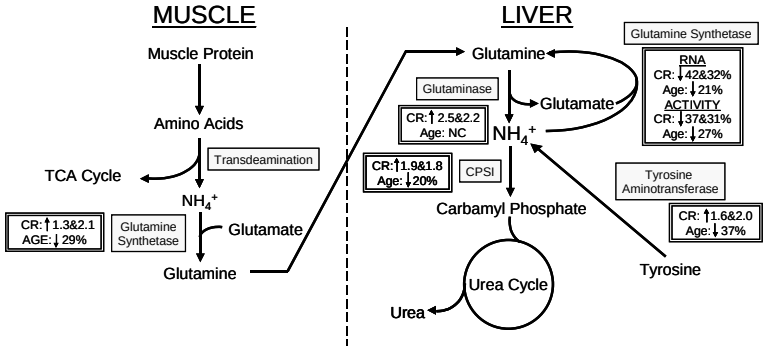

Figure 2. Summary of the effects of age and diet on muscle and liver nitrogen metabolism. In muscle and other extrahepatic tissues, the degradation of proteins to amino acids is utilized for generating metabolic energy. Transdeamination of amino acids produces tricarboxylic acid cycle intermediates and ammonia. Glutamine synthetase synthesizes glutamine from glutamate and ammonia. Glutamine is transported to the liver where glutaminase releases the ammonia, regenerating glutamate. CPSI converts this ammonia to carbamyl phosphate, which is converted to urea in the urea cycle. The amino group of excess tyrosine is released by TAT as ammonia, which is also detoxified beginning with the action of cpsI. In the figure, substrates are not boxed, enzyme names are in shaded boxes, and summaries of experimental results are in double bordered boxes. When two values are given following "CR", they represent the fold change in the young and old mice, respectively. The value after "Age" is the main effect of age. A down arrow indicates the percent decrease, an up arrow indicates the fold increase. The value given for age is a combination of both dietary groups. NC is no change.

14. CR AND HEPATIC ENERGY METABOLISM

In our microarray studies, CR modified the expression of a significant number of genes coding for key metabolic enzymes[6]. ST-CR increased expression of glutamate oxaloacetate transaminase 1 and decreased expression of pyruvate dehydrogenase E1α subunit. These changes are consistent with our conventional molecular-biological and biochemical studies showing that CR increases shuttling of nitrogen and carbon to the

liver from the peripheral tissues. It increases the enzymatic capacity of the liver for gluconeogenesis and the disposal of the byproducts of extrahepatic protein catabolism for energy production, while reducing the enzymatic capacity for glycolysis[121,125]. These CR effects are consistent with theories of aging, such as the oxidative stress theory, which postulate that the accumulation of damaged proteins contributes to the rate of aging[126].

CR increased fasting levels of the mRNA and activity of PEPCK and mRNA of G6pase[121]. The abundance of PEPCK mRNA was greater in the liver of young and old CR mice than it was in control mice of the same ages. PEPCK activity also was higher in the liver of CR mice. As discussed above, aging decreased the mRNA for PEPCK and G6Pase. In addition, when CR and control mice were fasted overnight and fed their normal daily ration of food, PEPCK mRNA and activity decreased within 1.5 hours of feeding in both control and CR mice. However, its mRNA abundance and activity increased rapidly thereafter, especially in CR mice. By 5 hours after feeding, PEPCK activity in CR mice was approximately twice that of controls. Similarly, G6Pase mRNA abundance was higher in CR mice for the 5 hours following feeding. G6Pase catalyzes the terminal step in hepatic glucose production, the hydrolysis of glucose 6-phosphate to glucose and inorganic phosphate (Fig. 1). This step leads to the release of glucose from the liver into the circulation when blood glucose levels would otherwise fall.

Together, these results suggest that the enzymatic capacity for gluconeogenesis returns rapidly after feeding. Thus, higher levels of peripheral tissue turnover persist in CR mice, even after feeding. These mice are at approximate weight equilibrium[127]. Therefore, in CR mice feeding is accompanied by intensified protein biosynthetic activity followed immediately by peripheral protein turnover. CR mice are approximately 4 times more insulin sensitive than control mice[120].

Consistent with the interpretation offered above, CR and age decreased the expression of glutamine synthetase activity and mRNA in the liver, while age decreased and CR increased its expression in muscle (Fig. 2). These differential effects should lead to a transfer of carbon and nitrogen in the form of glutamine from the periphery to the liver, where it would increase the hepatic pool of glutamine. The increase in glutaminase expression would increase hepatic catabolism of glutamine, producing glutamate and ammonia. mRNA levels closely reflect the levels of glutaminase activity[128,129]. Ammonia production by glutaminase is closely coupled to urea synthesis by CPSI. CPSI mRNA levels in young and old CR mice were twice that of control mice[121,125]. CR leads to coordinate induction of carbamylphosphate synthase-1 transcription, mRNA, protein, and activity[130]. The resulting glutamate accumulation would fuel CR enhanced gluconeogenesis.

These data support the interpretation that CR leads to enhanced carbon flux from amino acid degradation in the peripheral tissues to the liver. This amino acid degradation extends to tyrosine, an amino acid that requires a liver specific enzyme, TAT, for catabolism[121]. TAT degradation of tyrosine is well known to provide ketogenic and gluconeogenic substrates to the liver when glucose is limiting and amino acids are utilized as a major source of energy. Aging decreased TAT mRNA in the liver by an average of 37%. TAT mRNA in CR mice was approximately double the level in control mice. The age-related changes in nitrogen metabolizing enzymes are consistent with a decrease in catabolism of extrahepatic protein for energy. CR appears to enhance the capacity for mobilizing and transporting carbon and nitrogen products of muscle protein catabolism to the liver. CR mice also have enhanced hepatic capacity for the biosynthesis of glucose from this carbon, and for the detoxification of this nitrogen.

15. CONCLUSIONS

While the physiological and structural studies of the liver suggest that it ages well, the molecular biology and biochemistry of the liver indicate that it undergoes changes with age that have serious systemic effects. Genome-wide microarray and conventional molecular and biochemical studies indicate that there is an age-related shift in liver toward a state associated with oncogenesis, fibrosis, cirrhosis, and unhealthful apolipoprotein and fatty acid biosynthesis. Evidence was found for age-related increases in inflammation, cellular stress, and fibrosis; and for reduced capacity for apoptosis, negative cell-growth control, and phase I and II xenobiotic metabolism. LT- and ST-CR reversed the majority of these changes. LT-CR also produced CR-specific changes in signal transduction-associated gene expression known to lead to enhanced longevity. Evidence for a CR-related increase in the turnover and renewal of peripheral protein also was found. In addition, healthful changes in apolipoprotein and fatty acid biosynthesis-related gene expression were found. LT- and ST-CR produced changes in gene expression associated with enhanced anti-proliferative growth control, increased apoptosis and reduced chemical carcinogenesis. Together these studies make it clear that aging and its mitigation by CR are multifaceted processes which affect many aspects of liver function at the molecular level. It also appears that unbiased, exploratory approaches such

as the genome-wide microarray studies described here are providing new and valuable insights into these processes.

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17. ABBREVIATIONS

CR, caloric restriction; G6pase, glucose-6-phosphatase; GH, growth hormone; GST-II, glutathione S-transferase-II; IGF-1; insulin-like growth factor-1; LT-CR, long-term CR; PEPCK, phosphoenolpyruvate carboxykinase; ST-CR, short-term CR.

18. AFFILIATIONS

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