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Editorial

Aberrant Insulin Receptor Signaling and Amino Acid Homeostasis as a Major Cause of Oxidative Stress in Aging

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ABSTRACT

The mechanisms leading to the increase in free radical-derived oxidative stress in "normal aging" remains obscure. Here we present our perspective on studies from different fields that reveal a previously unnoticed vicious cycle of oxidative stress. The plasma cysteine concentrations during starvation in the night and early morning hours (the postabsorptive state) decreases with age. This decrease is associated with a decrease in tissue concentrations of the cysteine derivative and quantitatively important antioxidant glutathione. The decrease in cysteine reflects changes in the autophagic protein catabolism that normally ensures free amino acid homeostasis during starvation. Autophagy is negatively regulated by the insulin receptor signaling cascade that is enhanced by oxidative stress in the absence of insulin. This synopsis of seemingly unrelated processes reveals a novel mechanism of progressive oxidative stress in which decreasing antioxidant concentrations and increasing basal (postabsorptive) insulin receptor signaling activity compromise not only the autophagic protein catabolism but also the activity of FOXO transcription factors (*i.e.*, two functions that were found to have an impact on lifespan in several animal models of aging). In addition, the aging-related decrease in glutathione levels is likely to facilitate certain "secondary" disease-related mechanisms of oxidative stress. Studies on cysteine supplementation show therapeutic promise. *Antioxid. Redox Signal.* 10, 661–678.

INTRODUCTION

A LARGE BODY OF EVIDENCE suggests that oxidative stress is one of the key factors that limit our lifespan and compromise the quality of life in old age. A series of longevity strains of worms and fruit flies collectively suggests that an increase in oxidative stress resistance is often associated with an increase in lifespan (79, 116, 122, 138, 147, 184). An increase in superoxide dismutase (Mn-SOD) has been implicated in lifespan extension in the *daf-2* mutant of *Caenorhabditis elegans* (79), and catalase was shown to be required for lifespan extension in *daf-C* and *clk-1* mutants for C. *elegans* (184). In *Drosophila*, an increase in lifespan was found in strains with extra copies of genes of SOD and catalase (138, 147), and the long living *mth* mutant of *Drosophila* was shown to have an increased resistance to a free radical generator (116). Similarly, the increased longevity of the $p66^{shc}$ mouse mutant was associated with an increased resistance to oxidative stress (122). Last but not least, the maximum longevity of various mammalian species was found to be negatively correlated with steady state levels of DNA damage (10). Taken together, these findings strongly support the free radical theory of aging that was proposed >50 years ago (68).

It has been noted, however, that oxygen radicals and related reactive oxygen species (ROS) are not generally hazardous but play a positive role in various physiological signaling processes, provided they are produced in strictly controlled quantities (reviewed in refs. 15 and 42). Superoxide dismutase (119) and several other enzymes and antioxidative compounds provide an effective means to counterbalance the potentially damaging effects of ROS. It is the disturbance of this delicate balance between ROS and antioxidants that leads to stress conditions called oxidative stress (171).

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Several lines of evidence indicate that such a shift in balance towards oxidative stress occurs also in the course of normal aging. Work by many laboratories has shown that lipid peroxidation and the oxidative damage of protein and DNA increase with age (8, 26, 27, 30, 51, 62, 65, 83, 93, 110, 117, 129, 134, 149, 154, 173, 176, 188, 193, 207). This age-related increase in oxidative damage is typically associated with a corresponding decrease in the concentrations of antioxidants, including serum and tissue levels of vitamin E and plasma concentrations of vitamin C (8, 35, 104, 167) and intracellular glutathione concentrations in various tissues from rats, mice, and guinea pigs (4, 55, 140, 141, 143, 160). Glutathione is quantitatively the most important scavenger of free radicals and, together with glutathione peroxidase, an important substrate for the removal of hydrogen peroxide. It is not known, however, how much ROS concentrations increase as a consequence of the age-related decrease in intracellular glutathione concentrations, and to what extent the age-related decrease in glutathione concentrations actually accounts for the progressive increase in various manifestations of oxidative damage and for the decrease in other antioxidative compounds. Nevertheless, the age-related decrease in glutathione levels and the corresponding increase in ROS concentrations render elderly subjects increasingly vulnerable to oxidation and facilitate the development of "secondary oxidative stress" by mitochondrial DNA mutation (162) or various "disease-related" mechanisms of ROS production.

This editorial review summarizes evidence from both human and experimental animal studies showing that the insulin receptor signaling pathway plays a decisive role in the regulation of amino acid homeostasis under starving conditions (*i.e.*, in the postabsorptive state) and is likely to account at least partly for the aging-related decrease in the postabsorptive plasma cysteine concentration. As this decrease is associated with an aging-related decrease in intracellular glutathione concentrations, the redox sensitive insulin receptor signaling cascade appears to be a key element in a vicious cycle of oxidative stress, as schematically shown in Fig. 1. To illustrate this point, we are presenting here a mosaic of short overviews on the various parts of this cycle. There are still a few gaps in this mosaic and many details require more experimentation. It was felt, however, that this editorial review may be helpful and timely.

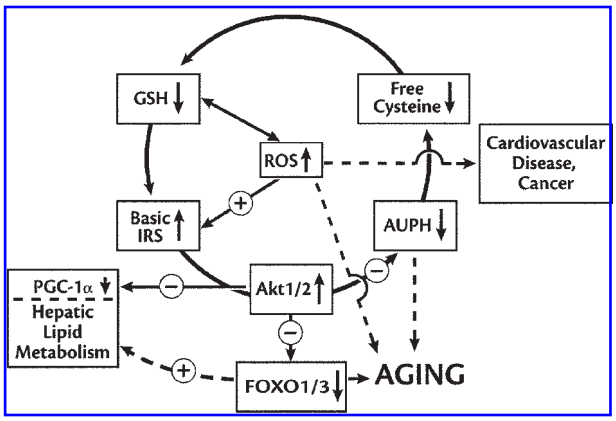

FIG. 1. A vicious cycle causing oxidative stress in aging. The insulin-independent oxidative enhancement of insulin receptor signaling activity (basic IRS) leads to an impairment of autophagic activity (AUPH) and amino acid homeostasis associated with a decrease in the postabsorptive plasma cysteine concentration. This causes a decrease in intracellular glutathione (GSH) and a corresponding increase in ROS concentrations that lead ultimately to a further upregulation of the insulin-independent *basic* insulin receptor signaling cascade. It may be noted that all the changes described by the arrows in this scheme happen primarily during the night and early morning hours [i.e., in the postabsorptive (starved) condition].

CHANGES IN GLUTATHIONE CONCENTRATION DURING STARVATION AND IN THE COURSE OF AGING

The tripeptide glutathione typically shows a high turnover along well-defined metabolic pathways (120). In line with the circadian diet-dependent variation in plasma cysteine concentrations (19), the glutathione concentrations in liver and plasma show a strong circadian variation and an even stronger decrease by ≥50% upon extensive starvation (19, 85). This finding suggests that the organism may be most vulnerable against ROS in the postabsorptive state and that the greatest impact of oxidative stress on aging-related parameters in humans is likely to take place at night and in the early morning hours. Other organs showed little or no circadian variation but a marked decrease after 48 h of starvation (85). A circadian variation in intracellular glutathione concentrations has also been reported for human bone marrow, but the differences were in this case relatively small (174).

An age-related decrease in the intracellular glutathione concentration has been reported for the liver and kidney from rats (4), brain tissues from rats and mice (143, 160), retinal glia cells from guinea pigs (141), and spleen cells from mice (55). In many instances, a decrease in intracellular glutathione concentration has been associated with an oxidative shift in glutathione redox status (reviewed in refs. 41 and 45). Age-related changes in the intracellular glutathione redox status have been reported for rat skeletal muscle tissue (133), liver, kidney, and brain from rats and mice (4, 57, 143) mouse brain (28), rat brain (45, 82), and mouse macrophages (127). In humans, an age-related oxidative shift in glutathione redox status has been shown in skeletal muscle tissue (145) and in peripheral blood mononuclear cells (72, 109).

The glutathione concentration in plasma is extremely small in comparison to both the intracellular glutathione concentration and the plasma cysteine concentration. Based on studies on rats, it has been suggested that variations in plasma glutathione concentration reflect variations in hepatic glutathione concentration (108). In line with the age-related decrease in intracellular glutathione levels, the plasma glutathione level also decreases with age (87, 159, 199). In view of the role of the plasma cysteine concentration as a critical determinant of the intracellular (notably intrahepatic) glutathione level (see below) and because of the export of glutathione into the plasma, it is reassuring to see that the plasma glutathione concentration was found to be significantly correlated with the plasma cysteine concentration in a study of young healthy human subjects (86). Studies in humans indicated that the plasma glutathione concentration is lower in the morning than in the afternoon (19, 49), but follows the diurnal variation of plasma cysteine with

a delay of several hours (19). Like plasma glutathione, the plasma concentration of glutathione disulfide is also significantly correlated with the tissue glutathione concentration, but its redox status is markedly more oxidized than in the tissue and independent of the cysteine/cystine redox status in the plasma (88).

Although the intracellular glutathione concentration in the liver is influenced by various factors, including γ-glutamyl cysteine ligase activity or environmental toxins and other xenobiotic substances (172), the hepatic glutathione concentration is determined to a large extent by the availability of its precursor amino acid cysteine. In mice, intravenous injection of cysteine derivatives such as *N*-acetylcysteine or glutathione was found to cause within 2 h a significant increase in the intracellular glutathione concentration in the liver but not in kidneys, spleen, or lung (85). It was therefore of interest to determine whether the postabsorptive plasma concentration of nonprotein thiol (acid-soluble thiol) or its major constituent cysteine may significantly decrease with age.

AGE-RELATED DECREASE IN POSTABSORPTIVE CYSTEINE AND ASPARAGINE CONCENTRATIONS INDICATIVE OF CHANGES IN AMINO ACID HOMEOSTASIS

A study of 219 healthy human subjects between the third and the ninth decade of life revealed a significant decrease in the postabsorptive plasma concentrations of nonprotein thiol and of asparagine (64, 75; Fig. 2). Nonprotein thiol can be considered in first approximation as a measure of plasma cysteine because the plasma concentrations of glutathione or other low molecular weight thiol compounds are very low in comparison to the plasma cysteine concentration. The age-related decrease of the mean plasma cysteine concentration has been confirmed by high pressure liquid chromatography (HPLC) (87).

The age-related decrease in plasma cysteine is associated with an equally significant increase in its oxidized derivative cystine (64, 87; Fig. 2). It was therefore tempting to speculate that the decrease in cysteine may result from enhanced oxidation into cystine, possibly due to an age-related increase in ROS. As persuasive as this argument may be, it is not supported by evidence. Middle-aged obese and hyperlipidemic patients show a significant decrease in plasma levels of nonprotein thiol (mainly cysteine) together with a decrease in asparagine, but no increase in the plasma cystine concentration (Fig. 2).

REGULATION OF POSTABSORPTIVE CYSTEINE AND ASPARAGINE HOMEOSTASIS BY AUTOPHAGY

As cysteine and asparagine are protein-forming amino acids, the body has at all times a relatively large reservoir of both amino acids. Young healthy individuals are able to maintain an adequate amount of free amino acids, including cysteine and asparagine, in the postabsorptive (starved) state through a tightly regulated mechanism of protein catabolism called autophagy (111, 170). To prevent excessive and potentially harm-

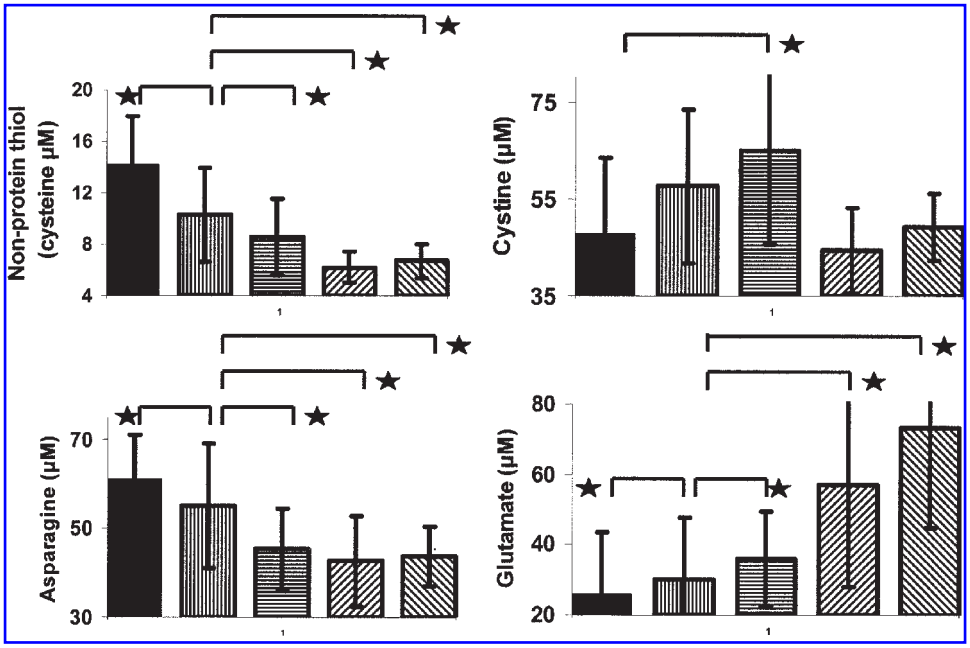

FIG. 2. Age- and disease-related changes in certain postabsorptive plasma amino acid levels (±S.D). Corresponding male and female subjects were not significantly different and have therefore been combined. Stars (★) indicate a significant difference between the indicated groups. Healthy subjects, age 19–29 years (n=93) ■; healthy subjects, age 30–69 years (n=116) ▥; healthy subjects, age ≥70 years (n=52) ⊟; obese subjects, age 42.0±11.8 years (n=49) ▨; Hyperlipidemic subjects, age 42.5±10.4 years (n=47) ▧. Data on obese and hyperlipidemic subjects are taken from the study in ref. 76. Other data are unpublished data from Wulf Dröge (see also ref. 64).

ful autocatabolism, autophagy is negatively controlled by the same signaling pathway that positively controls the rate of protein synthesis [*i.e.*, the insulin receptor signaling cascade including its key regulator, the target of rapamycin (TOR, mTOR in mammals) (33, 43, 136)]. Because mTOR is also activated by increasing concentrations of free amino acids and because mTOR negatively controls autophagic activity, this system provides an almost perfect autoregulatory loop which stops autophagy when free amino acid concentrations increase and reach a certain level (Fig. 3).

The concomitant decrease in both nonprotein thiol and asparagine in elderly, obese, and hyperlipidemic subjects is best explained by the interpretation that the postabsorptive (autophagic) protein catabolism is markedly impaired in all these conditions. This interpretation is supported by animal studies showing that autophagic activity is enhanced by the injection of an antilipolytic agent (16) or by dietary restriction (39). Another hypothetical mechanistic link between low plasma thiol (cysteine) levels and hyperlipidemia is discussed below.

In humans, the insulin- and amino acid-sensitive postabsorptive (autophagic) net protein catabolism in the peripheral (mostly skeletal muscle) tissue can be conveniently measured by determining the amino acid exchange rate across the lower extremities, as defined by the difference between the plasma amino acid concentrations in the femoral artery and femoral vein, multiplied by the blood flow (17, 181, 196). Amino acid exchange studies have shown that the peripheral tissues (mainly skeletal muscle) take up amino acids during the postprandial (fed) state and release amino acids in the postabsorptive (fasted) state (*i.e.*, in a state with relatively low plasma insulin and amino acid levels). This postabsorptive release of amino acids is strongly inhibited by infusion of insulin or by exogenous supply of amino acids, suggesting that it is mainly mediated by the lysosomal/autophagic mechanism of protein catabolism (17, 37, 54, 58, 126, 150, 181, 196).

Postabsorptive amino acid exchange studies in young healthy human subjects revealed a mean release of asparagine into the blood of 23.4 ± 3.7 nmol min^{-1} 100 ml $tissue^{-1}$ (78) which approximately corresponds to 8.2 μmoles min^{-1} in a person of 70 kg (~35 kg muscle mass). Given the venous plasma asparagine concentration of 60 μM (see Fig. 2) and ~3 L total plasma volume, the mean postabsorptive tissue asparagine release would be sufficient to replace the entire plasma asparagine pool about every 22 min. This is about twice as fast as for most other amino acids (not shown). Conversely, the urinary excretion of asparagine (83.5 μmoles hr^{-1} , see ref.132) is sufficient to release the entire plasma asparagine pool every 129 min, which is again exceptionally fast in comparison with most other amino acids. Irrespective of other metabolic/catabolic processes, these exceptionally high values of tissue release and urinary excretion relative to the plasma concentration are indicative of a high turnover of the plasma asparagine pool. This may explain why the plasma asparagine concentration is more profoundly affected by changes in the (autophagic) amino acid homeostasis than other amino acids except cysteine (see Fig. 2). Unfortunately, the corresponding tissue release and urinary excretion data for cysteine have not yet been determined.

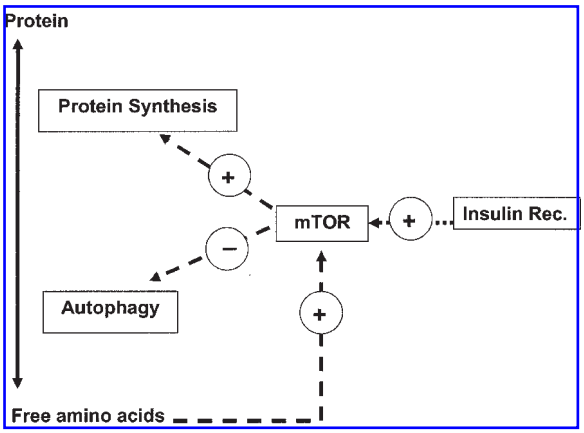

FIG. 3. Regulation of amino acid homeostasis during starvation by autophagy. Autophagy serves as a mechanism of amino acid homeostasis by converting proteins into free amino acids at a strictly regulated rate which is downregulated by free amino acids in a concentration-dependent way. Autophagy is also negatively regulated by the insulin receptor signaling cascade.

Amino acid exchange studies in humans have also shown that the postabsorptive (autophagic) release of amino acids by the peripheral tissues is associated, and quantitatively correlated, with a corresponding uptake of one single amino acid, glutamate (77). The postabsorptive glutamate uptake, in turn, leads to a corresponding decrease in venous plasma glutamate concentration, implying that any decrease in postabsorptive protein catabolism is associated with an increase in the postabsorptive venous glutamate concentration (63). The glutamate data in Fig. 2 confirm this point: The decrease in plasma nonprotein thiol and asparagine concentrations in seemingly healthy elderly subjects and middle-aged obese and hyperlipidemic patients was in all three cases associated with a significant increase in the postabsorptive venous plasma glutamate concentration. The increase in plasma glutamate is, therefore, further supportive evidence for a decrease in postabsorptive (autophagic) protein catabolism.

Taken together, there is evidence from different laboratories showing that the mean plasma cysteine concentration in the postabsorptive (starving) condition decreases with age. This decrease is associated with a similar significant decrease in asparagine and a corresponding increase in the postabsorptive plasma glutamate concentration. These changes are indicative of an aberrant amino acid homeostasis and best explained by the assumption that the postabsorptive (autophagic) protein catabolism decreases with age (see below).

CLOSING THE VICIOUS CYCLE: REDOX RESPONSIVENESS OF THE INSULIN RECEPTOR SIGNALING CASCADE

The weak point in the homeostatic control is that the autophagic activity is regulated not only by the free amino acid levels but also by the insulin receptor signaling cascade (Fig 3). The postabsorptive (starved) state is typically associated with low amino acid concentrations and low insulin levels. Several lines of evidence have shown, however, that even in the

absence of insulin, the activity of the insulin receptor signaling pathway is abnormally increased by oxidative stress. The age-related increase in oxidative stress provides therefore an explanation for the age-related change in the homeostatic control of cysteine and asparagine.

The *basic* activity of the insulin receptor signaling pathway in the absence of insulin is weak but is increased under oxidative conditions. Activation of the insulin receptor involves its autophosphorylation and is typically followed by phosphorylation of several target proteins in the signaling cascade. This signaling cascade is negatively regulated by several phosphatases including protein tyrosine phosphatase 1B (PTB 1B), phosphatase, and tensin homologue on chromosome 10 (PTEN), and SH2-domain-containing inositol phosphatase (SHIP2), all of which are inactivated under moderately oxidative conditions (18, 48, 59). PTP 1B, a phosphatase prominently involved in the insulin receptor signaling pathway, is one of the molecularly best characterized redox-sensitive signaling proteins (12, 13). Biochemical evidence indicates that the inhibition of its catalytic activity can proceed by either of two ways (Fig. 4). Hydrogen peroxide converts the catalytically relevant cysteine moiety into cysteine sulfenic acid (Cys-SOH), which interacts spontaneously with glutathione to form a catalytically inactive mixed protein–glutathione disulfide. Alternatively, the redox-sensitive cysteine residue may be converted directly by glutathione disulfide into the inactive disulfide (Fig. 4). The glutathionylated protein may be converted back into the catalytically active reduced form by an oxidoreductase such as glutaredoxin.

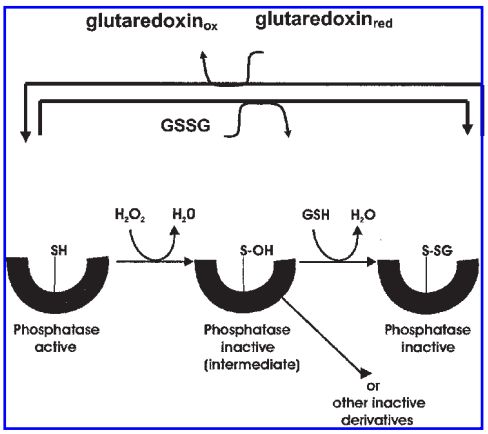

FIG. 4. Oxidative inactivation of phosphatases. Phosphatases typically contain a redox sensitive cysteine residue (SH) in their catalytic center. Upon oxidation by hydrogen peroxide, the phosphatase forms a catalytically inactive sulfenic acid derivative (S-OH) which can be further converted into the inactive glutathionylated derivative (S-SG) or other inactive derivatives. The glutathionylated protein can be reactivated by an oxidoreductase such as glutaredoxin.

It has been estimated that a 30 mV change in redox status is sufficient to cause a ten-fold change in the ratio of a protein dithiol–disulfide motif and a corresponding change in protein function (88). Although these glutathionylation processes involve relatively slow nonenzymatic thiol–disulfide exchange reactions, they may be facilitated by the persistence of the age-related changes in the thiol/disulfide redox status. The details of these processes such as compartmentalization, timing, and/or duration are still under investigation (50, 164).

In contrast to the functional inactivation of the phosphatases, the *basic* insulin receptor tyrosine kinase activity itself is strongly increased by low concentrations of hydrogen peroxide or by an oxidative shift in the glutathione redox status (165). This effect of hydrogen peroxide was shown to act directly on the cytoplasmic kinase domain and works, therefore, also in the absence of insulin. As the activity of the insulin receptor signaling pathway is determined by a balance between kinase and phosphatase activities, the oxidative activation of the kinase and the simultaneous inactivation of phosphatases, taken together, tend to enhance synergistically the upregulation of the signaling pathway.

Several lines of evidence support the *in vivo* relevance of these regulatory processes. A substantial fraction of tyrosine phosphatase activity in human adipose tissue has been shown to exist in an inactive oxidized form that can be reactivated to various degrees by biochemical reduction *in vitro*, indicating that reversible oxidation plays a role in protein tyrosine phosphatase regulation *in vivo* (206). Treatment of mice with cysteine or *N*-acetylcysteine for a period of 1 week was shown to cause a strong dose-dependent increase in plasma glucose concentration (192). In another study in mice, overexpression of glutathione peroxidase 1 (GPX1) was shown to decrease both the autophosphorylation of the hepatic insulin receptor and the clearance of glucose from the blood (118). A correlation between increased glutathione peroxidase and decreased glucose clearance has also been observed in a study of pregnant women (29). Between time of entry and the third trimester of pregnancy, the women showed a significant increase in glutathione peroxidase activity together with a significant increase in postabsorptive insulin and glucose concentrations. Individual changes in insulin and glucose levels were significantly correlated with the individual changes in glutathione peroxidase. On the average, the postabsorptive HOMA-R index (*i.e.*, the product of the insulin and glucose levels divided by 405, according to the homeostatic model of insulin responsiveness) showed an increase of ~1.6 points during this period (29). (The apparent decrease in postabsorptive insulin receptor signaling may serve the purpose to ensure optimum autophagy, adequate free amino acid homeostasis, and optimal glutathione levels during pregnancy. Enhanced autophagy may also be required for the lactating mother to produce relatively large quantities of cysteine-rich milk proteins). A similar increase in the postabsorptive HOMA-R index by ~2 points was observed in young obese nondiabetic women after oral supplementation of *N*-acetylcysteine (76).

As phosphatase PTB1B was shown to be glutathionylated by glutathione disulfide (12, 13), and because an oxidative shift in glutathione redox status was shown to increase insulin receptor autophosphorylation (165), it is concluded that the age-related decrease in the postabsorptive plasma cysteine concentration leads to a vicious cycle of progressively increasing oxidative stress, as schematically illustrated in Fig. 1.

Irrespective of these ROS-independent effects, the age-related decrease in plasma cysteine and intracellular glutathione concentrations inevitably compromises the ROS scavenging capacity and is likely to cause an age-related increase in ROS con-

centrations at least in the postabsorptive period (Fig. 1). This process provides an explanation for the observed increase in ROS-mediated structural damage in old age. In addition, the age-related increase in ROS concentrations is expected to further alter the set point of the redox-responsive insulin-independent *basic* insulin receptor signaling cascade and may thereby contribute to the vicious cycle (Fig. 1).

Such changes would inevitably compromise the activation of certain Akt-inhibitable factors such as FOXO 1 and peroxisome proliferator-activated receptor-γ coactivator 1 (PGC-1α), as discussed below. Other redox-responsive signaling cascades are involved in the stimulation of certain inflammatory cytokines that have also been implicated in the mechanism of aging (46) and carcinogenesis (177).

LIFESPAN EXTENSION BY IMPAIRMENT OF THE INSULIN RECEPTOR SIGNALING CASCADE: THE "INSULIN RECEPTOR PARADOX"

In support of the hypothetical scheme in Fig. 1, several longevity strains of *C. elegans* and *Drosophila* were found to involve mutations in components of the insulin receptor signaling pathway (81, 92, 95, 125). Some of these mutants showed a lifespan 2.5 times greater than that of the wild type. The increased lifespan in these mutants indicated that the insulin receptor is capable of exerting important negative effects which seriously compromise the lifespan of the species. In clinical medicine, however, the insulin receptor is commonly known for its positive function as a key regulator of glucose clearance, protein synthesis, and other important metabolic functions. This "insulin receptor paradox" raises the question whether the negative effects on lifespan may also be relevant in humans. The more detailed analysis shows that the indicated positive effects are mostly related to the postprandial (fed) state, whereas the observed negative effects are related to functions that typically operate in the postabsorptive (starved) condition as they are inhibited by the insulin receptor signaling cascade Activation of the insulin receptor involves its autophosphorylation and leads to sequential activation of other protein and lipid kinases, including phosphatidyl inositol 3-kinase (PI3K), phosphoinositide-dependent protein kinase 1 (PDK1), the serine/threonine kinases Akt1/2 (PKB), and the target of rapamycin (TOR, or mTOR in mammals). Akt1 and/or Akt2 stimulate many typically insulin-dependent functions including protein synthesis, but downregulate the autophagic/lysosomal protein catabolism, (44, 136, 183), and elicit the phosphorylation and inhibition of the forkhead transcription factor FOXO 1 (23, 152). Members of the FOXO transcription factors were shown to have an impact on lifespan in *C. elegans* (128) and *Drosophila* (81). In addition, Akt2 phosphorylates and inhibits the transcriptional coactivator PGC-1α, which is a global regulator of the postabsorptive hepatic metabolism and works in close association with FOXO 1 (113, 152). In mice, PGC-1α was required for the normal expression of several mitochondrial genes in the liver, skeletal muscle, heart, brain, and brown fat (reviewed in ref. 113). In the liver, PGC-1α and β are strongly induced upon fasting (202, reviewed in ref. 115). As PGC-1α promotes hepatic fatty acid oxidation in the starved condition and shifts fuel usage from glucose to fat, an aberrant activation of Akt2 may facilitate the development of hyperlipidemia and obesity. This and the schematic model in Fig. 1 may thus explain the association between low plasma thiol (cysteine) concentrations and hyperlipidemia or obesity (Fig. 2), as discussed below.

The importance of autophagy for lifespan extension in animals has been underscored by two independent genetic studies in *C. elegans* (60, 121). In two independent studies on mice, loss of autophagy in the central nervous system causes neurodegeneration and a shortened lifespan (67, 103). Autophagy has several important functions which are potentially relevant to the aging process in both humans and animals (33, 43, 111, 117). By removing damaged mitochondria and other forms of cellular waste, autophagy plays a key role in the maintenance of cellular integrity (174). It has important roles in development, immune defense, programmed cell death, tumor suppression, and prevention of neuron degeneration (115, 153, 182). Mice with a deletion of one copy of the autophagy gene beclin.1 (Becn1) or the Beclin 1 cofactor Bif1 showed a markedly increased incidence of tumors, indicating that autophagy may act as an important *in vivo* tumor suppressor (182, 203). Other well-known tumor suppressors, including the phosphatase PTEN, enhance autophagy by inhibiting the insulin receptor signaling activity. Perhaps most importantly, autophagy plays a role in amino acid homeostasis by converting protein into free amino acids at a rate which is downregulated either by increasing free amino acid concentrations or by insulin, as schematically illustrated in Fig. 3. The importance of amino acid homeostasis has been clearly demonstrated in *Saccharomyces cerevisiae* and *Dictyostelium discoideum*, where autophagy is essential for cellular survival at times of limited amino acid availability (101, 139, 190). The consequences of inadequate amino acid homeostasis in other species including humans have not yet been systematically investigated.

CHANGES IN AUTOPHAGIC ACTIVITY DURING STARVATION AND IN THE COURSE OF AGING

Autophagic protein catabolism is inevitably a destructive process that is strictly and negatively controlled by the same mechanism that positively controls protein synthesis [*i.e.*, the insulin signaling cascade and its key regulators Akt and TOR/mTOR (see Fig. 3)]. TOR/mTOR is upregulated not only by the insulin receptor cascade but also by an increase in amino acid concentrations (43). The strong inhibition of autophagy by insulin and elevated amino acid concentrations implicates that autophagy is optimally activated only under conditions of starvation (*i.e.*, in the postabsorptive state), and suppressed in the postprandial state (43). Electron microscopic studies in experimental animals have confirmed that the maximum rate of autophagic proteolysis occurs in the fasting condition (36).

The age-related accumulation of damaged mitochondria and other forms of biological "waste" in skeletal muscle fibers, neurons, and other post-mitotic cells has been interpreted as an in-

dication for an age-related decline in autophagic activity (33). In support of this conclusion, several experimental animal studies have shown that the formation of autophagosomes decreases with age (36, 180, 186). Electron microscopic studies and measurements of amino acid release in rats have shown that the highest rates of postabsorptive proteolysis and the greatest sensitivity to changes in amino acid concentrations were seen at 6 months of age and declined thereafter (36). The rate of proteolysis in the presence of high concentrations of amino acids and the inhibitory effect of insulin on the postabsorptive protein catabolism were not significantly altered by age, whereas the stimulatory effect of glucagon was shown to be blunted (33). Taken together, the available evidence is best explained by the interpretation that the autophagic activity in old age is not fully derepressed in the absence of insulin, although it is still subject to downregulation by insulin and high amino acid concentrations.

Taken together, evidence from both human and experimental animal studies indicates that the aberrant activity of the insulin receptor signaling pathway under starving conditions (*i.e.*, in the postabsorptive state) plays a decisive role in the aging process by preventing adequate autophagy and amino acid homeostasis. It thereby accounts at least partly for the aging-related decrease in the postabsorptive plasma cysteine concentration. As this decrease is associated with an aging-related decrease in intracellular glutathione concentrations, the insulin receptor signaling cascade appears to be a key element in the vicious cycle of aging-related oxidative stress, as illustrated in Fig. 1).

AGE-RELATED DECREASE IN THE PLASMA ALBUMIN CONCENTRATION

Due to its free thiol group at Cys 34 and its plasma concentration of ~600 μM, albumin is another quantitatively important redox buffer of the blood. In studies on healthy subjects and cancer patients, the plasma albumin concentration was significantly correlated with the plasma thiol/disulfide redox status (64). A marked decrease in plasma albumin level was typically seen in elderly subjects and in practically all catabolic conditions, including cancer cachexia and HIV infection (14, 169, reviewed in Ref. 44). A placebo-controlled trial on human immunodeficiency virus (HIV)-infected patients (22) and an unblinded study on cancer patients (64) showed that cysteine supplementation increases the (otherwise low) plasma albumin levels at least in these conditions. As albumin exists in the plasma in both the reduced and the oxidized (*i.e.*, mixed disulfide) form, and as the oxidized forms of albumin have a higher catabolic rate (105), the effect of cysteine supplementation is tentatively explained by the conversion of oxidized albumin into its more stable reduced form.

In a study on healthy elderly subjects, a low plasma albumin level was correlated with a low 10-year survival rate and a loss of skeletal muscle mass. Amongst patients with wasting syndrome, the plasma albumin level was shown to be a strong predictor of survival (44). However, it is not clear from these studies whether the decrease in plasma albumin is a causative factor in its own right or merely an epiphenomenon related to an aberrant plasma cysteine homeostasis.

RESPONSE OF REDOX SENSITIVE SIGNALING PATHWAYS TO CHANGES IN THIOL/DISULFIDE REDOX STATUS

As ROS production by certain inducible NAD(P)H oxidases is known to play a role in various redox-sensitive signaling pathways, it was to be expected that an abnormal elevation of ROS concentrations in cells and tissues is associated not only with an abnormal increase in oxidative damage of cellular constituents but also with changes in the set points of important physiological signaling pathways and aberrant gene expression (15, 42). There is a strong possibility, for example, that the age-related increase in ROS levels may account for age-related changes in the steady state levels of certain cytokines, such as interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α) (reviewed in Ref. 45). These cytokines are proteins with a hormone-like function. Certain changes in signaling pathways and gene expression may be as important, or even more important, than the direct structural damage resulting from aging-related oxidative stress.

The two transcription factors which were first shown to be stimulated by ROS, nuclear factor kappa B (NF-κB) and activator protein-1 (AP-1), were also found to be stimulated by an oxidative shift in the glutathione redox status (56). The transcription factor NF-κB is known to control the expression of several inflammatory cytokines including TNF-α and IL-6. Aging is associated with increased circulating levels of certain inflammatory cytokines, including TNF-α and IL-6, and has, therefore, been interpreted by some authors as a low-grade inflammatory condition (24). The age-related increase in the steady state level of IL-6 mRNA and IL-6 production in the brain (154, 200) is a case in point. *In vivo* and *in vitro* studies indicate that the increase in IL-6 expression results to some extent from enhanced binding of NF-κB to the IL-6 promoter in microglial cells (200, 201). In human dendritic cells, NF-κB has also been implicated in the oxidative upregulation of TNF-α and IL-8 (195). In elderly subjects, high plasma TNF-α levels are correlated with increased morbidity, mortality, Alzheimer's disease, atherosclerosis, and decreased muscle mass and muscle strength (44). In combination with interferon-γ, TNF-α was shown to downregulate MyoD, a transcription factor essential for repairing damaged muscle. Hyperlipidemic patients who have, on the average, markedly decreased postabsorptive nonprotein thiol concentrations (Fig. 1) were also found to have a significantly higher expression of inflammatory and oxidative stress-related genes, including that of TNF-α (21). In frail elderly subjects, the plasma TNF-α level was significantly correlated with the plasma cystine level (71). Last but not least, NF-κ-B and TNF-α have been implicated in the development of inflammation-associated cancer (44, 177). In several independent studies, TNF-α concentrations was downregulated by cysteine supplementation (44, 71). Treatment with *N*-acetylcystine was shown to suppress NFκ-B activation in patients with sepsis (148).

The signaling pathways of NFκB and AP-1 involve various protein tyrosine kinase species and are counter-regulated by protein tyrosine phosphatases, the activity of which is controlled by a redox-sensitive cysteine moiety (18, 48). At least some of these phosphatases are sensitive to changes of the glutathione

redox status (42). As ROS-mediated oxidative stress may include both an increase in structural damage of cellular constituents as well as an abnormal induction of signaling pathways and gene expression, it is reasonable to assume that any change in the thiol/disulfide redox status may also be sensed, at least to some extent, as oxidative stress.

THE THIOL/DISULFIDE REDOX STATUS IN CARDIOVASCULAR DISEASE

The age-related decrease in glutathione concentration inevitably compromises the ROS scavenging capacity, at least during the postabsorptive period and facilitates thereby certain "secondary" disease-related mechanisms of oxidative stress. A prominent case in point is the group of cardiovascular diseases that are responsible for a large percentage of aging-related mortality in humans. Impairment of endothelial function is a critical step in the pathogenesis of atherosclerosis (2, 146) and is typically associated with increased oxidative stress (61, 157, 158, 187). The aggravation of disease-related mechanisms of oxidative stress such as xanthine oxidase-dependent ROS production or aberrant NADPH oxidase-mediated ROS production by the general age-related decrease in glutathione level provides an explanation for the age-related increase in the incidence of cardiovascular diseases.

Patients with cardiovascular disease have, on the average, a significantly more oxidized plasma thiol/disulfide redox status (41, 89). Atrial fibrillation, the most common cardiac arrhythmia, was found to be significantly associated with oxidative stress as indicated by the plasma redox status of both cysteine and glutathione (131). A study of middle-aged individuals at risk of cardiovascular disease has also shown that an oxidative shift of the glutathione redox status is correlated with a change in carotid intima media thickness (6, 89). A study on normal- and hyperlipidemic human subjects with and without coronary heart disease revealed an inverse correlation between LDL-cholesterol and plasma nonprotein thiol and a positive correlation between HDL-cholesterol and plasma thiol concentrations (98). Another study revealed a dose-related increase of HDL-cholesterol levels after *N*-acetylcysteine supplementation (52). The plasma concentration of cysteine disulfide (cystine), in contrast, showed a significant positive correlation with the expression of the pro-inflammatory enzyme cyclooxygenase 2 (Cox-2) (20). In hypercholesterolemic aorta, decreased glutathione concentrations are significantly correlated with endothelial dysfunction (1). Several independent reports illustrated the important role of glutathione in controlling various aspects of the inflammatory process, including permeability and cell adhesion (102, 137, 204). Inhibition of glutathione biosynthesis in murine macrophages was found to increase the cell mediated oxidation of low density lipoprotein (LDL) and the release of superoxide radicals by up to 32% (60, 156). Ox-LDL, in turn, was shown to induce the production of the inflammatory cytokine TNF-α by macrophages in a process involving the redox-reactive transcription factor AP-1 (90).

Several intervention studies using glutathione or the glutathione precursor *N*-acetylcysteine have been performed to demonstrate causality. In a placebo-controlled double blind clinical trial of 40 patients with peripheral artery disease, intravenous administration of glutathione improved macrocirculatory and microcirculatory parameters (5). In another study of 16 patients, intracoronary infusion of *N*-acetylcysteine was found to augment acetylcholine-mediated microvascular dilation, indicative of enhanced endothelial-dependent vasomotion. Coronary vascular resistance was decreased and coronary blood flow significantly increased (3). In independent studies of patients with end-stage renal failure, *N*-acetylcysteine has been shown to reduce cardiovascular events (185) and to improve pulse pressure and endothelial function (166). Finally, *N*-acetylcysteine was shown to prevent accelerated atherosclerosis in an animal model (84).

ROS scavenge nitric oxide (155) and cause an aberrant stimulation of redox-sensitive transcription factors such as NF-κB, which functions as a pivotal transcription factor in chronic inflammation (11). Inflammatory processes play a key role in the development of vascular disease (114). In addition, ROS-mediated oxidative stress increases platelet activation and aggregation (66, 194). Elevated ROS concentrations have been associated with hypertension (100) and hyperlipidemia (135).

All these oxidative processes are facilitated by the general age-related decrease in glutathione concentrations. It should be noted, however, that the availability of cysteine may not be the only determinant that limits intracellular glutathione levels in cardiovascular diseases (94). Under conditions of cysteine sufficiency, intracellular glutathione concentrations can be increased by transcriptional upregulation of γ-glutamyl-cysteine ligase expression, the key enzyme in glutathione biosynthesis (123). Polymorphisms in the γ-glutamyl-cysteine ligase gene are associated with coronary endothelial vasomotor dysfunction (130).

ASSOCIATION BETWEEN LOW POSTABSORPTIVE CYSTEINE CONCENTRATION AND ABERRANT LIPID METABOLISM

The hepatic lipid metabolism is strongly regulated by the transcriptional coactivator, peroxisome proliferator-activated PGC-1α. PGC-1α is strongly induced upon fasting (115, 202), and inhibited through phosphorylation by protein kinase Akt2 (PKBβ) [*i.e.*, by activation of the insulin receptor signaling cascade (113) (see Fig. 1)]. Accordingly, PGC-1α is mainly active in the postabsorptive state. As PGC-1α stimulates fatty acid β-oxidation and shifts fuel usage from glucose to lipids (115), an aberrant increase in postabsorptive insulin receptor signaling activity provides a plausible explanation for the observed association between low postabsorptive plasma thiol (cysteine) levels and hyperlipidemia or obesity (see Fig. 2). Whereas in the diabetic state, the liver is unresponsive to insulin with regard to the postprandial suppression of glucose output, it continues to produce large amounts of lipids as expected from an activated insulin receptor cascade (38, 40). Three independent placebo-controlled clinical studies on nondiabetic obese patients (76) and healthy subjects (97, 106) have previously shown that treatment with *N*-acetylcysteine (76, 97) or a cysteine-rich protein (106), respectively, leads to a significant decrease in body fat.

AGE-RELATED INCREASE IN PLASMA CYSTINE (CYSTEINE DISULFIDE) CONCENTRATION

Blood is a relatively oxidative environment where cysteine is constantly oxidized by several different mechanisms (41, 74, 189). As the insulin-stimulated protein synthetic activity in the postprandial state determines decisively the clearance rate of dietary cysteine from the plasma, it determines to a large extent how much cysteine is converted into cystine. The age-related increase in plasma cystine concentration is, therefore, a predictable consequence of the well-known decrease in *postprandial* insulin responsiveness (7, 54, 198) and the correspondingly decreased rate of postprandial protein synthesis (34, 53, 142). A similar increase in plasma cystine concentration can be experimentally induced in healthy human subjects by a program of intense physical exercise (96), a method which is known to cause a decrease in insulin responsiveness (99, 151, 191). That the increased formation of cystine during the postprandial state can account for an increased cystine concentration in the postabsorptive plasma is tentatively explained by an extremely low rate of cystine clearance, due to the fact that most cells and tissues exhibit high transport activity for reduced cysteine but relatively low transport activity for its large disulfide derivative, cystine (163). In frail elderly subjects, the plasma cystine level was found to be significantly correlated with the plasma TNF-α level [*i.e.*, an inflammatory cytokine which is induced by the redox-responsive transcription factor NF-κB (71)].

That the age-related decrease in plasma cysteine concentration is associated with a corresponding decrease in intracellular glutathione concentration in spite of the age-related increase in plasma cystine level is also best explained by the differential membrane transport activities for cysteine and cystine. Although the x^-_c transport system for cystine and glutamate anions is clearly detectable and even inducible in some cell types (163), its relative contribution to the cellular supply of cysteine remains unclear.

EFFECTS OF CYSTEINE SUPPLEMENTATION ON SURROGATE PARAMETERS OF AGING

A few clinical investigations have already shown that cysteine supplementation exerts positive effects on certain functional parameters which are typically deteriorating in the course of aging and may be viewed as surrogate parameters of aging (45). One of the hallmarks of aging is the massive loss of muscle mass and muscle function which is often associated with psychological stress, compromised physical and social function, and financial burden (reviewed in Ref. 64). Physical exercise has been considered as a therapeutic tool to increase muscle mass and muscle function but was also found to cause the oxidation of glutathione in the blood (161, 168). This oxidation was ameliorated by treatment with *N*-acetylcysteine (168). The explorative analysis of the effect of *N*-acetylcysteine on skeletal muscle functions in a placebo-controlled double-blind study on frail elderly subjects in the context of a 6-week program of physical exercise (71) revealed that the mean of the exercise-induced changes in eleven different parameters of skeletal muscle function in the placebo group was significantly correlated with the sum of plasma thiol (cysteine) and asparagine concentrations (mean of measurements at 3 weeks and at the end of the exercise program, ($r = 0.49$, $p < 0.05$)). The correlation of the mean changes in the four parameters, knee extension strength, knee flexion strength, ankle extension strength, and hand grip strength, with the plasma concentrations of thiol plus asparagine ($r = 0.56$, $p < 0.02$) is shown in Fig. 5A. This association between exercise benefit and the individual level of amino acid homeostasis was completely abrogated by the concomitant treatment with *N*-acetylcysteine. Although the *mean* knee extension strength of *all* subjects under test was significantly increased by cysteine supplementation (71), the more detailed analysis revealed that the benefit of *N*-acetylcysteine treatment was only seen in persons with lower than median plasma thiol and asparagine concentrations (Fig. 5B). Persons with higher than median values of thiol plus asparagines [mean value 55.7 $\pm$ 3.8 μM ($\pm$ S.D)], that is, values close to those of medium-aged healthy subjects (see Fig. 2), showed a substantial exercise-induced increase in knee extension strength, both with and without *N*-acetylcysteine treatment, whereas the subjects with low amino acid homeostasis (mean thiol plus asparagine level 42.2 $\pm$ 5.9 μM) did not benefit at all from the muscle exercise program unless supplemented with *N*-acetylcysteine (Fig. 5B). Similar results were seen when groups with higher and lower than median plasma arginine were compared (71). In view of the results of Fig. 5, it is reasonable to assume that the previously observed correlation with the plasma arginine level (71) reflects the importance of the state of amino acid homeostasis in general rather than a particular function of the amino acid arginine.

The data of Fig. 5 support the notion that the benefits of cysteine supplementation (*i.e.*, an intervention designed to decrease the insulin receptor signaling activity) may depend on the level of amino acid homeostasis in a given individual. This conclusion leads to the question whether this level of amino acid homeostasis as indicated by the postabsorptive thiol and asparagine concentrations is a relatively constant property of a given individual or subject to longitudinal variations. It was therefore of interest to determine the longitudinal changes of the thiol plus asparagine concentrations in the individual elderly subjects from the trial of Ref. 71, (*i.e.*, same subjects as in Fig. 5) during the 12-week observation period. The data (Fig. 6) illustrate that approximately half of the subjects who started at baseline examination with thiol plus asparagine concentrations in the lowest tertile ($<$46.99 μM) moved into a higher tertile during the following 3 weeks, whereas 33% of subjects in the intermediate tertile moved into the lowest tertile during the following 3 weeks, and $\sim$40% of the subjects in the intermediate or highest tertile shifted into the lowest tertile at some time during the 12-week observation period. This relatively strong longitudinal variation suggests that cysteine supplementation should be given to all subjects and not only to those with low thiol plus asparagine concentrations in order to ensure for all subjects a substantial improvement in muscle function in response to physical activity.

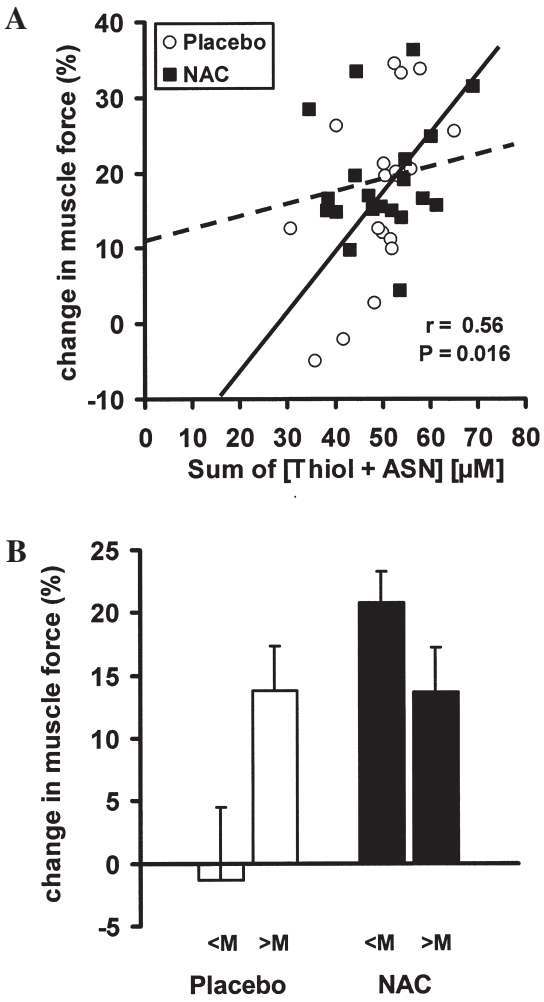

FIG. 5. Correlation between muscle function and amino acid homeostasis—effect of cysteine supplementation. The details of this study have been described in Ref. 71. Changes in muscle force have been determined in frail geriatric patients during a 6-week program of physical exercise and a 6-week follow-up period. The subjects were treated for 6 weeks with a daily dose of 1.8 g *N*-acetylcysteine or placebo. Indicated amino acid concentrations are the sum of thiol (cysteine) and asparagine (ASN) and represent the mean of two measurements obtained during and immediately after the 6-week period of physical exercise. The indicated changes in muscle force are the mean of changes during the 6-week exercise period and the changes during the total 12-week observation period. (**A**) Correlation between the mean of the changes in leg extension, leg flexion, ankle extension, and hand grip versus the sum of thiol and asparagine concentrations. (**B**) Mean changes in knee extension strength for the following subgroups of patients: (a) placebo group with thiol plus asparagine levels < median (51.3 μM); (b) placebo group with > median thiol plus asparagine; (c) *N*-acetylcysteine treated group with < median thiol plus asparagine; (d) *N*-acetylcysteine treated group with > median thiol plus asparagine. Subjects with a low level of amino acid homeostasis show practically no improvement in response to skeletal muscle activity (physical exercise) unless supplemented with an additional source of cysteine.

Unfortunately, all these data and conclusions are based on a single clinical study and need to be confirmed. The weight of the evidence may be strengthened, however, by the total body of complementary information described in this editorial review.

CYSTEINE SUPPLEMENTATION, CALORIE RESTRICTION, AND OTHER DIETARY STRATEGIES TO MODULATE AUTOPHAGY AND OXIDATIVE STRESS

In various animal species, including mammals, restriction of dietary calories was shown to result in significant lifespan extension (175, 197). Lifespan extension in diet-restricted rats was associated with increased blood glutathione levels (107). In two independent studies, calorie restriction increased autophagic activity (39, 124), and in several studies of mice, rats, and fruit flies, lifespan extension of caloric-restricted animals was associated with a decrease in oxidative tissue damage (65, 205). Insulin attenuated at least some of these effects (31). These findings raise the question whether the method of balancing cysteine supplementation could simply be replaced by calorie restriction.

The downside of calorie restriction is that any episode of hypoglycemia induces hypoglycemic response factors, including cortisol, glugacon, and adrenalin (epinephrine). These hypoglycemic hormones have a powerful catabolic effect on skeletal muscle protein which may involve the proteasomal mechanism of proteolysis (32). Because plasma glucocorticoid levels increase in many catabolic conditions, it has been suggested that glucocorticoids may generally play an important role in skeletal muscle wasting (32, 46, 70, 112). Despite the impressive lifespan-extending effects of calorie restriction in several animal models, there is general agreement that this method ought to be replaced by a better one as soon as the underlying mechanisms are understood.

An important implication of the mechanistic scheme in Fig. 1 is that it may lead to better strategies of slowing this vicious cycle of age-related oxidative stress. Dietary cysteine supplementation is just one obvious strategy. Theoretically, simply increasing the water intake during the night may facilitate autophagic activity by decreasing the plasma amino acid concentration. This and other possibilities remain to be tested.

EFFECT OF DIETARY CREATINE

Another factor of consequence is the dietary intake of creatine. Creatine monohydrate has been extensively used by athletes in doses of up to 20 g per day to increase their muscle mass. Beef, for comparison, contains ~5 g creatine per kg. As muscle tissue may contain up to 30 m*M* creatine phosphate and the muscle mass accounts for almost half of our body weight, creatine is one of the quantitatively most important low mo-

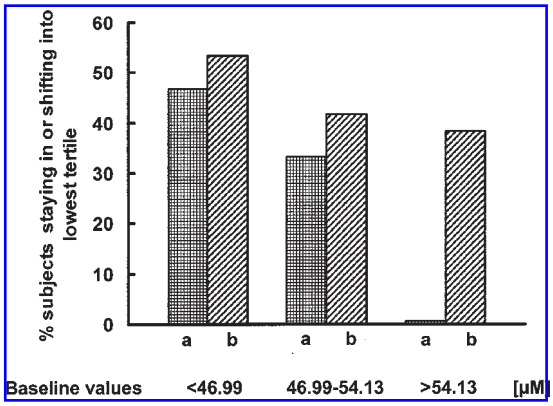

FIG. 6. Longitudinal changes in thiol plus asparagine values. To determine the longitudinal variation of the postabsorptive amino acid homeostasis, we grouped the frail elderly subjects from the study of ref. 71 (see Fig. 5) according to their thiol plus asparagine values at baseline examination as being in the lowest, intermediate, or highest tertile. The data show the proportion of subjects of each tertile which either remained in, or shifted into, the lowest tertile (**a**) as tested in the subsequent blood test after 3 weeks and (**b**) as determined in any of the following three blood tests. The data show that nearly half of the subjects who started in the lowest tertile shifted out of this low range, and that close to 40% of the subjects in the intermediate and highest tertile moved into the lowest tertile at least at some point during the observation period, indicative of a substantial degree of longitudinal variation.

lecular weight metabolites in our body. Creatine is a natural constituent of skeletal muscle and brain tissues and plays an important role in the energy metabolism of these tissues (9, 47, 73, 80, 178, 179, 208). The endogenous capacity to synthesize creatine within our body is not always sufficient to satisfy the needs (25). Several authors described pathological consequences of creatine deficient states of the central nervous system (47, 178, 179) and muscle tissues (9).

However, simultaneous administration of creatine was found to completely reverse the increase in postabsorptive HOMA-R index by *N*-acetylcysteine (76). This is tentatively explained by the fact that the insulin receptor tyrosine kinase reaction requires adenosine 5′-triphosphate (ATP) as a co-substrate and is inhibited by adenosine 5′-diphosphate (ADP) as one of its products (165). In the cytoplasm of skeletal muscle cells, ADP is rapidly converted into ATP by creatine kinase in combination with phosphocreatine. High dietary intake of creatine may therefore enhance the phosphorylation events in the insulin receptor signaling pathway.

Accordingly, a carefully balanced amount of dietary cysteine supplementation in combination with a well-balanced amount of beef or other sources of dietary creatine may be used to adjust the individual postabsorptive insulin receptor signaling activity to the desired level. A low level of postabsorptive insulin receptor signaling is a prerequisite for amino acid homeostasis and autophagic removal of cellular waste. However, as autophagy is a catabolic process, it has to be carefully balanced to avoid loss of body cell mass. Major changes in insulin receptor signaling may also compromise other insulin-responsive functions such as glucose clearance and the regulation of cell survival vs. apoptosis in certain cells and tissues. Ideally, cysteine supplementation should, therefore, be well balanced and adjusted to the individual metabolic state.

NEED FOR A SIMPLE DIAGNOSTIC TEST

To establish the need for cysteine supplementation is not a trivial task. Analysis of postabsorptive venous plasma concentrations of reduced cysteine is complicated by the fact that standard amino acid analyzers determine cystine but not reduced cysteine. Reduced cysteine can be determined by HPLC after derivatization, but processing of the blood would have to start soon after drawing the blood because cysteine is rapidly oxidized into cystine. Simple routine diagnostic tests for postabsorptive plasma cysteine concentrations will therefore not be available in the near future.

The intracellular glutathione concentration in the liver is most strongly affected by changes in cysteine availability and represents one of the largest glutathione pools in the body. In addition, a change in the hepatic glutathione level may be one of the functionally most relevant consequences of inadequate cysteine homeostasis. However, liver biopsies are not available for routine tests.

With regard to the plasma glutathione concentration, there is reason to believe that this glutathione pool has no function other than being an overflow of the highly concentrated intracellular glutathione in the liver (108). However, as the intracellular glutathione concentration in the liver is significantly correlated with the sum of the reduced plus oxidized plasma glutathione concentrations (89), the plasma level of "total glutathione" may be a relatively robust indicator for changes in the hepatic glutathione concentration. Time will tell whether the plasma glutathione level may be the simple routine diagnostic test one would be looking for.

Whole blood glutathione measurements reflect mainly red blood cell glutathione. A study of community-based elderly subjects revealed that relatively high levels of whole blood glutathione were significantly correlated with a fewer number of illnesses, lower cholesterol, and lower blood pressure (91). However, because of its dependence on different confounding factors, the whole blood glutathione measurement is not a meaningful diagnostic test to determine the cysteine requirement of the individual subject.

Regardless of which of these techniques are being used, the data in Fig. 6 suggest that a given individual would have to be tested repeatedly to obtain meaningful information.

CONCLUDING REMARKS

Taken together, this mosaic of information from different fields of research reveals a vicious cycle in which oxidative stress is caused by an aberrant insulin-independent (*postab-*

sorptive) insulin receptor signaling activity and the resulting impairment of amino acid homeostasis. The aberrant increase in the *postabsorptive* insulin receptor signaling activity, in turn, can be induced by oxidative stress as illustrated in Fig. 1. The available evidence strongly suggests that the *postabsorptive* period (*i.e.*, the starved condition) determines decisively the burden of oxidative stress and some of its most important consequences because glutathione levels in the liver and possibly other cells and tissues reach their lowest value during this period. The aging-related decrease in glutathione concentrations and thiol/disulfide redox status and the corresponding increase in oxidative stress are associated with and may result from an age-related decrease in the *postabsorptive* plasma concentration of nonprotein thiol and its major constituent cysteine. The decrease in *postabsorptive* cysteine, in turn, appears to reflect a decrease in autophagic activity as a key mechanism in amino acid homeostasis under starving conditions.

Glutathione is one of the quantitatively most important radical scavengers and co-substrate for the enzymatic removal of hydrogen peroxide. As ROS are produced at a highly regulated rate by numerous NAD(P)H oxidases and play a role in important signaling pathways, the age-related decrease in glutathione concentrations alters the set points of the corresponding physiological signals at least to some extent. The widely observed age-related increase in inflammatory cytokines and an aberrant increase in insulin independent (basic) insulin receptor signaling activity are interpreted to be consequences of this effect.

A low insulin receptor signaling activity in the *postabsorptive* period is physiologically important as it sets the stage for maximal autophagy and for maximal activity of FOXO transcription factors and hepatic PGC-1α, both of which are inhibited by insulin through Akt-dependent phosphorylation. An aberrant increase in postabsorptive insulin receptor signaling activity is expected to compromise all these functions.

Autophagy is known to play a role in the maintenance of cellular integrity by removing damaged mitochondria and other forms of cellular waste, and an inadequate autophagic removal of cellular waste may play an important role in human aging. In *Saccharomyces cerevisiae* and *Dictyostelium discoideum*, autophagy was also shown to be essential for free amino acid homeostasis and survival under starving conditions. As the quality of amino acid homeostasis determines the plasma concentration of reduced cysteine in the starved condition and the autophagic activity is negatively controlled by the insulin receptor signaling pathway, this signaling cascade plays a critical role in the vicious cycle of oxidative stress, as illustrated in Fig. 1.

Genetic studies on a series of longevity mutants in worms, fruit flies, and mice have convincingly shown that the insulin receptor signaling cascade and the insulin-inhibitable FOXO transcription factor activity and autophagic protein catabolism have a decisive influence on the lifespan of these species. In view of the spectacular lifespan extension in some of these mutants, it is reasonable to hypothesize that the current maximum lifespan of the human species of ~120 years can be substantially extended and the quality of life in old age markedly improved once the underlying mechanism of longevity will be understood in greater detail and new interventions will be clinically established. This editorial review is hoped to shed some light.

The various details of the scheme in Fig. 1 are supported by numerous studies in humans and experimental animals. A series of animal studies has shown that autophagic protein catabolism is rigorously controlled by the insulin receptor signaling cascade and by the concentration of free amino acids. Complementary studies in humans have shown that a substantial rate of insulin sensitive protein catabolism indicative of autophagy occurs in the postabsorptive (starving) condition. As typical for autophagy, this catabolism is downregulated by increasing amino acid levels and leads to a controlled efflux of free amino acids as a key mechanism of amino acid homeostasis.

Evidence for an age-related decline in autophagic activity has been obtained in animal studies, but not yet in humans. However, the age-related decline in the mean postabsorptive plasma thiol, cysteine, and asparagine concentrations in humans may be indicative of an age-related decline in autophagic activity and amino acid homeostasis. The insulin-inhibitable postabsorptive protein catabolism in human peripheral tissues (mainly skeletal muscle) has been extensively investigated by amino acid exchange studies in several laboratories. Unfortunately, there are no reports about aging-related changes in the insulin and amino acid-sensitive postabsorptive protein catabolism, and there are practically no quantitative data on the postabsorptive release of reduced cysteine into the plasma. This has been hampered by the difficulty in dealing with this easily oxidizable amino acid.

The causal relationship between changes in cysteine availability and postabsorptive (basal) insulin signaling activity as expressed by the HOMA-R index has been demonstrated in one clinical trial and is supported by indirect evidence from many complementary laboratory experiments and clinical studies.

The various findings summarized in this editorial review reveal new perspectives but also limitations. One implication of the scheme in Fig. 1 is that the age-related increase in oxidative stress may be ameliorated by dietary strategies which aim at increasing the postabsorptive autophagic activity. A major limitation is the difficulty to reconcile the need for the autophagic removal of cellular waste and maintenance of amino acid homeostasis, on the one hand, with the anabolic processes needed to maintain body cell mass and to minimize the age-related loss of skeletal muscle mass and muscle function, on the other hand. There is some clinical evidence, however, showing that a well-balanced cysteine supplementation can be effective in improving skeletal muscle function in frail elderly subjects. This finding is based on one study and needs to be confirmed.

Reports about adverse effects of superoptimal cysteine supplementation are scarce. In a recent study of mice, high-dose *N*-acetylcysteine treatment was found to cause pulmonary arterial hypertension that mimicked the effects of chronic hypoxia (144). This effect appears to be related to the hypoxia-mimetic effects of *N*-acetylcysteine treatment in humans (74). More systematic studies on potentially adverse effects of cysteine supplementation and the determination of a safe dose range are clearly needed, but will inevitably be restricted to experimental animals.

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ABBREVIATIONS

ADP, adenosine 5′-diphosphate; Akt/PKB, protein kinase B; AP-1, activator protein 1; ATP, adenosine 5′-triphosphate; Becn1, beclin-1; Cox-2, cyclooxygenase 2; Cys-SOH, cysteine sulfenic acid; FOXO, forkhead transcription factor; GPX1, glutathione peroxidase 1; HDL, high-density lipoprotein; HIV, human immunodeficiency virus; HOMA-R, homeostatic model of insulin responsiveness; HPLC, high pressure liquid chromatography; IL-6, interleukin 6; LDL, low-density lipoprotein; (m)TOR, (mammalian) target of rapamycin; NF-κB, nuclear factor kappa B; OxLDL, oxidized LDL; PDK, phosphoinositide-dependent kinase; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1+; PTB 1B, protein tyrosine phosphatase 1B; PTEN, phosphatase and tensin homologue on chromosome 10; ROS, reactive oxygen species; SHIP2, SH2-domain containing inositol phosphatase; SOD, superoxide dismutase; TNF-α, tumor necrosis factor α.

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