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Alpha-Ketoglutarate: Physiological Functions and Applications

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Abstract

Alpha-ketoglutarate (AKG) is a key molecule in the Krebs cycle determining the overall rate of the citric acid cycle of the organism. It is a nitrogen scavenger and a source of glutamate and glutamine that stimulates protein synthesis and inhibits protein degradation in muscles. AKG as a precursor of glutamate and glutamine is a central metabolic fuel for cells of the gastrointestinal tract as well. AKG can decrease protein catabolism and increase protein synthesis to enhance bone tissue formation in the skeletal muscles and can be used in clinical applications. In addition to these health benefits, a recent study has shown that AKG can extend the lifespan of adult *Caenorhabditis elegans* by inhibiting ATP synthase and TOR. AKG not only extends lifespan, but also delays age-related disease. In this review, we will summarize the advances in AKG research field, in the content of its physiological functions and applications.

Key Words: Alpha-ketoglutarate, Functions, Lifespan extension, Applications

INTRODUCTION

Several decades ago, the list of key nutrients that may influence metabolic processes was limited studied. Currently, the list includes fatty acids, vitamins, microelements, nucleic acids and specific amino acids. Common research in nutrient support is beginning to investigate exerting organ-specific effects by modulating metabolic processes rather than by simply improving nutrition. Alpha-ketoglutarate (AKG), also referred to as 2-ketoglutaric acid, 2-oxoglutamate, 2-oxoglutaric acid, oxoglutaric acid and 2-oxopentanedioic acid (Harrison and Pierzynowski, 2008), is a rate-determining intermediate in the tricarboxylic acid (TCA) and has a crucial role in cellular energy metabolism. In cellular metabolism, the generation and decomposition of AKG involved in a variety of metabolic pathways. In the TCA cycle, AKG is decarboxylated to succinyl-CoA and CO_2 by AKG dehydrogenase (encoded by ogdh-1), a key control point of the TCA cycle. Otherwise, AKG can be generated from isocitrate by oxidative decarboxylation catalysed by isocitrate dehydrogenase (IDH). Also, AKG can be produced anaplerotically from glutamate by oxidative deamination using glutamate dehydrogenase, and as a product of pyridoxal phosphate-dependent trans-amination reactions in which glutamate is a common amino donor. AKG can dissolve well in water, does not show toxic properties and its water solutions characterize has high stability.

AKG supplementation in human adult stage is sufficient whereas it is found to be insufficient in the senescent stage (Chin *et al*., 2014). In the cellular metabolism, it is impossible to utilize AKG from the TCA cycle in the synthesis of amino acids, for this to occur, one must provide AKG as a pure dietary supplement. It was demonstrated that AKG was significantly better absorbed from the upper small intestine than from the distal sections (Dąbek *et al*., 2005). Low pH, Fe^{2+} and/or SO^{2-}_4 ions can enhance AKG absorption. AKG has a short lifetime, is probably dependent on quick metabolism in the enteorcyetes and liver (Dąbek *et al*., 2005). Over 60% of enteral AKG passes through the intestine in different forms and is not oxidized to the degree of 100% as glutamine and glutamate (Junghans *et al*., 2006). In the enterocytes, AKG is converted into proline, leucine and other amino acids (Lambert *et al*., 2006). Moreover, enteric feeding of AKG supplements can significantly increase circulating plasma levels of such hormones as insulin, growth hormone and insulin like growth factor-1 (IGF-1) (Colomb *et al*., 2004; Cynober, 2004; Son *et al*., 2007) and all derivatives of AKG (e.g. glutamine or glutamate) are immediately converted to CO_2 during their passage across the gut epithelium (Harrison and Pierzynowski, 2008). Precisely because AKG play crucial role in cellular energy metabolism and participate in a variety of metabolic pathways, in this review,

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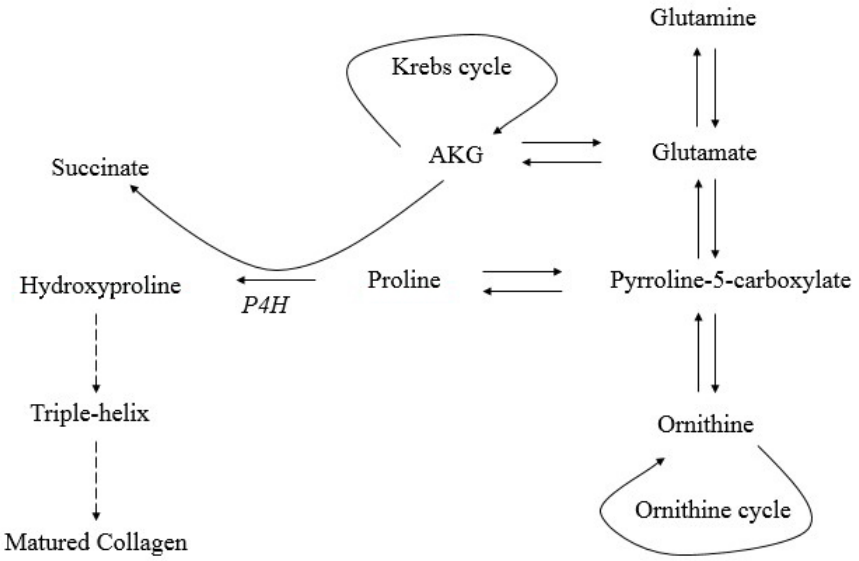

Fig. 1. Mechanism of AKG in collagen production.

we will summarize generally the advances in AKG research field to promote the understanding of AKG and calling for more research focus on AKG.

PHYSIOLOGICAL FUNCTIONS

AKG can modulate protein synthesis and bone development

In the cellular metabolism, AKG provides an important source of glutamine and glutamate that stimulates protein synthesis, inhibits protein degradation in muscle, and constitutes an important metabolic fuel for cells of the gastrointestinal tract (Hixt and Müller, 1996; Jones *et al*., 1999). Glutamine is an energy source for all types of cells in the organism constituting more than 60% of the total amino acid pool, so AKG as a precursor of glutamine, is a main source of energy for intestinal cells and a preferred substrate for both enterocytes and other rapidly dividing cells. In addition, glutamate, released from nerve fibers in bone tissue, is synthesized by the reductive amination of AKG in peri-vein hepatocytes (Stoll *et al*., 1991) and can give rise to an increase in proline synthesis, which plays a central role in the synthesis of collagen (Kristensen *et al*., 2002). In the liver, glutamine serves as a precursor for ureagenesis, gluconeogenesis and acute phase protein synthesis (Espat *et al*., 1996; Alpers, 2006), plays an important role in the inter-organ flow of nitrogen and carbon. Glutamine has traditionally been considered to be a non-essential amino acid in health, but in catabolic states and stress, it is an essential fuel source for cells of the gastrointestinal tract, rapidly dividing leucocytes and macrophages in the immune system and can be rapidly depleted despite the significant release from muscle tissue (Śliwa *et al*., 2009). Otherwise, it was also shown that AKG can improve absorption of Fe^{2+}. Thus, AKG and its derivatives can play a role as a Fe^{2+} absorption enhancer both in rapidly growing animals and humans with Fe^{2+} insufficiency (Dąbek *et al*., 2005). Furthermore, AKG, ascorbate and Fe^{2+} steer hydroxylation of peptide-bound proline to hydroxyproline via prolyl hydrolase, increasing the conversion of pro-collagen to collagen and bone matrix formation (Tocaj *et al*., 2003). Therefore, AKG is an important source of amino acids for collagen synthesis in the cell and organism.

It has been demonstrated that AKG is involved in collagen metabolism through a variety of mechanisms. The main mechanism is presented in Fig. 1. First, AKG is a cofactor of prolyl-4-hydroxylase (P4H). P4H is located within the endoplasmic reticulum (ER), and catalyze the formation of 4-hydroxyproline, which is crucial for the formation of the collagen triple helix. Incomplete hydroxylation of proline residues within the repeated amino acid motif: any amino acid-proline-glycine (X-Pro-Gly), results in incomplete formation of the collagen triple helix. Incorrectly folded triple helices are not secreted into cytoplasm, and are subsequently degraded in the ER (Lamande and Bateman, 1999; Myllyharju, 2003). Second, AKG contributes to facilitate collagen synthesis by increasing the pool of proline residues via glutamate (Panosyan *et al*., 2004; Wu *et al*., 2004; Dakshayani and Subramanian, 2006; Lambert *et al*., 2006; Rani *et al*., 2012; Korkmaz *et al*., 2007; Son *et al*., 2007) and about 25% of the dietary AKG is converted to proline in the enterocytes (Kristensen *et al*., 2002). Proline is a primary substrate for collagen synthesis, and plays a central role in collagen metabolism. As seen in Fig. 1, proline is formed through the conversion of pyrroline 5-carboxylate (P5C), an intermediate in the inter-conversion of proline, ornithine and glutamate. Recently, it was reported that in addition to being a source of proline residues through the P5C-pathway, P5C activates collagen production through the activation of prolidase, a key enzyme in proline recyling (Son *et al*., 2007). This is a significant finding, because the P5C-pathway is a minor contributor to the proline pool during collagen synthesis; the major source of proline is through recycling of proline from collagen degradation products (Isemura *et al*., 1979; Myara *et al*., 1984; Bissonnette *et al*., 1993; Palka and Phang, 1997; Karna *et al*., 2013). In this regard, AKG, which is a precursor of

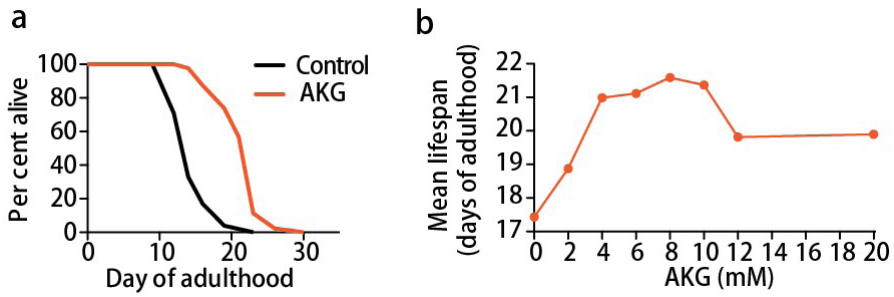

Fig. 2. AKG extends the adult lifespan of *C. elegans*. (A) AKG extends the lifespan of adult worms. (B) Dose-response curve of the AKG effect on longevity.

P5C, also has a close relationship to proline metabolism in the cell and organism. In a study performed in growing pigs, it was displayed that enteral AKG administration increased the level of proline in the portal and arterial blood by 45% and 20%, respectively, when compared to animals that were not given AKG. Through improved proline and hydroxyproline formation, enteral AKG is believed to enhance bone tissue formation (Bellon *et al*., 1995; Kristensen *et al*., 2002).

Another mechanisms of AKG influence on bone tissue results from its impact on the endocrine system of the organism. Glutamine and glutamate is transformed in ornithine and then to arginine (Pierzynowski and Sjodin, 1998). Both ornithine and arginine stimulate the secretion of growth hormone (GH) and insulin-like growth factor I (IGF-I) (Harrison *et al*., 2004; Fayh *et al*., 2007). The osteotropic effect of functional axis GH-IGF-I is widely known and well described (Giustina *et al*., 2008; Tritos and Biller, 2009). AKG may also affect bone structure by the interaction of glutamate-glutamate receptors (GluR). The presence of GluR has been confirmed on osteoblasts (Gu *et al*., 2002) and osteoclasts (Mentaverri *et al*., 2003), whereas Genever *et al* (Spencer *et al*., 2007) reported its significance in bone tissue metabolism. Additionally, there is a preliminary evidence to show that dietary AKG counteracts the bone losses in rats with experimental osteopenia induced by ovariectomy (Bieńko *et al*., 2002; Radzki *et al*., 2002) and fundectomy (Dobrowolski *et al*., 2008). Although we can infer the importance of AKG in collagen metabolism based on these studies, the direct effects of AKG on collagen production have yet to be reported.

AKG can stabilize immune system homeostasis

AKG is also called the immune nutrient factor and it play an important role in the general immune metabolism (Abcouwer, 2000; Ziegler and Daignault, 2000; Yeh *et al*., 2004). It is already known that AKG is an important source of glutamine and glutamate, is defined as glutamine homologue and derivative (Pesty *et al*., 1997; Tapiero *et al*., 2002). Glutamine is an important fuel for lymphocytes and macrophages (Parry-Billings *et al*., 1990). Macrophages and neutrophils are involved in the early, non-specific host-defence responses and play an important role in the pathophysiology and/or protection against sepsis (Sawyer *et al*., 1989; Zimmerman and Ringer, 1992). Previous reports showed that during inflammatory states such as sepsis and injury, the consumption of glutamine by circulating and immune cells increases (Ashkanazi *et al*., 1980; Roth *et al*., 1982; Hammarqvist *et al*., 1989). Studies have revealed that supplemental glutamine augments the in vitro bactericidal activity of neutrophils in burned or postoperative patients (Ogle *et al*., 1994; Furukawa *et al*., 2000). Parry-Billings *et al*. (1990) (Parry-Billings *et al*., 1990) reported that depressed glutamine concentrations were associated with reduced phagocytosis by murine peritoneal macrophages. The study by Gianotti *et al*. (1995) (Gianotti *et al*., 1995) showed that oral glutamine supplementation decreases bacterial translocation in experimental gut-origin sepsis. Thus, AKG as glutamine homologue has immuno-enhancing properties, can maintain a gut barrier, increase immune cells and the activity of neutrophils and phagocytosis, reduce bacterial translocation in vivo (Le Boucher and Cynober, 1997; Danbolt, 2001; MacFie and McNaught, 2002; Salvalaggio and Campos, 2002).

AKG can modulate aging

A recent study (Chin *et al*., 2014) shows that AKG can extend the lifespan of adult *Caenorhabditis elegans* by inhibiting ATP synthase and TOR. They discovered that the tricarboxylic acid cycle intermediate AKG delays ageing and extends the lifespan of *C. elegans* by ~ 50% (Fig. 2A) with a concentration-dependent manner of 8 mM AKG producing the maximal lifespan extension in wild-type N2 worms (Fig. 2B). Chin *et al* (Chin *et al*., 2014) also demonstrated that AKG not only extends lifespan, but also delays age-related phenotypes, such as the decline in rapid, coordinated body movement. In this study, it reported that AKG has greater potential values in aging. Thus, we would like to generally describe the mechanism how AKG inhibits ATP synthase and TOR to extend the lifespan in the organisms.

Mitochondrial ATP synthase is a significant ubiquitous enzyme in energy metabolism of virtually all living cells (Abrahams *et al*., 1994; Boyer, 1997). It is a membrane-bound rotary motor enzyme that is a key energy carrier for cellular energy metabolism. Chin *et al* (Chin *et al*., 2014) provided evidence that the lifespan increase by AKG requires ATP synthase subunit β and is dependent on target of rapamycin (TOR) downstream. They used a small-molecule target identification strategy termed drug affinity responsive target stability (DARTS) (Lomenick *et al*., 2009), found the ATP synthase subunit β is a novel binding protein of AKG. They discovered AKG inhibits ATP synthase, leads to reduced ATP content, decreased oxygen consumption, and increased autophagy in both *C. elegans* and mammalian cells, similar to ATP synthase 2 (ATP-2) knockdown. Together, the direct binding of ATP-2 by AKG, the related enzymatic inhibition, reduction in ATP levels and oxy-

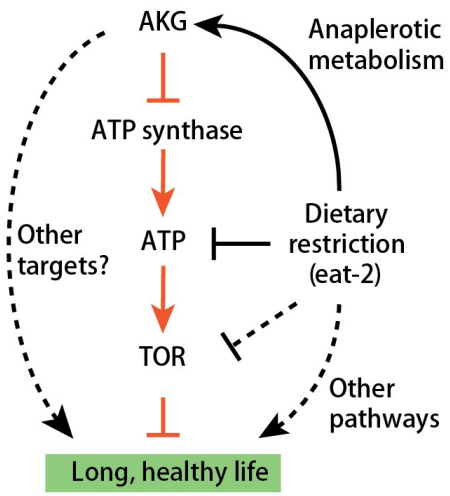

Fig. 3. Model of α-KG-mediated longevity.

gen consumption, lifespan analysis, and other similarities to ATP-2 knockdown, they inferred AKG probably extends lifespan primarily by targeting ATP-2. In addition, previous studies also has shown that complete loss of mitochondrial function is detrimental, but partial suppression of the electron transport chain has been demonstrated to extend *C. elegans* lifespan (Tsang *et al*., 2001; Dillin *et al*., 2002; Lee *et al*., 2003; Curran and Ruvkun, 2007). Thus, AKG can inhibit the ATP synthase, so to achieve the effect of prolonging life is completely possible.

Target of rapamycin (TOR), belongs to a conserved group of serine/threonine kinases from the phosphatidylinositol kinase-related kinase (PIKK) family, regulates growth and metabolism in all eukaryotic cells. Previous researches have demonstrated that inhibition of TOR activity can delay the aging process, as evidenced by increased life span in yeast (Kaeberlein *et al*., 2007), worms (Vellai *et al*., 2003; Hansen *et al*., 2007), flies (Kapahi *et al*., 2004; Luong *et al*., 2006), and mice (Selman *et al*., 2009) with mutations in TOR pathway components. AKG does not interact with TOR directly and mainly decreases TOR pathway activity through the inhibition of ATP synthase (Fig. 3). AKG longevity partially depends on AMPK and FoxO (Urban *et al*., 2007). The AMP-activated protein kinase (AMPK) is an evolutionarily conserved cellular energy sensor with key roles in aging and lifespan (Hardie *et al*., 2012; Huang *et al*., 2013). AMPK is activated when the AMP/ATP ratio is high and subsequently, activated AMPK inhibits TOR signaling by activating phosphorylation of the TOR suppressor TSC2, sequentially adjusting the cell's metabolic program to energy status (Toivonen *et al*., 2007). Fork head box 'Other' (FoxO) proteins, a subgroup of the Fork head transcription factor family, have an pivotal role in mediating the impacts of insulin and growth factors on diverse physiological functions, including cell proliferation, apoptosis and metabolism (Brunet, 2004; Barthel *et al*., 2005; Gross *et al*., 2008; Wang *et al*., 2014; Webb and Brunet, 2014). Consistent with the implicate of TOR in AKG longevity, the FoxO, a transcription factor PHA-4, which is required to extend lifespan in response to reduced TOR signaling (Sheaffer *et al*., 2008), is likewise essential for AKG-induced longevity. In addition, autophagy, which is activated both by TOR inhibition (Wullschleger *et al*., 2006; Stanfel *et al*., 2009) and by dietary restriction (Meléndez *et al*., 2003), is significantly increased in worms treated with AKG. Therefore, AKG treatment and TOR inactivation extend lifespan either through the same pathway (with AKG acting on or upstream of TOR), or through independent mechanisms or parallel pathways that converge on a downstream effector (Chin *et al*., 2014).

Furthermore, physiological increases in AKG levels have been shown in starved yeast and bacteria (Brauer *et al*., 2006), in the liver of starved pigeons (Kaminsky *et al*., 1982), and in humans after physical exercise (Brugnara *et al*., 2012). The biochemical basis for this increase of AKG is explained by starvation based anaplerotic gluconeogenesis, which activates glutamate-linked transaminases in the liver to generate carbon derived from amino acid catabolism. Consistent with this idea, Chin *et al* (Chin *et al*., 2014) observed that AKG levels are elevated in starved *C. elegans* and AKG does not extend the lifespan of dietary-restricted animals. These findings indicated a model in which AKG is a key metabolite mediating lifespan extension by starvation/dietary restriction (Fig. 3). It demonstrated new molecular links between a common metabolite, a universal cellular energy generator and dietary restriction in the regulation of organismal lifespan, thus indicated new strategies for the prevention and treatment of aging and age-related diseases.

THE APPLICATION OF AKG IN ANIMALS

AKG has been given to pigs (Kowalik *et al*., 2005; Andersen *et al*., 2008), turkeys (Tatara *et al*., 2005a; Tatara *et al*., 2005b), rats (Bieńko *et al*., 2002; Radzki *et al*., 2002) and sheep (Harrison *et al*., 2004; Tatara *et al*., 2007) with effects on the skeletal system and protein synthesis. Considering current knowledge of AKG, its metabolites and functions, it can be concluded that improved bone quality may be induced by higher glutamate synthesis and its utilization as signaling molecule in bone metabolism regulation (Stoll *et al*., 1991; Chenu, 2002a; Chenu, 2002b; Taylor, 2002). The other mechanism that may be involved in bone metabolism regulation by AKG is increased collagen formation as the result of higher proline synthesis and its following conversion to hydroxyproline, which was previously introduced (Kristensen *et al*., 2002).

In studies on animals, AKG administration has generated positive effects on skeletal development and homeostasis maintenance (Kowalik *et al*., 2005; Tatara *et al*., 2005a; Tatara *et al*., 2005b). In AKG-treated animals, significant increase of weight, length, bone mineral density, bone mineral content, cross-sectional area, second moment of inertia, mean relative wall thickness, cortical index, maximum elastic strength and ultimate strength of the bones was associated with improved serum concentration of IGF-1 and serum BAP activity when compared to the control group (Śliwa, 2010). Results of long bone analysis in slaughter pigs treated during 21 and 24 days of neonatal life with AKG has shown its positive effects on length, cortical bone mineral density, maximum elastic strength, ultimate strength and Young's modulus that was connected with elevated plasma estrogen level (Andersen *et al*., 2008). In studies on growing turkeys, 14-week long administration with AKG eliminated neurectomy-induced osteopenia of radius increasing its weight, volumetric bone mineral density, the cross-sectional area, second moment of inertia, mean relative wall thickness, maximum elastic strength and ultimate

strength (Tatara *et al*., 2005a). These advantageous effects were combined with higher serum concentration of proline and leucine in comparison to the control group birds (Tatara *et al*., 2005a). In other studies on sheep, two week long neonatal treatment with AKG improved the trabecular bone mineral density, cortical bone mineral density and maximum elastic strength of femur as well as increasing weight, length, cortical bone mineral density, maximum elastic strength and the moments of maximum elastic strength and ultimate strength (Harrison *et al*., 2004; Tatara *et al*., 2007).

The similar influence of AKG administration on bone tissue was also observed in studies performed on humans (Tocaj *et al*., 2003; Fayh *et al*., 2007). It can facilitate muscle protein synthesis in post-operative patients (Wernerman *et al*., 1990), to improve amino acid metabolism in haemo-dialysed patients (Riedel *et al*., 1996), and to accelerate the transport of organic anions in the kidneys (Welborn *et al*., 1998), when AKG was given as a supplement. Use of AKG or calcium-AKG as dietary supplements has mainly been studied on hospitalized adult humans, who are well nourished and have a normal functional metabolism (Pierzynowski *et al*., 2007). In clinical studies on septic, traumatic or surgical patients, AKG has been found to display beneficial effects by improving the body weight gain, nitrogen balance. A recent study has shown the potential usefulness of AKG treatment in preserving bone mass as well as lowering bone turnover in post-menopausal women (Tocaj *et al*., 2003). Results suggest a link between enteral AKG and an increase in oestrogen levels. Some studies have also reported that AKG is an efficient nutritional support in trauma situations, especially after burns (Wernerman *et al*., 1990; Le Boucher *et al*., 1997). Therefore, AKG can be an alternative for elderly patients after trauma and surgery and for people who execute intensive, but the short duration physical effort (Neu *et al*., 1996). It also is known that AKG has a beneficial effect on nitrogen metabolism (Wirén and Permert, 2002) and in reducing toxicity levels of ammonium ions as a protective agent for kidney function in the body (Stoll *et al*., 1991; Welborn *et al*., 1998; Velvizhi *et al*., 2002). In addition, Schlegel *et al* (Schlegel *et al*., 2000) observed that AKG supplementation can limit bacterial dissemination and metabolic changes after injury in rats and thus may be useful in protection of gut mucosa. Therefore, a number of studies have revealed the beneficial effects of AKG in human and animals.

SUMMARY AND FUTURE OUTLOOK

On the whole, the physiological significance of AKG are multi-directional and not all metabolic pathways have been well established. The mechanisms of AKG action on the skeletal system is associated with glutamate receptor activation, bone collagen production via proline and possible anti-catabolic and anabolic effects of 17b-oestradiol (Andersen *et al*., 2008), and is probably multifactorial. In addition, the positive influence of AKG might be expected to improve chest function and internal organ protection of premature and low birthweight newborns (Tatara *et al*., 2007). The present findings may have important clinical implications, motivating for the testing of AKG in prevention and therapy of metabolic bone disorders in human and animals. Therefore, further studies are needed to understand the function of AKG, clarify of the mechanism of AKG and explore the potential application in human society or other fields.

In the aspect of aging, some exciting discoveries indicated that TORC1 is involved in a large number of human diseases, including diabetes, obesity, heart disease, and cancer (Inoki and Guan, 2006; Katewa and Kapahi, 2011). Aging is a common risk factor for these diseases, and it has been revealed that the mechanism of the link between cellular senescence, diseases and organismal aging is via TOR (Kapahi and Zid, 2004; Blagosklonny, 2006). Therefore, inhibition of TOR function by metabolism of AKG indicated that AKG may play an important role in tumor suppress.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

REFERENCES

Abcouwer, S. F. (2000) Effects of glutamine on immune cells. *Nutrition* **16**, 67-69.

Abrahams, J. P., Leslie, A. G., Lutter, R. and Walker, J. E. (1994) Structure at 2.8 A resolution of F1-ATPase from bovine heart mitochondria. *Nature* **370**, 621-628.

Alpers, D. H. (2006) Glutamine: do the data support the cause for glutamine supplementation in humans? *Gastroenterology* **130**, S106-S116.

Andersen, N. K., Tatara, M. R., Krupski, W., Majcher, P. and Harrison, A. P. (2008) The long-term effect of alpha-ketoglutarate, given early in postnatal life, on both growth and various bone parameters in pigs. *J. Anim. Physiol. Anim. Nutr.* **92**, 519-528.

Ashkanazi, J., Carpertier, Y. and Michelsen, C. (1980) Muscle and plasma amino acids following injury. *Ann. Surg.* **192**, 78-85.

Barthel, A., Schmoll, D. and Unterman, T. G. (2005) FoxO proteins in insulin action and metabolism. *Trends Endocrinol. Metab.* **16**, 183-189.

Bellon, G., Chaqour, B., Wegrowski, Y., Monboisse, J. C. and Borel, J. P. (1995) Glutamine increases collagen gene transcription in cultured human fibroblasts. *Biochim. Biophys. Acta* **1268**, 311-323.

Bieńko, M., Radzki, R., Puzio, I., Filip, R., Pierzynowski, S. and Studziński, T. (2002) The influence of alpha-ketoglutarate (AKG) on mineralization of femur in rats with established osteopenia. *Acta Orthop. Scand.* **73**, 52.

Bissonnette, R., Friedmann, D., Giroux, J. M., Dolenga, M., Hechtman, P., Der Kaloustian, V. M. and Dubuc, R. (1993) Prolidase deficiency: a multisystemic hereditary disorder. *J. Am. Acad. Dermatol.* **29**, 818-821.

Blagosklonny, M. V. (2006) Aging and immortality: quasi-programmed senescence and its pharmacologic inhibition. *Cell Cycle* **5**, 2087-2102.

Boyer, P. D. (1997) The ATP synthase-a splendid molecular machine. *Annu. Rev. Biochem.* **66**, 717-749.

Brauer, M. J., Yuan, J., Bennett, B. D., Lu, W., Kimball, E., Botstein, D. and Rabinowitz, J. D. (2006) Conservation of the metabolomic response to starvation across two divergent microbes. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 19302-19307.

Brugnara, L., Vinaixa, M., Murillo, S., Samino, S., Rodriguez, M. A.,

Beltran, A., Lerin, C., Davison, G., Correig, X. and Novials, A. (2012) Metabolomics approach for analyzing the effects of exercise in subjects with type 1 diabetes mellitus. *PloS one* **7**, e40600.
Brunet, A. (2004) [The multiple roles of FOXO transcription factors]. *Med. Sci.* **20**, 856-859.
Chenu, C. (2002a) Glutamatergic innervation in bone. *Microsc. Res. Tech.* **58**, 70-76.
Chenu, C. (2002b) Glutamatergic regulation of bone remodeling. *J. Musculoskelet Neuronal Interact.* **2**, 282-284.
Chin, R. M., Fu, X., Pai, M. Y., Vergnes, L., Hwang, H., Deng, G., Diep, S., Lomenick, B., Meli, V. S. and Monsalve, G. C. (2014) The metabolite α-ketoglutarate extends lifespan by inhibiting ATP synthase and TOR. *Nature* **510**, 397-401.
Colomb, V., Dabbas, M., Goulet, O., Talbotec, C., Corriol, O. and Ricour, C. (2004) Prepubertal growth in children with long-term parenteral nutrition. *Horm. Res. Paediatr.* **58**, 2-6.
Curran, S. P. and Ruvkun, G. (2007) Lifespan regulation by evolutionarily conserved genes essential for viability. *PLoS Genet.* **3**, e56.
Cynober, L. (2004) Ornithine α-ketoglutarate as a potent precursor of arginine and nitric oxide: a new job for an old friend. *J. Nutr.* **134**, 2858S-2862S.
Dąbek, M., Kruszewska, D., Filip, R., Hotowy, A., Pierzynowski, Ł., Wojtasz-Pająk, A., Szymanczyk, S., Valverde Piedra, J., Werpachowska, E. and Pierzynowski, S. (2005) α-Ketoglutarate (AKG) absorption from pig intestine and plasma pharmacokinetics. *J. Anim. Physiol. Anim. Nutr.* **89**, 419-426.
Dakshayani, K. and Subramanian, P. (2006) α-ketoglutarate modulates the circadian patterns of lipid peroxidation and antioxidant status during N-nitrosodiethylamine-induced hepatocarcinogenesis in rats. *J. Med. Food* **9**, 90-97.
Danbolt, N. C. (2001) Glutamate uptake. *Prog. Neurobiol.* **65**, 1-105.
Dillin, A., Hsu, A. L., Arantes-Oliveira, N., Lehrer-Graiwer, J., Hsin, H., Fraser, A. G., Kamath, R. S., Ahringer, J. and Kenyon, C. (2002) Rates of behavior and aging specified by mitochondrial function during development. *Science* **298**, 2398-2401.
Dobrowolski, P. J., Piersiak, T., Surve, V. V., Kruszewska, D., Gawron, A., Pacuska, P., Håkanson, R. and Pierzynowski, S. G. (2008) Dietary α-ketoglutarate reduces gastrectomy-evoked loss of calvaria and trabecular bone in female rats. *Scand J. Gastroenterol.* **43**, 551-558.
Espat, N. J., Watkins, K. T., Lind, D. S., Weis, J. K., Copeland, E. M. and Souba, W. W. (1996) Dietary modulation of amino acid transport in rat and human liver. *J. Surg. Res.* **63**, 263-268.
Fayh, A. P., Friedman, R., Sapata, K. B. and Oliveira, A. R. (2007) [Effect of L-arginine supplementation on secretion of human growth hormone and insulin-like growth factor in adults]. *Arq. Bras. Endocrinol. Metabol.* **51**, 587-592.
Furukawa, S., Saito, H., Inoue, T., Matsuda, T., Fukatsu, K., Han, I., Ikeda, S. and Hidemura, A. (2000) Supplemental glutamine augments phagocytosis and reactive oxygen intermediate production by neutrophils and monocytes from postoperative patients in vitro. *Nutrition* **16**, 323-329.
Gianotti, L., Alexander, J. W., Gennari, R., Pyles, T. and Babcock, G. F. (1995) Oral glutamine decreases bacterial translocation and improves survival in experimental gut-origin sepsis. *J. Parenter. Enteral Nutr.* **19**, 69-74.
Giustina, A., Mazziotti, G. and Canalis, E. (2008) Growth hormone, insulin-like growth factors, and the skeleton. *Endocr. Rev.* **29**, 535-559.
Gross, D. N., van den Heuvel, A. P. and Birnbaum, M. J. (2008) The role of FoxO in the regulation of metabolism. *Oncogene* **27**, 2320-2336.
Gu, Y., Genever, P., Skerry, T. and Publicover, S. (2002) The NMDA type glutamate receptors expressed by primary rat osteoblasts have the same electrophysiological characteristics as neuronal receptors. *Calcif. Tissue Int.* **70**, 194-203.
Hammarqvist, F., Wernerman, J., Ali, R., von der Decken, A. and Vinnars, E. (1989) Addition of glutamine to total parenteral nutrition after elective abdominal surgery spares free glutamine in muscle, counteracts the fall in muscle protein synthesis, and improves nitrogen balance. *Ann. Surg.* **209**, 455-461.
Hansen, M., Taubert, S., Crawford, D., Libina, N., Lee, S. J. and Kenyon, C. (2007) Lifespan extension by conditions that inhibit translation in Caenorhabditis elegans. *Aging Cell* **6**, 95-110.
Hardie, D. G., Ross, F. A. and Hawley, S. A. (2012) AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nat. Rev. Mol. Cell Biol.* **13**, 251-262.
Harrison, A. P. and Pierzynowski, S. (2008) Biological effects of 2-oxoglutarate with particular emphasis on the regulation of protein, mineral and lipid absorption/metabolism, muscle performance, kidney function, bone formation and cancerogenesis, all viewed from a healthy ageing perspective state of the art-review article. *J. Physiol. Pharmacol.* **59**, 91-106.
Harrison, A. P., Tygesen, M. P., Sawa-Wojtanowicz, B., Husted, S. and Tatara, M. (2004) α-Ketoglutarate treatment early in postnatal life improves bone density in lambs at slaughter. *Bone* **35**, 204-209.
Hixt, U. and Müller, H. (1996) L-alanyl-glutamine-a glutamine dipeptide for paraenteral nutrition. *Environ. Health Perspect.* **2**, 72-76.
Huang, X., Liu, J., Withers, B. R., Samide, A. J., Leggas, M. and Dickson, R. C. (2013) Reducing signs of aging and increasing lifespan by drug synergy. *Aging Cell* **12**, 652-660.
Inoki, K. and Guan, K.-L. (2006) Complexity of the TOR signaling network. *Trends Cell Biol.* **16**, 206-212.
Isemura, M., Hanyu, T., Gejyo, F., Nakazawa, R., Igarashi, R., Matsuo, S., Ikeda, K. and Sato, Y. (1979) Prolidase deficiency with imidodipeptiduria. A familial case with and without clinical symptoms. *Clin. Chim. Acta* **93**, 401-407.
Jones, C., Allan Palmer, T. and Griffiths, R. (1999) Randomized clinical outcome study of critically ill patients given glutamine-supplemented enteral nutrition. *Nutrition* **15**, 108-115.
Junghans, P., Derno, M., Pierzynowski, S., Hennig, U., Eberhard Rudolph, P. and Souffrant, W. B. (2006) Intraduodenal infusion of α-ketoglutarate decreases whole body energy expenditure in growing pigs. *Clin. Nutr.* **25**, 489-496.
Kaeberlein, M., Burtner, C. R. and Kennedy, B. K. (2007) Recent developments in yeast aging. *PLoS Genet.* **3**, e84.
Kaminsky, Y. G., Kosenko, E. A. and Kondrashova, M. N. (1982) Metabolites of citric acid cycle, carbohydrate and phosphorus metabolism, and related reactions, redox and phosphorylating states of hepatic tissue, liver mitochondria and cytosol of the pigeon, under normal feeding and natural nocturnal fasting conditions. *Comp. Biochem. Physiol. B.* **73**, 957-963.
Kapahi, P. and Zid, B. (2004) TOR pathway: linking nutrient sensing to life span. *Sci. Aging Knowledge Environ.* **2004**, pe34.
Kapahi, P., Zid, B. M., Harper, T., Koslover, D., Sapin, V. and Benzer, S. (2004) Regulation of lifespan in drosophila by modulation of genes in the TOR signaling pathway. *Curr. Biol.* **14**, 885-890.
Karna, E., Szoka, L. and Palka, J. A. (2013) The mechanism of hydralazine-induced collagen biosynthesis in cultured fibroblasts. *Naunyn Schmiedebergs Arch.Pharmacol.* **386**, 303-309.
Katewa, S. D. and Kapahi, P. (2011) Role of TOR signaling in aging and related biological processes in Drosophila melanogaster. *Exp Gerontol.* **46**, 382-390.
Korkmaz, A., Yurdakok, M., Yigit, S. and Tekinalp, G. (2007) Long-term enteral glutamine supplementation in very low birth weight infants: effects on growth parameters. *Turk J. Pediatr.* **49**, 37-44.
Kowalik, S., Śliwa, E., Tatara, M. R., Krupski, W., Majcher, P. and Studziński, T. (2005) Influence of alpha-ketoglutarate on mineral density and geometrical and mechanical parameters of femora during postnatal life in piglets. *Bull. Vet. Inst. Pulawy* **49**, 107-111.
Kristensen, N. B., Jungvid, H., Fernández, J. A. and Pierzynowski, S. (2002) Absorption and metabolism of α-ketoglutarate in growing pigs. *J. Anim. Physiol. Anim. Nutr.* **86**, 239-245.
Lamande, S. R. and Bateman, J. F. (1999) Procollagen folding and assembly: the role of endoplasmic reticulum enzymes and molecular chaperones. *Semin. Cell Dev. Biol.* **10**, 455-464.
Lambert, B. D., Filip, R., Stoll, B., Junghans, P., Derno, M., Hennig, U., Souffrant, W. B., Pierzynowski, S. and Burrin, D. G. (2006) First-pass metabolism limits the intestinal absorption of enteral α-ketoglutarate in young pigs. *J. Nutr.* **136**, 2779-2784.
Le Boucher, J., Coudray-Lucas, C., Lasnier, E., Jardel, A., Ekindjian, O. G. and Cynober, L. A. (1997) Enteral administration of ornithine alpha-ketoglutarate or arginine alpha-ketoglutarate: a comparative study of their effects on glutamine pools in burn-injured rats. *Crit.*

Care Med. **25**, 293-298.
Le Boucher, J. and Cynober, L. A. (1997) Ornithine alpha-ketoglutarate: the puzzle. *Nutrition* **14**, 870-873.
Lee, S. S., Lee, R. Y., Fraser, A. G., Kamath, R. S., Ahringer, J. and Ruvkun, G. (2003) A systematic RNAi screen identifies a critical role for mitochondria in *C. elegans* longevity. *Nat. Genet.* **33**, 40-48.
Lomenick, B., Hao, R., Jonai, N., Chin, R. M., Aghajan, M., Warburton, S., Wang, J., Wu, R. P., Gomez, F. and Loo, J. A. (2009) Target identification using drug affinity responsive target stability (DARTS) *Proc. Natl. Acad. Sci. U.S.A.* **106**, 21984-21989.
Luong, N., Davies, C. R., Wessells, R. J., Graham, S. M., King, M. T., Veech, R., Bodmer, R. and Oldham, S. M. (2006) Activated FOXO-mediated insulin resistance is blocked by reduction of TOR activity. *Cell Metab.* **4**, 133-142.
MacFie, J. and McNaught, C. (2002) Glutamine and gut barrier function. *Nutrition* **18**, 433-434.
Meléndez, A., Tallóczy, Z., Seaman, M., Eskelinen, E. L., Hall, D. H. and Levine, B. (2003) Autophagy genes are essential for dauer development and life-span extension in *C. elegans*. *Science* **301**, 1387-1391.
Mentaverri, R., Kamel, S., Wattel, A., Prouillet, C., Sevenet, N., Petit, J., Tordjmann, T. and Brazier, M. (2003) Regulation of bone resorption and osteoclast survival by nitric oxide: Possible involvement of NMDA-receptor. *J. Cell. Biochem.* **88**, 1145-1156.
Myara, I., Charpentier, C. and Lemonnier, A. (1984) Prolidase and prolidase deficiency. *Life Sci.* **34**, 1985-1998.
Myllyharju, J. (2003) Prolyl 4-hydroxylases, the key enzymes of collagen biosynthesis. *Matrix Biol.* **22**, 15-24.
Neu, J., Shenoy, V. and Chakrabarti, R. (1996) Glutamine nutrition and metabolism: where do we go from here? *FASEB J.* **10**, 829-837.
Ogle, C. K., Ogle, J. D., Mao, J. X., Simon, J., Noel, J. G., Li, B.-G. and Alexander, J. W. (1994) Effect of glutamine on phagocytosis and bacterial killing by normal and pediatric burn patient neutrophils. *J. Parenter. Enteral Nutr.* **18**, 128-133.
Palka, J. A. and Phang, J. M. (1997) Prolidase activity in fibroblasts is regulated by interaction of extracellular matrix with cell surface integrin receptors. *J. Cell. Biochem.* **67**, 166-175.
Panosyan, E. H., Grigoryan, R. S., Avramis, I. A., Seibel, N. L., Gaynon, P. S., Siegel, S. E., Fingert, H. J. and AVRAMIS, V. I. (2004) Deamination of glutamine is a prerequisite for optimal asparagine deamination by asparaginases in vivo (CCG-1961) *Anticancer Res.* **24**, 1121-1126.
Parry-Billings, M., Calder, P., Newsholme, E. and Evans, J. (1990) Does glutamine contribute to immunosuppression after major burns? *Lancet* **336**, 523-525.
Pesty, F. H., Sultan, F. and Braun, B. (1997) Glutamine homologues and derivatives: A limiting factor in current artificial nutrition? *Nutrition* **13**, 575-577.
Pierzynowski, S. and Sjodin, A. (1998) Perspectives of glutamine and its derivatives as feed additives for farm animals. *J. Anim. Feed Sci.* **7**, 79-91.
Pierzynowski, S. G., Filip, R. and Harrison, A. (2007) Effect of feed supplementation with alpha-ketoglutarate, combined with vitamin B~ 6 or C, on the performance and haemoglobin and amino acid levels in growing rats. *Bull. Vet. Inst. Pulawy* **51**, 289-296.
Radzki, R., Bienko, M., Puzio, I., Filip, R., Pierzynowski, S. and Studzinski, T. (2002) The effect of alpha-ketoglutarate (AKG) on mineralization of femur in ovariectomized rats. *Acta Orthop. Scand.* **73**, 52.
Rani, R. S., Leela, A. C. and Rao, G. N. (2012) Effect of dielectric constant on protonation equilibria of l-aspartic acid and ethylenediamine in 1, 2-propanediol-water mixtures. *P. Natl A Sci. India A.* **82**, 313-316.
Riedel, E., Nundel, M. and Hampl, H. (1996) alpha-Ketoglutarate application in hemodialysis patients improves amino acid metabolism. *Nephron* **74**, 261-265.
Roth, E., Funovics, J., Mühlbacher, F., Schemper, M., Mauritz, W., Sporn, P. and Fritsch, A. (1982) Metabolic disorders in severe abdominal sepsis: glutamine deficiency in skeletal muscle. *Clin. Nutr.* **1**, 25-41.
Salvalaggio, P. R. and Campos, A. C. (2002) Bacterial translocation and glutamine. *Nutrition* **18**, 435-437.
Sawyer, D. W., Donowitz, G. R. and Mandell, G. L. (1989) Polymorphonuclear neutrophils: an effective antimicrobial force. **11**, S1532-S1544.
Schlegel, L., Coudray-Lucas, C., Barbut, F., Le Boucher, J., Jardel, A., Zarrabian, S. and Cynober, L. (2000) Bacterial dissemination and metabolic changes in rats induced by endotoxemia following intestinal E. coli overgrowth are reduced by ornithine α-ketoglutarate administration. *J. Nutr.* **130**, 2897-2902.
Selman, C., Tullet, J. M., Wieser, D., Irvine, E., Lingard, S. J., Choudhury, A. I., Claret, M., Al-Qassab, H., Carmignac, D. and Ramadani, F. (2009) Ribosomal protein S6 kinase 1 signaling regulates mammalian life span. *Science* **326**, 140-144.
Sheaffer, K. L., Updike, D. L. and Mango, S. E. (2008) The target of rapamycin pathway antagonizes pha-4/FoxA to control development and aging. *Curr. Biol.* **18**, 1355-1364.
Śliwa, E. (2010) 2-Oxoglutaric acid administration diminishes fundectomy-induced osteopenia in pigs. *J. Anim. Physiol. Anim. Nutr.* **94**, e86-e95.
Śliwa, E., Dobrowolski, P., Tatara, M., Piersiak, T., Siwicki, A., Rokita, E. and Pierzynowski, S. (2009) Alpha-ketoglutarate protects the liver of piglets exposed during prenatal life to chronic excess of dexamethasone from metabolic and structural changes. *J. Anim. Physiol. Anim. Nutr.* **93**, 192-202.
Son, E. D., Choi, G. H., Kim, H., Lee, B., Chang, I. S. and Hwang, J. S. (2007) Alpha-ketoglutarate stimulates procollagen production in cultured human dermal fibroblasts, and decreases UVB-induced wrinkle formation following topical application on the dorsal skin of hairless mice. *Biol. Pharm. Bull.* **30**, 1395-1399.
Spencer, G. J., McGrath, C. J. and Genever, P. G. (2007) Current perspectives on NMDA-type glutamate signalling in bone. *Int. J. Biochem. Cell Biol.* **39**, 1089-1104.
Stanfel, M. N., Shamieh, L. S., Kaeberlein, M. and Kennedy, B. K. (2009) The TOR pathway comes of age. *Biochim. Biophys. Acta* **1790**, 1067-1074.
Stoll, B., McNelly, S., Buscher, H. P. and Häussinger, D. (1991) Functional hepatocyte heterogeneity in glutamate, aspartate and α-ketoglutarate uptake: A histoautoradiographical study. *Hepatology* **13**, 247-253.
Tapiero, H., Mathe, G., Couvreur, P. and Tew, K. (2002) II. Glutamine and glutamate. *Biomed. Pharmacother.* **56**, 446-457.
Tatara, M., Brodzki, A., Krupski, W., Silmanowicz, P., Majcher, P., Pierzynowski, S. and Studziński, T. (2005a) Effects of alpha-ketoglutarate on bone homeostasis and plasma amino acids in turkeys. *Poult Sci.* **84**, 1604-1609.
Tatara, M., Tygesen, M. P., Sawa-Wojtanowicz, B., Krupski, W., Majcher, P. and Harrison, A. P. (2007) Bone development: The effect of short-term alpha-ketoglutarate administration on long-term mechanical properties of ribs in ram lambs. *Small Ruminant Res.* **67**, 179-183.
Tatara, M. R., Silmanowicz, P., Majcher, P., Krupski, W. and Studziński, T. (2005b) Influence of alpha-ketoglutarate on cortical bone atrophy after denervation of the humerus in turkey. *Bull. Vet. Inst. Pulawy* **49**, 113-116.
Taylor, A. (2002) Osteoblastic glutamate receptor function regulates bone formation and resorption. *J. Musculoskelet. Neuronal Interact.* **2**, 285-290.
Tocaj, A., Filip, R., Lindergard, B., Wernerman, J., Studzinski, T., Ohman, K. and Pierzynowski, S. (2003) Alpha-ketoglutarate (AKG) inhibit osteoporosis development in postmenopausal women. In Journal of Bone and Mineral Research, Vol. 18, pp. S267-S267. Amer Soc Bone & Mineral Res 2025 M St, Nw, Ste 800, Washington, Dc 20036-3309 USA.
Toivonen, J. M., Walker, G. A., Martinez-Diaz, P., Bjedov, I., Driege, Y., Jacobs, H. T., Gems, D. and Partridge, L. (2007) No influence of Indy on lifespan in Drosophila after correction for genetic and cytoplasmic background effects. *PLoS Genet.* **3**, e95.
Tritos, N. A. and Biller, B. M. (2009) Growth hormone and bone. *Curr. Opin. Endocrinol. Diabetes Obes.* **16**, 415-422.
Tsang, W. Y., Sayles, L. C., Grad, L. I., Pilgrim, D. B. and Lemire, B. D. (2001) Mitochondrial respiratory chain deficiency in *Caenorhabditis elegans* results in developmental arrest and increased life span. *J. Biol. Chem.* **276**, 32240-32246.
Urban, J., Soulard, A., Huber, A., Lippman, S., Mukhopadhyay, D., De-

loche, O., Wanke, V., Anrather, D., Ammerer, G. and Riezman, H. (2007) Sch9 Is a Major Target of TORC1 in *Saccharomyces cerevisiae*. *Mol. Cell* **26**, 663-674.

Vellai, T., Takacs-Vellai, K., Zhang, Y., Kovacs, A. L., Orosz, L. and Müller, F. (2003) Influence of TOR kinase on lifespan in *C. elegans*. *Nature* **426**, 620.

Velvizhi, S., Dakshayani, K. B. and Subramanian, P. (2002) Effects of α-ketoglutarate on antioxidants and lipid peroxidation products in rats treated with ammonium acetate. *Nutrition* **18**, 747-750.

Wang, Y., Zhou, Y. and Graves, D. T. (2014) FOXO transcription factors: their clinical significance and regulation. *Biomed. Res. Int.* **2014**, 925350.

Webb, A. E. and Brunet, A. (2014) FOXO transcription factors: key regulators of cellular quality control. *Trends Biochem. Sci.* **39**, 159-169.

Welborn, J. R., Shpun, S., Dantzler, W. H. and Wright, S. H. (1998) Effect of α-ketoglutarate on organic anion transport in single rabbit renal proximal tubules. *Am. J. Physiol. Renal Physiol.* **274**, F165-F174.

Wernerman, J., Hammarqvist, F. and Vinnars, E. (1990) α-Ketoglutarate and postoperative muscle catabolism. *Lancet* **335**, 701-703.

Wirén, M. and Permert, J. (2002) α-ketoglutarate-supplemented enteral nutrition: effects on postoperative nitrogen balance and muscle catabolism. *Nutrition* **18**, 725-728.

Wu, G., Fang, Y. Z., Yang, S., Lupton, J. R. and Turner, N. D. (2004) Glutathione metabolism and its implications for health. *J. Nutr.* **134**, 489-492.

Wullschleger, S., Loewith, R. and Hall, M. N. (2006) TOR signaling in growth and metabolism. *Cell* **124**, 471-484.

Yeh, S. L., Lai, Y. N., Shang, H. F., Lin, M. T. and Chen, W. J. (2004) Effects of glutamine supplementation on innate immune response in rats with gut-derived sepsis. *Br. J. Nutr.* **91**, 423-430.

Ziegler, T. R. and Daignault, N. M. (2000) Glutamine regulation of human immune cell function. *Nutrition* **16**, 458-459.

Zimmerman, J. J. and Ringer, T. V. (1992) Inflammatory host responses in sepsis. *Crit. Care Clin.* **8**, 163-189.