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EFFECT OF AGING AND CALORIC RESTRICTION ON INTESTINAL SUGAR AND AMINO ACID TRANSPORT

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1. ABSTRACT

The incidence of intestinal nutrient malabsorption increases with age. Therefore, an important question is whether there are age-related changes in intestinal nutrient absorption which may contribute to a decline in absorptive capacity. Sugar and amino acid transport per mg intestine generally decreases with age. The proximate mechanism underlying this age-related decrease in transport activity is a decrease in number of transporters per mg. This reduction in transporter number can be caused by age-related changes in cell proliferation rates which, in turn, can alter the ratio of absorptive to nonabsorptive cells. The age-related change in proliferation rates typically increases intestinal mass. There seems to be no age-related changes in the steady state levels of transporter mRNA. Aging also modestly impairs the ability of intestinal nutrient transport systems to adapt to changes in dietary conditions. Caloric restriction is the only procedure known to consistently increase the lifespan of mammals. Chronic caloric restriction markedly enhances intestinal nutrient transport per mg without affecting intestinal mass. Since body weight decreases with caloric restriction, there is a dramatic increase in intestinal absorptive capacity normalized to body weight. This suggests that an increase in intestinal nutrient absorption may be a critical adaptation to caloric restriction. There is a need to perform *in vivo* transport studies during senescence, to distinguish between acute and chronic effects of caloric restriction, and to identify hormones that may mediate aging and caloric restriction effects on intestinal nutrient transport.

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2. INTRODUCTION

Death by starvation has reached epidemic proportions among older Americans (1). Nutrition surveys found malnutrition in 6% of men and 5% of women between the ages of 70 and 80 years, and in 12% of men and 8% of women over the age of 80 (see review by Rolls (2)). Bond and Levitt (3) showed that 33% of active, clinically-well individuals over the age of 65 malabsorbed a portion of a 100 g carbohydrate meal. This indicates that the mean carbohydrate absorptive capacity fell progressively from age 65. Xylose absorption also decreases with advancing age (4, 5, 6). The elderly also malabsorb peptides and amino acids. Serum albumin concentrations are significantly lower in the elderly than in young humans even when their dietary protein intake is similar (7), suggesting that peptides and amino acids may be malabsorbed in the elderly.

There are many possible causes of malnutrition, ranging from behavioral factors like decreased appetite to pathological conditions like malabsorption. One of the most neglected areas of study in elderly nutrition is the effect of aging on intestinal absorption and on the ability of the small intestine to regulate absorption of nutrients. This information is important since decreases in rates of transport may contribute to malabsorption and to malnutrition. In addition, the rate of intestinal absorption of sugars and their site of absorption largely determine postprandial plasma glucose concentrations.

The effect of aging on glucose tolerance is quite evident after consumption of a meal (8). For each decade after middle age, the level of fasting plasma glucose increases by 0.05 to 0.1 mM, but the postprandial plasma glucose level increases much more, by 0.4 to 1.1 mM. Thus, older people often are considered diabetic based on postprandial plasma glucose level, and not on fasting glucose level. If aging

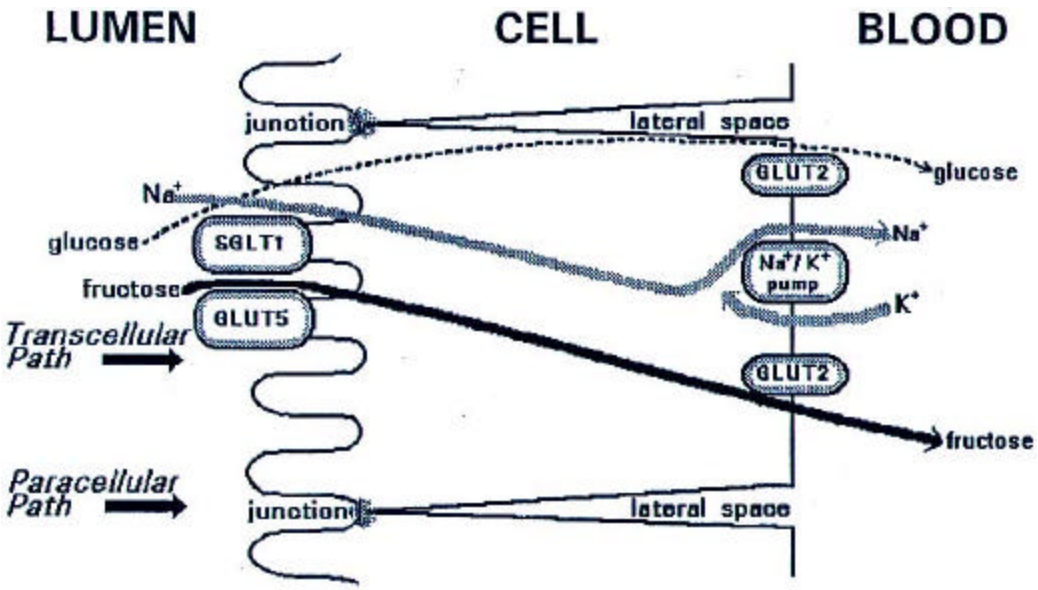

Figure 1. Schematic diagram of an intestinal cell showing location of the Na^+-dependent glucose (SGLT1) and Na^+-independent fructose (GLUT5) transporters in the brushborder, and Na^+-independent glucose and fructose (GLUT2) transporter in the basolateral membrane. Nutrient transport via the transcellular path entails crossing the brushborder and basolateral membranes in series, that via the paracellular path need only cross the tight junction. Paracellular transport accounts for only a small fraction of total intestinal nutrient transport.

results in adaptive mechanisms which alter the rate of intestinal sugar absorption and move the main absorptive site, then, the subsequent rise of plasma glucose will be affected. For example, adaptive mechanisms in chronic diabetes result in a dramatic increase in total intestinal absorptive capacity for glucose (9). Similar adaptive mechanisms during aging could result in an age-related change in postprandial plasma glucose tolerance.

In this brief review, I will mainly focus on the effect of aging on intestinal sugar and amino acid absorption, and primarily on studies done in the last 15 years since Holt (10), Thomson and Keelan (11) and Vinardell (12) wrote reviews on aging and intestinal absorption. This review excludes studies on development (neonatal to adult). Holt and Balint (13) reviewed the effect of aging on intestinal lipid absorption. I will describe the nonspecific or specific mechanisms underlying the effects of aging on transport, and the reader is referred to a recent review by Ferraris and Diamond (14) which gives a detailed explanation of these mechanisms. Finally, I will also review studies on the effect of chronic caloric restriction on absorption of nutrients, since caloric restriction is a well accepted procedure for extending lifespan and postponing aging. I will end with suggestions for future research.

2.1 Basis for normalization.

Because investigators use a variety of methods to determine rates of intestinal nutrient absorption, it is important for the reader to keep track of how absorption rates are normalized. Briefly, the intestinal transport rate of a nutrient may be normalized to the weight (or protein content) of intestinal tissue or cell or membrane, to length or surface area of intestinal tissue, or to the entire small intestine. The last expression is often referred to as intestinal absorptive capacity or *SUM***J**. When normalized in this manner, a change in the transport rate may mean a change in transport activity by the intestinal absorptive cell as well as a change in intestinal mass. Similarly, a change in transport per cm of intestine would mean a change in transport activity in that segment of intestine, as well as a change in the intestinal mass per cm. Because of macroscopic (folds) and microscopic (villi and microvilli) elaborations, the actual surface area of the absorptive mucosa is difficult to measure (see Ref. 15 for a detailed discussion). In almost all cases, absorption normalized to surface area refers only to serosal (the nonabsorptive side), and not mucosal, surface area. A change in transport expressed per unit weight reflects only a change in functional activity independent of a change in mass. Hence, if aging were to have no effect on transport activity per unit weight, but were to induce a two-fold increase in intestinal weight, studies normalizing to weight would report no age-related change in transport, but studies normalizing to surface area or intestinal length would report an increase.

2.2 Intestinal sugar and amino acid transporters.

Monosaccharides and amino acids are transported from the intestinal lumen across the epithelial cells and into the blood, mainly through the transcellular pathway (figure 1). These nutrients are initially absorbed by carriers in the brushborder membrane facing the lumen and are subsequently transported from intracellular compartments across the basolateral membrane by a different set of carriers. Almost all nutrient transport studies in aging determined rates of absorption across the brushborder membrane into the cell, or across the epithelial layer into the blood (or serosal space). Both transport rates can be determined experimentally by radioisotope tracer techniques.

Absorption of glucose and galactose across the brushborder membrane of intestinal cells is Na^+-dependent and

is mediated by SGLT1 (16) (figure 1). The absorption of fructose by GLUT5 is Na^+-independent (17). All three sugars cross the basolateral membrane via another member of the facilitative glucose transporter family, GLUT2 (18). There are many more transport systems for absorption of amino acids, seven (five are Na^+-dependent) for the brushborder membrane and five (two are Na^+-dependent) for the basolateral membrane (19).

3. EFFECT OF AGING ON SUGAR TRANSPORT

There are conflicting reports on the effect of aging on intestinal sugar transport. Intestinal glucose brushborder transport per mg measured *in vitro* changed little with age ($\leq$ 24 months old) until measured in senescent (30 months old) mice whose glucose uptake was greater than those in younger mice (20). Wallis *et al* (21) also found no age-related change in glucose transport rate per mg protein across the brush border membrane of human enterocytes. Both studies measured initial uptake rate under incubation conditions with minimal unstirred layers, suggesting that the transport function of intestinal cells remain largely unaffected by age. Transepithelial glucose and fructose transport was found to increase in old (24 months old) compared to those in young (4 months old) rats (22). Since these transepithelial measurements expressed uptake in terms of mucosal surface area, transport may have increased because intestinal mucosa from old mice may have more absorptive cells, or because each cell transported more sugar.

In contrast to these findings, most studies find intestinal sugar transport to decrease with age. Esposito *et al* (23, 24) found net transintestinal glucose transport per mg *in vivo* and *in vitro* to decrease significantly with age and demonstrated the absence of an overshoot when measuring brushborder membrane uptake of glucose in aged rats. Age-related reductions in glucose transport and this absence of an overshoot, which is indicative of Na^+-independence, were each confirmed by Vincenzini *et al* (25) in brushborder membrane vesicles from aged humans, and by Lindi *et al* (26) and Treves *et al* (27) in aged rats. The ability to concentrate glucose in the intestinal cell or in the serosal medium also diminished with age (23). Hirata *et al* (28) used two independent methods (glucose transport in brushborder membrane vesicles, and glucose-induced changes in transepithelial electrical potentials) to demonstrate age-related reductions in glucose transport by rat small intestine. Active, but not passive, absorption of 3-O-methylglucose per mg also decreased with age by about two fold *in vivo* (29) as well as *in vitro* using everted sacs and brushborder membrane vesicles in rats (30). Chen *et al* (31) found age-dependent decreases in 3-O-methylglucose transepithelial transport and Ferraris *et al* (32) found age-dependent decreases in transmembrane transport per mg in mice. These studies concluded that a decrease in number of glucose transporters is the probable mechanism underlying the decrease in active glucose transport.

4. EFFECT OF AGING ON AMINO ACID TRANSPORT

There are considerably fewer studies on the effect of aging on intestinal amino acid transport. There were modest decreases in rates of tyrosine, phenylalanine, tryptophan and histidine uptakes per mg in everted rings (33) or brushborder membrane vesicles (27, 34) of aging rat jejunum. Chen *et al* (31) found similar decreases in transepithelial tyrosine transport per g, but only in 36-, and not in 24- or 30-month old mice. Ferraris *et al* (32) found no statistically significant age-related changes in transapical membrane transport of five amino acids between 7- and 24-month old mice, although they found transport of each amino acid to exhibit a decreasing trend with age.

Probably there are several reasons why studies on age-dependence of transport activity differ in their conclusions. *First* and probably most important, is the method of normalization of uptake rates. An excellent case in point is the study of Goodlad and Wright (35) who found absorption per intestine or per unit length of intestine to increase with age in rats. The main mechanism underlying this increase is a marked increase in intestinal length and weight per cm, even though absorption per mg decreased. Review of literature showed that many studies reporting decreased intestinal transport with age use methods that normalize uptake to intestinal weight or to amount of protein (32). On the other hand, some studies which reported increased intestinal transport with age instead normalized uptake to length. *Second*, the choice of age is critical. Age-related decreases in uptake remain rather modest until well past the median lifespan. In many rodents that have a median lifespan of about 24 mo, significant changes in uptake become easily discernible only at 27 months or later (29, 31, 32). *Third*, initial transmembrane uptake rates should be measured at substrate concentrations yielding V_{max} and should be corrected for diffusive uptake. This is due to the fact that uptake measured at low concentrations is subject to significant unstirred layer effects, while uptake at overly high concentrations may contain a large diffusive component (see Karasov and Diamond (36) for a detailed discussion).

5. PROXIMATE MECHANISMS OF AGE-RELATED DECREASES IN TRANSPORT RATES

There are a number of mechanisms through which age can alter nutrient transport rates.

5.1 Nonspecific mechanisms.

There are a large number of studies on age-related changes in intestinal surface area, cell proliferation rate and membrane permeability, each of which can nonspecifically alter intestinal transport. A detailed discussion of these important parameters is beyond the scope of this review.

Almost all studies find intestinal mass to increase with age all the way through senescence. Early studies as exemplified by Moog (37) suggest an increase in mouse intestinal weight with age. While some of the increase is due to an increase in amounts of connective tissue, there is also an increase in the number of villi, thereby increasing mucosal mass and absorptive surface area in rats (38). In rats, most of the increase in villus epithelium is in the distal gut regions (22, 39, 40), and the increase is sufficient enough to compensate for age-dependent loss in jejunal function (41). Ferraris *et al* (32) showed that the age-dependent increase in tissue weight per cm in the ileum is tightly correlated with age-dependent increases

in villus heights in this region. This suggests that changes in intestinal weights due to age can be accounted for by changes in amount of intestinal mucosa. In humans, age has no significant effect on intestinal surface area (42). An increase in surface area would enhance the transport per cm or per intestine for all nutrients, and is an easy parameter to measure.

Because cells in the small intestine turnover every 3 - 5 days, a change in intestinal mass is the likely outcome of a change in the rate of intestinal cell proliferation. There is a vast literature on intestinal cell proliferation rate in aging, perhaps because of its cancer implications. This subject is beyond the scope of this review but a review by Atillasoy and Holt (43) suggest that there is a state of hyperproliferation of epithelial cells of the small intestine, especially in aging rodents. In rats, Holt and Yeh (44) found a significant increase in intestinal cell production rate, especially in distal regions. Goodlad and Wright (35) found modest or insignificant increases in the proliferation of intestinal cells while Ecknauer *et al* (38) found cell production rate to be independent of age. In mice, Ferraris and Vinnakota (45) found rates of enterocyte turnover and migration rate along the crypt/villus axis to be independent of age, except in the distal region where there was a modest and statistically borderline increase in migration rate. Levels of polyamines, factors thought to be required for growth of the gastrointestinal tract, are much higher in aged than in young rats (46).

Age-related changes in proliferation rates may not only affect mass and surface area, but also the ratio of absorptive to nonabsorptive cells (14). If this ratio increases, there is a corresponding increase in nutrient absorption rate per mg tissue or cell protein. If this ratio decreases, the absorption rate per mg also decreases.

A change in ratio of absorptive to nonabsorptive cells is difficult to demonstrate experimentally. We have attempted to detect changes in this ratio by *in situ* hybridization of SGLT1 and GLUT5 mRNA, but failed to obtain any significant difference. There is probably only a single study able to demonstrate a change in ratio of differentiated (probably absorptive) to undifferentiated cells in aged rats (47). Holt *et al* assayed for enzyme activity in cryostat sections collected perpendicular to the crypt villus axis of the small intestine. They concluded that reduced enzyme specific activities in mucosal homogenates from aging animals is due to an increase in the proportion of relatively undifferentiated villus cells. This approach cannot be used in transport studies which requires a much higher number of cells for transport assays. Ligand binding to nutrient transporters, a method used by Ferraris and Diamond (48) to demonstrate diet-induced changes in site density of SGLT1 transporters along the crypt/villus axis, cannot detect small changes in ratios.

A potentially important factor affecting transport is an age-related change in passive permeability of intestinal cells which would nonspecifically change transport rate. The permeability of the rat small intestine to high molecular weight probes is independent of age. However, the permeability to low molecular weight probes increases modestly with age (49). In humans (50, 51) and mice (32), however, there is no age-dependent change in passive permeability.

There are other nonspecific mechanisms which have either a direct (e.g. change in membrane potential) or indirect (e.g. intestinal motility) effect on transport, but these factors have either not been studied or were found to be independent of age. The absence of an overshoot in studies utilizing brushborder membrane vesicles from aged animals suggest age-related changes in the electrochemical gradient for sodium (25, 26, 27). In human small intestine, motility is independent of age (52).

5.2 Specific mechanisms.

An often mentioned mechanism underlying age-related decreases in intestinal glucose transport is a decrease in the number of Na^+-dependent glucose carriers (30, 31). Ferraris *et al* (32) provided direct evidence in support of this hypothesis by finding that the number of specific, glucose-protectable phlorizin sites (presumably the number of glucose carriers since phlorizin competitively binds to the glucose carrier) decreases with age. The decrease in site number is tightly correlated with the decrease in intestinal glucose transport. In mice, the age-related decrease in transporter number is modest until senescence ($\geq$ 27 months old), which reflects the difficulty in getting good evidence for age-related decreases in transport. Paradoxically, Western blots show no significant difference in amounts of SGLT1 in brushborder membranes of aged and young mice (32), presumably because changes in amounts of protein are too small to detect by scanning densitometry. It is also possible that similar amounts of immunoreactive SGLT1 are actually found in both ages, but that SGLT1 from aged mice are less efficient in binding phlorizin and in transporting glucose. Steady state levels of SGLT1, GLUT5 and GLUT2 mRNAs as shown by Northern blot analysis or by RT-PCR (Casirola *et al* in press), are similar. This suggests that age-related decreases in transporter activity are not correlated with changes in steady state levels of mRNA coding for those transporters.

6. REGULATION OF SUGAR AND AMINO ACID TRANSPORT BY DIET

Aging reduces the ability of physiological systems to adapt to changes in the environment (53). The small intestine of young adults can easily adapt to acute and chronic changes in diet by altering the number of brushborder (54) and basolateral (55) sugar transporters. The adaptation occurs 1 - 2 days after the change in diet.

The adaptive capability of the small intestine in the aged has been challenged in several studies, though only a couple of these are on sugar and amino acid transporters. Young adults adapt to dietary calcium concentrations by increasing intestinal transport when dietary levels are low, and by decreasing transport when its levels are high. The larger the change in dietary levels, the greater the change in transport rate. Armbrecht *et al* (56) demonstrated that the amplitude of this intestinal adaptation progressively decreased with age, so that in rats 12 months of age, rates of calcium uptake and levels of calcium binding protein no longer changed with dietary calcium

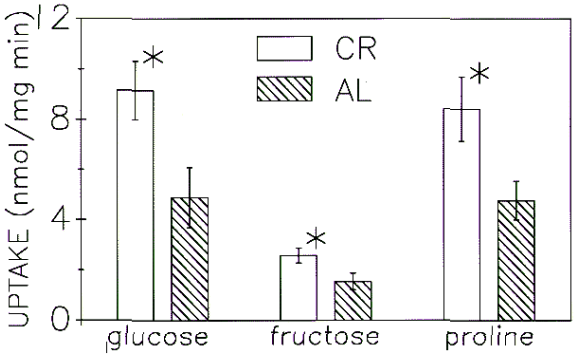

Figure 2. Transmembrane transport of glucose, fructose and proline was each greater (*) in the small intestine of calorie-restricted (CR) than in that of *ad libitum* (AL) fed, same age (24 months old) mice.

levels. Similarly, rates of intestinal sugar uptake and site density of sugar transporters are directly proportional to dietary carbohydrate concentrations (54, 56, 57). Rates of intestinal transport of nonessential amino acids are also directly proportional to levels of dietary protein (56, 57). The transport rates of essential or potentially toxic amino acids, however, may be inversely proportional to dietary protein levels (see Ferraris and Diamond (58) for a detailed discussion). In young adults, the time course of this adaptive change is rapid and occurs within 1 - 2 days (59). For Na^+-dependent glucose transport, the time course of diet-induced changes in uptake is mainly due to cell migration times, because only crypt cells perceive the dietary signal for glucose transporter regulation (48). In order for intestinal glucose transport activity to change, these crypt cells have to first migrate to replace mature enterocytes lining the villus. This is due to the fact that mature cells cannot be reprogrammed to change their SGLT-1 mediated transport activity (48). In contrast, intestinal fructose transport adapts much more rapidly to changes in dietary fructose level, probably because mature cells can also change their GLUT5-mediated transport activity.

The time course of adaptive change in Na^+-dependent glucose transport basically remains unchanged in aged mice (45), because cell migration rates in most intestinal regions are also independent of age. The time course of adaptive changes in fructose, proline, aspartate and alanine uptakes was also independent of age, suggesting that aged mice adapt just as readily as young mice to changes in dietary levels of nutrients.

In mice, age seems to affect the amplitude of the change in sugar and nonessential amino acid transport in response to a change in the dietary carbohydrate and protein levels, respectively (60). Young adult mice enhance their sugar transport by about two-fold in the proximal intestine. In contrast, aged mice enhance their transport by only 1.5 fold despite the fact that their baseline transport rate was already lower than that of young mice. In contrast to the distal region of small intestine of young mice, that of aged mice also failed to respond to a change in dietary nutrient levels. Although the age-related differences in the amplitude change were modest, they were consistent from one transporter to another, and are similar to age-impaired adaptations in levels of hepatic glycolytic enzymes, of intestinal calcium transporters, and of pancreatic enzymes to changes in levels of dietary nutrients (56, 61, 62).

7. EFFECT OF CALORIC RESTRICTION ON INTESTINAL NUTRIENT TRANSPORT

Caloric restriction is the balanced reduction of the protein, carbohydrate and fat content of the diet without reduction of its micronutrient (vitamins and minerals) content. In practice, an animal on a calorie-restricted diet consumes an amount of food at 60 - 70% that of an animal fed to satiety (*ad libitum*). Over 60 years of research in rodents and other small animals have shown that caloric restriction can dramatically extend lifespan, maintain vitality, and reduce the incidence of age-associated disease (63). How do absorptive systems in the small intestine adapt to lifelong caloric restriction and therefore a chronic reduction in amounts of luminal nutrients that need to be absorbed? A striking development in age-related changes in intestinal nutrient transport has been the recent finding that intestinal nutrient transport (normalized to weight, length or entire intestine) differs markedly between chronically calorie-restricted and *ad libitum* fed mice (64).

This effect of caloric restriction on intestinal nutrient transport is best elucidated by estimating the total intestinal absorptive capacity (*SUM***J**). This is done by integrating the *in vitro* uptake per cm along the length of the small intestine, then expressing *SUM***J** as a function of metabolic body weight ($BW^{0.75}$) to correct for calorie restriction -induced differences in metabolic mass (since metabolic rate increases with body weight as $BW^{0.75}$ among mammals). Casirola *et al* found ***SUMJ*** for sugars and amino acids to be about 50% higher in 24 months old calorie-restricted mice compared to same age controls (*ad libitum* fed). The ratio *SUM***J** /$BW^{0.75}$ was 80% greater in calorie-restricted mice, suggesting that their intestine has the potential to absorb nutrients at almost two-fold the rate in mice fed *ad libitum*. The proximate mechanism underlying this dramatic increase in *SUM***J** is not intestinal mass which is similar between calorie restricted and those fed *ad libitum*, but an equally dramatic increase in transport per mg of intestine (figure 2). Switching 24 months old mice fed *ad libitum* to CR for one month does not significantly change BW, *SUM***J**, and intestinal mass. Caloric restriction had no effect on intestinal permeability (49, 64).

Casirola *et al* (65) switched 32 months old calorie-restricted mice to one month of *ad libitum* feeding, and found BW to increase by 35%, and *SUM***J** to decrease by 30% compared to those in same age controls that remained calorie-restricted. Interestingly, they found no changes in mRNA levels of sugar transporters associated with changes in *SUM***J**. ***SUMJ*/BW$^{0.75}$** was over 50% greater in mice that remained calorie-restricted compared to same age mice switched to ad libitum feeding. Intestinal weights again remained similar, but uptake per mg of intestine decreased by 42% with the switch to *ad libitum* feeding. Calorie restricted mice switched to *ad libitum* feeding for only three days (the lifetime of most intestinal cells) had significant increases in body weight but no changes in *SUM***J**.

Cao and Ferraris (unpublished observations) tracked changes in *SUM***J** of fructose in young adult mice sacrificed 1, 2, 10, 24, and 270 days after CR. They found modest increases in *SUM*/**J** 24 days after caloric restriction. However, 270 d after caloric restriction, *SUM***J** and *SUM***J** */BW*$^{0.75}$ were 60 and 120%, respectively, greater in calorie-restricted mice as compared to same age controls fed *ad libitum*. As in the previous study, they found no changes in mRNA levels of fructose transporters associated with changes in *SUM***J**.

Caloric restriction seems to be associated with marked increases in intestinal absorptive capacity. This adaptive increase is developed over a long period of time and can be reversed by *ad libitum* feeding of a much shorter duration. However, changes in levels of transporter mRNA and in intestinal mass are not detected. Since the change *in SUM***J/BW**$^{0.75}$ is so dramatic, it indicates that calorie-restricted mice can absorb a much greater amount of nutrients per unit metabolic mass than *ad libitum* mice, and probably implies that an increase in intestinal nutrient absorption rates is a critical adaptation to caloric restriction.

8. PERSPECTIVE

It is not really known whether age-related changes in transport observed *in vitro* are physiological, hence, *in vivo* transport studies are needed. To increase the chance of detecting significant changes, these studies should use animals well past the median lifespan and should also measure age-related changes in intestinal mass. Studies are also needed to identify the mechanisms underlying age-related changes in nutrient absorption. Although Ferraris *et al* (32) have determined an age-related decrease in the number of specific phlorizin binding sites (glucose transporters) per mg, it is not known whether this decrease is due to fewer functional carriers per cell, or to a decrease in the ratio of absorptive to nonabsorptive cells. The latter is the more probable mechanism, since transport rates of amino acids and fructose tend to decrease with age as well.

Nutrition is the most powerful extrinsic factor which influence the aging process. This role has been clarified by such underfeeding paradigms as caloric restriction (66, 67). The effect of caloric restriction on intestinal sugar and amino acid transport has just been studied. Additional studies are required not only to determine the effect of caloric restriction on the transport of other amino acids, but also on transport of micronutrients as well. There is a need to distinguish acute and chronic effects of caloric restriction on intestinal transport of nutrients, as well as to identify hormones that may mediate the effects of caloric restriction on intestinal nutrient transport.

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