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Roles of heat-shock protein 70 in protecting against intestinal mucosal damage

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1. ABSTRACT

Heat shock proteins (HSPs) are remarkably conserved in all living organisms. The upregulation of expression of HSPs is triggered by a variety of physiological and environmental insults. HSPs play an important role in protecting against protein denaturation and subsequent celluar stress, which damages the intestinal mucosa and reduces the protective function of the mucosal barrier, resulting in the formation of stress ulcers. Heat shock protein 70 (HSP70) is the most widely studied of all HSPs and has numerous important chaperoning functions. Stress accelerates the synthesis of HSP70, which in turn inhibits the apoptosis of intestinal mucosal cells. In this article, we review the main classification of HSPs, the expression and regulation of HSPs and their roles in stress ulcers. We also discuss the role of functional amino acids in regulating the expression of HSPs (particularly HSP70) and protecting the intestinal mucosa and other tissues.

2. INTRODUCTION

HSPs are a class of functionally related proteins, whose expression is upregulated when cells are exposed to elevated temperatures or other stresses. As intracellular chaperones, HSPs are an evolutionarily conserved family of proteins in virtually all living organisms, from bacteria to humans (1). Despite their designation, most HSPs are expressed constitutively and perform essential functions, such as protecting organisms against morbidity and mortality (2, 3).

Stresses, including starvation, burns, wounds, and surgical operations, can result in remarkable alterations in the nutritional status, which leads to intestinal mucosal and stress ulcers, hepatic dysfunction, and even morbidity and mortality (4, 5). Since an organ's energy and redox status are closely related to its nutritional status, organ dysfunction may be further compromised. Malnutrition

and ongoing serum protein losses can have an adverse effect on intestinal absorption. The relative concentrations of amino acids are also altered in response to various forms of stress (6). For example, a major burn injury provokes a complex disruption of hormonal homeostasis that induces increases in: a) resting metabolic rate and oxygen consumption, b) lipolysis, and c) glucose turnover, as well as the loss of body mass and negative nitrogen balance.

In response to either elevated temperatures or several other metabolic insults, cells from all organisms respond by upregulating HSPs (7). Experimental evidence suggests that some amino acids regulate HSP expression, which is essential to prevent organ dysfunction. HSP70 is the most widely studied heat-shock protein and has several important chaperoning functions. Particularly, both arginine and/or glutamine in the intestine, in addition to their role as a major fuel for enterocytes, regulate the expression of HSPs (especially HSP70), facilitate cell proliferation, limit the inflammatory response and apoptosis, and modulate intermediary metabolism through specific transcription factors (8). The regulatory effects of amino acids, and particularly those of arginine and/or glutamine, have been extensively studied and the molecular mechanisms involved in protecting against damage to the intestinal mucosa appear to be diverse and complex.

3. HSPS AND HSP70

HSPs are members of a highly conserved family of proteins that possess a variety of functions, but which are best known for chaperoning and re-folding partially denatured proteins (9-11). In addition, HSPs have been shown to participate in protein assembly, secretion, trafficking, protein degradation, and the regulation of transcription factors and protein kinases. HSPs also either promote or protect against immunologically mediated disease.

HSPs are named according to their molecular weights. For example, HSP60, HSP70 and HSP90 (the most widely-studied HSPs) refer to families of heat shock proteins on the order of 60, 70 and 90 kilodaltons in size, respectively (1, 12). The dramatic upregulation of HSPs plays a key role in recovery from stress. HSP70, the major stress-induced HSP, has been found in the extracellular medium and is capable of protecting cells. The mechanism responsible for the release of HSP70 is controversial because this protein does not present a consensual secretory signal. HSP70 chaperones, together with their co-chaperones (e.g., DnaJ/Hsp40 and GrpE), make up a set of prominent cellular machines that assist with a wide range of protein-folding processes in almost all cellular compartments (9, 13). HSP70 has essential functions in preventing aggregation and assisting in the refolding of non-native proteins under both normal and stress conditions, and provides thermotolerance in animals challenged with heat stress (13). HSP70 also prevents protein folding during post-translational import into the mitochondria/chloroplast. Furthermore, HSPs are involved in protein import and translocation processes, and in facilitating the proteolytic degradation of unstable proteins by targeting these proteins to lysosomes or proteasomes (14).

The HSP70 family contains at least eight homologous chaperone proteins, including HSP72, HSP73, GRP75, GRP80, GRP80, etc. (15). Cytosolic 70-kDa HSPs are present in cells as two different, but closely related, gene products. These are the stress-inducible form, HSP72 (known as HSP70), and a constitutively expressed form, HSP73 (known as the 70-kDa heat shock cognate protein, HSC70). In contrast to HSP73, HSP72 is only found in inflammatory cells or damaged tissue cells under stress (16).

4. REGULATION OF HSP70 EXPRESSION

An important consideration regarding the regulation of HSP70 involves the apparent discordance between transcription of message and HSP70 translation. Some evidence suggests that transcriptional activation of the HSP70 gene is independent of protein synthesis (17). In addition, some data indicate that both transcriptional and posttranscriptional regulatory steps are required for HSP production (17, 18). Enhanced expression of the HSP70 gene persists in response to this condition, as initiated by the activation of heat shock factor 1 (HSF1) and the lesser-characterized HSF2 (19). A recent study indicated that the molecular mechanism of Gln-induced HSP70 expression appears to be mediated via enhancement of O-linked beta-N-acetylglucosamine modification and subsequent increases in the levels of endonuclear HSF-1 expression and HSF-1 transcription activity (20).

Nitric oxide (NO) is an important multifunctional biomolecule that is involved in a variety of physiological and pathological processes (21). While low physiological concentrations of NO can inhibit apoptosis, higher concentrations of NO may be toxic (21, 22). NO is also involved in the heat shock-induced activation of HSP70 synthesis (23). It has been reported that pretreatment of hepatocytes with NO altered the redox state accompanied by the oxidation of glutathione (GSH) and the formation of S-nitrosoglutathione (GSNO), both of which are involved in the induction of HSP70 mRNA. NO may stimulate HSP70 expression (24). The putative mechanisms for the action of NO are illustrated in Figure 1.

5. HSP70 AND PROTECTION OF THE INTESTINAL MUCOSA

As internal cytoprotectants, HSPs may play important roles in protecting the intestinal mucosa against cytotoxic agents and cellular stress, and this may lead to a better understanding of the mechanisms of cytoprotection and cellular repair, and present new strategies for therapy involving stress-responses in the intestinal mucosa (25, 26). In both *in vivo* and *in vitro* models, the members of the HSP70 family have been shown to be important factors protecting cells of the intestinal mucosa (27-30). HSP70 plays a role in maintaining intestinal epithelial cell structure and function under cellular stress and injury (28). For example, the perfusion of mice with glacial acetic acid

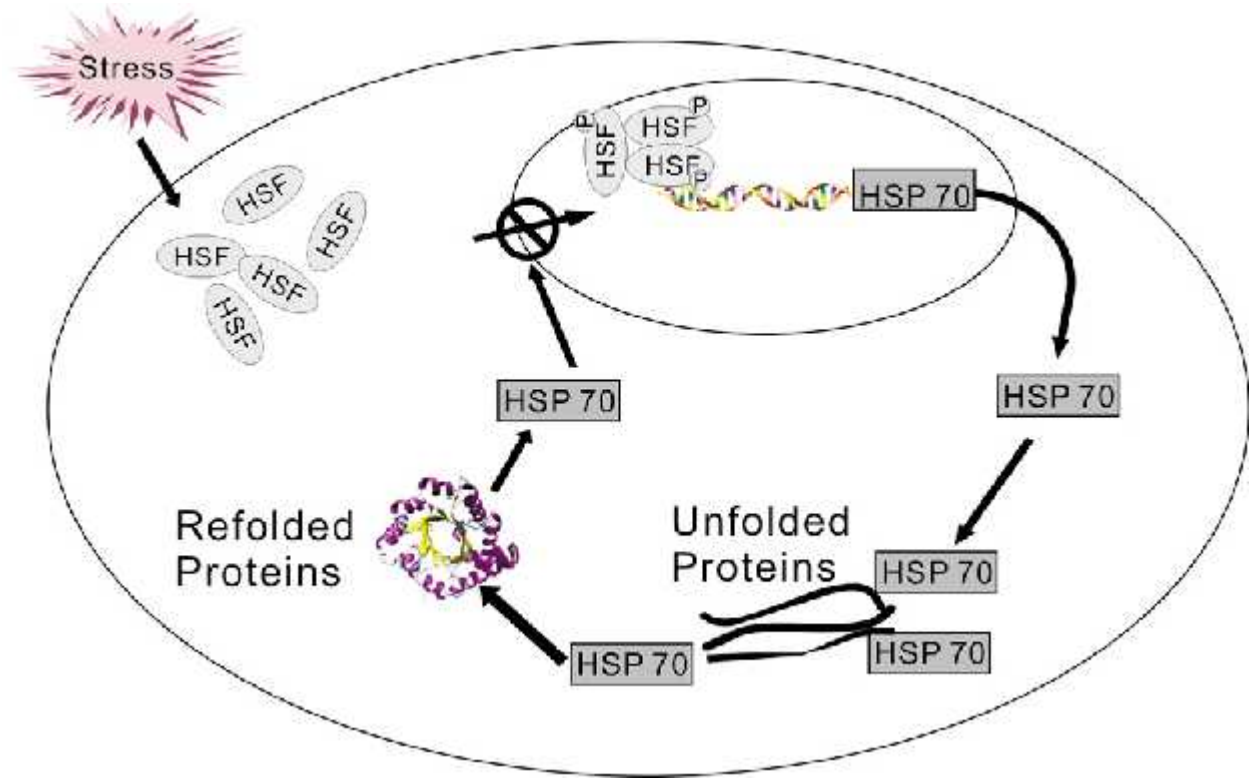

Figure 1. Enhanced expression of the HSP70 gene persists in response to the stress, as initiated by the activation of heat shock transcription factors (HSF), mainly HSF-1 and the lesser characterized HSF2. The accumulation of aggregated or denatured proteins in the cytosol appears to trigger the induction of HSF-1 as the first step in the stress response. Trimer formation of the phosphorylated HSF-1 then activates its movement into the nucleus where it stimulates the promoter lesion heat shock element (HSE) to induce HSP gene expression (20). ATP and NO may enhance HSP70 synthesis under conditions of the stress-induced activation.

induced an inflammatory response in the colonic mucosa and a high level of HSP70 expression was detected, while HSP60 and HSP90 were expressed at low levels. This result indicated that HSP70, but not HSP60 or HSP90, plays a key role in the inflammatory response (25).

Suboptimal nutritional status, including malnutrition and obesity, is associated with gastrointestinal diseases (31). Weaning causes dysfunction of the intestinal mucosa (32, 33). Early weaning has been shown to result in: a) the increased expression of genes (52-346%) related to oxidative stress and immune activation, and b) the decreased expression of genes (35-77%) related to macronutrient metabolism and cell proliferation in the gut (34). The expression of HSP70 was transiently increased in the stomach and duodenum postweaning, while its expression was transiently decreased in the ileum (35).

6. FUNCTIONAL AMINO ACIDS AND REGULATION OF HSPS EXPRESSION

6.1. Functional amino acids and HSP70

The relative concentrations of amino acids are altered in response to various forms of stress, such as sepsis, fevers, thermal burns, or malnutrition. For example, concentrations of amino acids in tissues (including plasma) undergo dynamic changes when there is a deficiency of any one of the essential amino acids, a dietary imbalance of amino acids, or an insufficient intake of protein (6). Functional amino acids (e.g., arginine, glutamine, glutamate, proline, leucine, cysteine and tryptophan) play important roles in regulating fetal and postnatal survival, growth and development (36). The results of recent studies have indicated that functional amino acids can beneficially modulate nutrient metabolism, protein turnover, and immune function.

Several drugs target the induction of HSP expression to protect the intestinal mucosa and other tissues. Amino acid analogs have been shown to enhance the expression of HSPs (37). Some amino acids exert a wide range of regulatory effects on gene expression through the activation of different signaling pathways and transcription factors, and several *cis* elements have been shown to respond to changes in extracellular amino acid concentrations. Particular attention has been directed to the effects of glutamine and arginine, which modulate several cell functions through the activation of various pathways in different tissues.

Functional amino acids also play essential roles in regulating the expression of HSPs under stress

conditions.

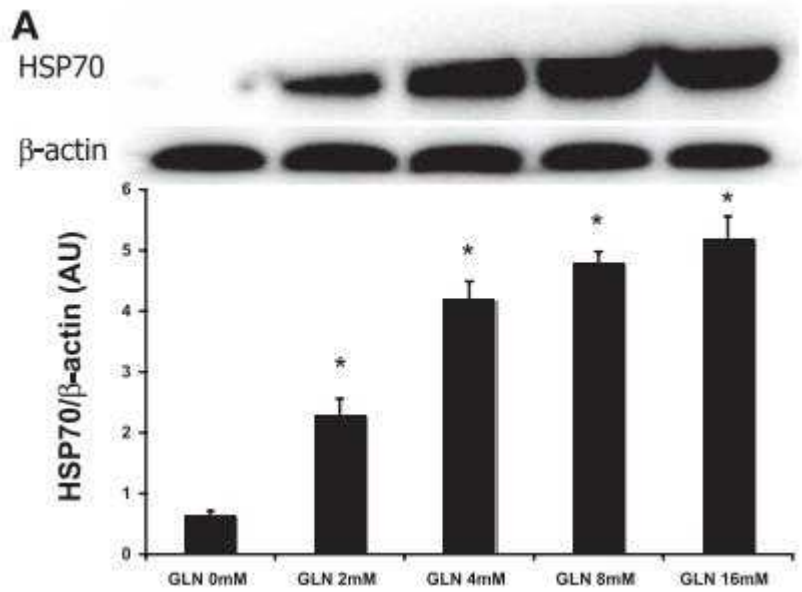

Figure 2. GLN increases HSP70 expression in intestinal cells after heat stress in a dose-dependent fashion (40).

Glutamine is the first clinically relevant pharmacological regulator of HSP expression (2). In the intestine, appropriate concentrations of both arginine and/or glutamine contribute to facilitate cell proliferation, to limit the inflammatory response and apoptosis, and to modulate intermediary metabolism through specific transcription factors (8).

6.2. Glutamine and HSP70 in protection of the intestinal mucosa

Glutamine is the most abundant free α-amino acid in animals and humans. It is mainly stored in skeletal muscle, where it comprises about 60% of all unbound amino acids. In addition to its role as a constituent of proteins and its importance in amino acid transamination, glutamine has regulatory role in immune and cell modulation (38). Glutamine is a conditionally essential amino acid, because it becomes insufficient under conditions of severe illness or injury where supplementation from the diet or other sources is necessary. Glutamine influences a variety of different molecular pathways (39). Glutamine deprivation reduces the proliferation of lymphocytes, influences the expression of surface activation markers on lymphocytes and monocytes, affects the production of cytokines, and stimulates apoptosis.

Numerous studies have demonstrated that treatment of chondrocytes with glutamine protected the cells from heat stress-induced apoptosis, and these chondro-protective effects of glutamine may be mediated by HSP70 (40). A proteomic analysis identified 23 proteins that were affected by glutamine starvation, including metabolic enzymes, proteins involved in the synthesis and degradation of RNA and proteins, and stress proteins. In U937 cells that had been cultivated with low levels of glutamine, the half-life of mRNA of HSP70 was drastically shortened (45min vs. 4 h) (41). As summarized in Figure 2, glutamine induced HSP-70 expression after heat stress in a dose-dependent manner (42).

The pathway by which glutamine enhances HSP70 is unknown. The effects of glutamine on the induction of HSP70 and cellular protection are mediated by metabolic and nonmetabolic mechanisms (43). For example, the mechanism of glutamine-mediated protection against injury appears to involve increases in a) nuclear HSF-1 content before stress; and b) HSF-1 promoter binding and phosphorylation (42). Animals and cells that lack the HSP70 gene or the HSF-1 gene show no survival benefit with glutamine administration (44). Glutamine stimulates the formation of HSP70 in monocytes by enhancing the stability of mRNA, influences the redox potential of cells by enhancing the formation of glutathione, induces cellular anabolic effects by increasing the cell volume, activates mitogen-activated protein kinases, and interacts with particular aminoacyl-transfer RNA synthetases in specific glutamine-sensing metabolism (39). Glutamine is a key substrate for the hexosamine biosynthetic pathway (HBP), which has been shown to induce HSP70. The mechanism by which the metabolism of glutamine via the HBP enhances HSP70 expression appears to be mediated via O-glycosylation, nuclear translocation, and transcriptional activation of Sp1 and HSF-1 (41).

Glutamine supplementation has been shown to reduce heat shock-induced cell death *in vitro*. This effect, together with the maintenance of cell growth, may play a key role in the prevention of intestinal mucosal atrophy (45). *In vivo*, glutamine supplementation is beneficial for intestinal health and development, thereby mitigating

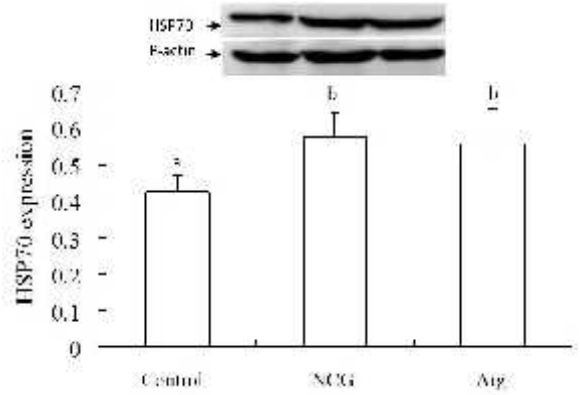

Figure 3. Dietary supplementation with L-arginine or N-carbamylglutamate enhances intestinal growth and HSP70 expression in weanling pigs (55).

diarrhea and improving growth performance. Dietary supplementation with glutamine increased the intestinal expression of genes (120–124%), including HSP70, that are necessary for cell growth and the removal of oxidants, while reducing the expression of genes (34-75%) that promote oxidative stress and immune activation (34). In a recent study, glutamine supplementation increased the expression of HSP70 mRNA and proteins in the duodenum and jejunum in weaning piglets, and the localization of HSP70 in the cytoplasm indicates that HSP70 plays a cytoprotective role in epithelial cell function and structure (46). Treatment with glutamine also enhanced HSP expression in colonic mucosa of rats with experimental inflammatory bowel disease (IBD), and this effect contributed to the cell-protective, antiapoptotic, and anti-inflammatory actions of glutamine against inflammatory injury (47).

Physiological levels of glutamine are necessary for maximum HSP70 accumulation under heat shock (48). When mice were subjected to cecal ligation and puncture (CLP)-induced sepsis and then treated with Gln (0.75 g/kg body weight) or a saline placebo 30 min after CLP, glutamine increased cellular abundances of HSP70 and HSP25 in a dose-dependent manner (47). Glutamine has also been used to aid recovery from intestinal surgery. Disorders of the small intestine, such as ulcers and bleeding (as in Crohn's disease), may also be ameliorated by supplementation with glutamine. For example, glutamine protects the gut mucosa against injury and promotes mucosal healing, and these effects are in part mediated by the induction of HSP70 (43). Moreover, preoperative administration of glutamine can enhance HSP70 expression and glutathione concentrations before islet transplantation and to attenuate ischemic damage in rat islets (49).

Available evidence shows that glutamine can be administered under clinical conditions as an oral, parenteral, or enteral supplement, either as a single amino acid or in the form of glutamine-containing dipeptides, to prevent mucositis/stomatitis and glutamine deficiency in critically ill patients (39). However, glutamine should not be considered a cure-all agent for gastro-intestinal disorders or any of the other diseased conditions. Rather, if there are indications of glutamine deficiency (e.g., low muscle mass, high levels of stress, persistent and/or severe intestinal disorder), it is reasonable to consider glutamine supplementation as a therapeutic strategy.

6.3. Arginine and HSP70 in protection of the intestinal mucosa

Arginine plays an important role in the intestine, where it is extensively metabolized, and enhances its immune-supportive function and mucosal repair (50). Arginine is classified as a conditionally essential amino acid that is required exogenously during catabolic disease states and periods of rapid growth, both of which are characterized by increased arginine utilization. In stressful situations, such as injury, burn, and sepsis, L-arginine may become an indispensable amino acid. In times of stress or severe injury, the endogenous supply of L-arginine may become critical for maintaining homeostasis.

The results of 2-DE combined with MALDI-TOF-MS and the antibody microarray technique have provided evidence that arginine deficiency modulates the HSP expression profiles of preconfluent Caco-2 cells differently than those in postconfluent differentiated cells (51). Arginine deprivation decreases cell proliferation and HSP expression, and enhances the cell's susceptibility to apoptosis (51). Supplementation with L-arginine is effective in inhibiting the intense apoptosis in fetal cells (52). In addition, arginine is a precursor of endogenous NO (53, 54). Supplementation with L-arginine prevents intestinal mucosal dysfunction regarding the involvement of the L-arginine/NO/HSP70 pathway to reduce intestinal mucosal injury (55). In support of this notion, we found that dietary supplementation with L-arginine or N-carbamylglutamate enhanced intestinal growth and the intestinal expression of HSP70 in weanling pigs (55). Furthermore, arginine prevents tissue dysfunction in weaned piglets and alleviates weaning-associated stress by inducing the intestinal expression of HSP70 in piglets (56; Figure 3). These effects of arginine are likely mediated, in part, by enhanced expression HSP70 (57).

6.4. Other effects of glutamine

Glutamine is metabolized to other amino acids, including ornithine, citrulline, arginine, glutamate, proline, aspartate, and alanine in the small intestine (58-60). Glutamine is a major metabolic fuel for the small-intestinal mucosa. In leukocytes, glutamine is a major source of energy and metabolized to glutamate, aspartate, and alanine (61). carbon dioxide. As described above, glutamine and glutamate are interconverted to each other in most cells (62), but the synthesis of glutamine from glutamate is limited in the small intestine or its epithelial cells (63-67). This necessitates adequate provision of glutamine in the diet to maintain the intestinal-mucosal integrity and function. Emerging evidence shows that like arginine (68, 69), glutamine can modulate metabolism of amino acids by bacteria in the lumen of the small intestine and protects the cells from viral infection (70, 71). Glutamine Results from our recent studies indicate that dietary supplementation with alpha-ketoglutarate (AKG), an intermediate of

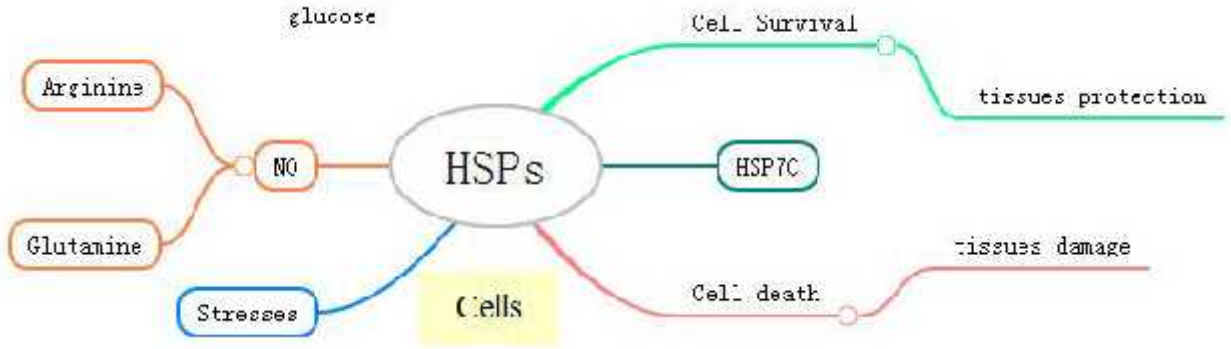

Figure 4. Functional amino acids in intestinal nutrition and health. The regulatory roles for amino acids, in particular arginine and glutamine, have been extensively studied. The underlying molecular mechanisms are complex and may involve HSP70 in protecting against intestinal mucosal damage. In the intestine, sufficient concentrations of both arginine and glutamine function to facilitate cell proliferation, limit the inflammatory response and apoptosis, and modulate intermediary metabolism through specific transcription factors.

glutamine catabolism, can beneficially regulate the intestinal expression of HSP70, intestinal energy metabolism, and intestinal function in young pigs (72-74).

Glutamine is a source of ornithine (a substrate for putrescine synthesis) and also stimulates the activity of ornithine decarboxylase (ODC), a key enzyme for polyamine synthesis, in intestinal epithelial cells (75, 76). Polyamines play a critical role in the glutamine-dependent induction of the intestinal epithelial heat shock response through the facilitation of HSF-1 binding to HSE (77). Polyamines can also promotes proliferation, migration and maturation of intestinal epithelial cells (75, 78-80).

A few studies have reported that amino acids other than arginine and glutamine have direct effects on HSP70. For example, glycine and alanine, but not glutamate, aspartate, leucine, or arginine, enhanced heat stress–induced HSP 72 expression (81). However, results of another study indicated that, glutamine, but not taurine or glycine, increased expression of HSP70 and that treatment with increasing concentrations of taurine or glycine did not improve cell survival in the same study (42; Figure 4). These different findings may be explained by different experimental conditions, including cell types, culture media, and the period of exposure.

Although plant and animal protein-based foods may contain relatively large amounts of glutamine and arginine (82, 83), dietary supplementation with these two amino acids can improve lactation performance (84). For example, arginine or glutamine is effective in increasing milk production by lactating sows (85-87). Interestingly, the expression of HSP70 in the neonatal intestine is regulated by consumption of milk (88). While the active components in milk are not known, amino acids (including glutamine and arginine) are likely candidates. This raises an important possibility that these functional nutrients may be crucial for improving intestinal health and preventing gut disease in neonates, including low-birth-weight infants and preterm infants (89, 90).

7. SUMMARY

Intestinal cells respond to either elevated temperatures or other metabolic insults by increasing the expression of HSP70. As a chaperone, HSP70 possesses a variety of functions, including the protection of the intestinal-mucosal integrity and the normal metabolism of epithelial cells. Amino acids, particularly arginine and glutamine, regulate the expression of HSPs (especially HSP70) to prevent intestinal dysfunction and maintain intestinal structure. These functionally amino acids are expected to play increasingly important roles in nutrition and health of animals and humans.

8. ACKNOWLEDGMENTS

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Abbreviations: AKG: alpha-ketoglutarate; CLP: cecal ligation and puncture; GSH: oxidation of glutathione; GSNO: S-nitrosoglutathione; AAs: amino acids; HBP: hexosamine biosynthetic pathway; HSP: Heat shock protein; HSP70: Heat shock protein 70; HSF: heat shock factor; IBD: inflammatory bowel disease; NO: Nitric oxide; ODC: ornithine decarboxylase

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