Nutritional interventions for patients with alkaptonuria: A minireview

Richard Imrich 1,2, Andrea Zatkova 1, Olga Lukacova 2, Jana Sedlakova 2, Elizabeth Zanova 2, Miroslav Vlcek 1,2, Adela Penesova 1,2, Zofia Radikova 1,2, Andrea Havranova 1,2, Lakshminarayan Ranganath 3

1 Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia; 2 National Institute of Rheumatic Diseases, Piestany, Slovakia; 3 Liverpool University Hospitals NHS Foundation Trust, Liverpool, UK
E-mail: ueenmri@savba.sk

Alkaptonuria (AKU, OMIM, No. 203500) is a rare, slow-progressing, irreversible, multisystemic disease resulting from a deficiency of the homogentisate 1,2-dioxygenase enzyme, which leads to the accumulation of homogentisic acid (HGA) and subsequent deposition as pigment in connective tissues called ochronosis. As a result, severe arthropathy of large joints and spondyloarthropathy with frequent fractures, ligament ruptures, and osteoporosis develops in AKU patients. Since 2020, the first-time treatment with nitisinone has become available in the European Union. Nitisinone significantly reduces HGA production and arrests ochronosis in AKU patients. However, blocking of the tyrosine metabolic pathway by the drug leads to tyrosine plasma and tissue concentrations increase. The nitisinone-induced hypertyrosinemia can lead to the development of corneal keratopathy, and once it develops, the treatment needs to be interrupted. A decrease in overall protein intake reduces the risk of the keratopathy during nitisinone-induced hypertyrosinemia in AKU patients. The low-protein diet is not only poorly tolerated by patients, but over longer periods, leads to a severe muscle loss and weight gain due to increased energy intake from carbohydrates and fats. Therefore, the development of novel nutritional approaches is required to prevent the adverse events due to nitisinone-induced hypertyrosinemia and the negative impact on skeletal muscle metabolism in AKU patients.

Key words: alkaptonuria, nitisinone, nutritional intervention

Alkaptonuria (AKU) is an autosomal recessive inherited condition (OMIM #203500) characterized by a lack of homogentisate 1,2 dioxygenase activity (EC:1.13.11.5), due to mutations at the homogentisate dioxygenase (HGD) gene loci leading to accumulation of homogentisic acid (HGA). HGA undergoes oxidation via benzoquinone acetate intermediary resulting in deposition of melanin-like pigment in joint and spine cartilage, tendons, and ligaments, in a process known as ochronosis. The incidence of AKU in the world is ranged from 1:250,000 to 1:500,000. In Slovakia, the incidence of this disease is approximately 1:19,000 with the highest frequency in specific regions of the Northern Slovakia.

Alkaptonuria genetics

In 1993, the gene encoding HGD enzyme was mapped to the 3q13.33 chromosome, thanks to a genetic analysis in the families of Slovak patients (Pollak et al. 1993). Shortly afterwards, the structure of the gene with its 14 exons was described and the first mutations in the patient’s families identified (Granadino et al. 1997). Currently, up to 212 variants
of this gene have been identified in about 530 patients around the world registered in the worldwide HGD mutation database http://hgddatabase.cvtisr.sk, created and managed by the Laboratory of Human Genetics of the Biomedical Research Center, Slovak Academy of Sciences (Zatkova et al. 2012; Nemethova et al. 2016). This autosomal recessive disease is a classic example of congenital metabolic disorder, and historically, the first disease, in which the principles of Mendelian heredity has been proposed (Garrod 1902). Patients are homozygotes or compound heterozygotes for variants/mutations in the HGD gene.

**Alkaptonuria diagnostics**

Although the genetic analysis of HGD mutation provides clinicians with supportive information, the cornerstone of AKU diagnostics is based on the detection of increased urinary HGA excretion. Currently, the gas chromatography with mass detection (GC-MS) or liquid chromatography tandem mass spectrometry (LC-MSMS) for quantitative determination of HGA in urine and serum represents a gold standard method for the biochemical confirmation of AKU (Jellum et al. 1989). The LC-MSMS allows a simultaneous determination of not only tyrosine and phenylalanine, but also other metabolites of the tyrosine metabolic pathway (Milan et al. 2019).

**Alkaptonuria clinical features**

During the first years of life, the HGA urinary excretion resulting in dark urine represents the only symptom of the disease. Brownish-black discoloration of diapers is often the first diagnostic clue in AKU. During the first two decades of life HGA deposits gradually develop in bradytrophic tissues such as in cartilages during the process called ochronosis. This progressively leads to the reduction of cartilage elasticity, increased fragility, and brittleness. Clinical and radiologic signs of the ochronotic arthropathy and spondyloarthropathy are present as early as in the third decade of life (Imrich et al. 2022). The ochronosis develops in non-musculoskeletal tissues, such as the heart, lungs, and kidneys, as well (Phornphutkul et al. 2002). The latter represents the main organ of HGA elimination by glomerular filtration and tubular secretion (Ranganath et al. 2020a). The deposition of the ochronotic pigment has also been detected in the adrenal glands, thyroid, testicles, and prostate; less in the spleen and the pancreas (Millucci et al. 2017). Typical blue-black spots appear on the eye sclera (Figure 1), ears (Figure 2), the skin of hands, face, eye area, and under arms.

The cartilage is vitreous, fragile, brittle, but there is no pulpung of the superficial layer, as is the case with osteoarthritis. Tiny fragments of altered cartilage accumulate in the articular fissure, where irritation, hyperaemia can occur. Intervertebral disc-space narrowing, the presence of osteophytes/hyperostosis, and calcification are the three most frequent radiologic features in AKU with the most affected spine region being lumbosacral, followed by the thoracic and cervical segments (Imrich et al. 2022). The AKU spondyloarthropathy often progresses to

![Figure 1. The deposition of ochronotic pigment in the conjunctiva in a 37-year-old man (up) and in the sclera of a 68-year-old woman (down) with alkaptonuria. Source: The Robert Gregory National Alkaptonuria Centre, Liverpool, United Kingdom.](image-url)
vertebral fusions. In the ochronotic arthropathy, several joints including knee, hip, and shoulders are affected by painful arthropathy requiring multiple joint replacements as early as in the fifth decade of the life (Taylor et al. 2017).

**Alkaptonuria treatment**

Before 2020, treatment options for AKU were a combination of a low-protein diet, high doses of vitamin C, physiotherapy, symptomatic NSAIDs, and surgical treatment of complications of arthropathy and heart valve disorders, respectively. Results of the SONIA 1 study showed that a dose of 8 mg of nitisinone per day produced the greatest reduction of serum and urinary HGA without safety issues (Ranganath et al. 2016). Furthermore, long-term treatment with nitisinone (10 mg) has been shown to be an effective and safe treatment for AKU leading not only to an arrest of clinical symptoms, but also to a regression of ochronosis in the eye and the ear (Ranganath et al. 2020b). Based on the results of the latter study, the European Medicines Agency approved nitisinone 10 mg daily as the first effective treatment in adult patients with AKU in October 2020. At present, nitisinone is registered by Swedish Orphan Biovitrum International under the trade name Orfadin in the European Union. Since September 2022, orfadin is fully reimbursed for treatment of adult patients with AKU in Slovakia.

**Mechanism of nitisinone action**

Nitisinone is the inhibitor of the enzyme p-hydroxyphenylpyruvate dioxygenase (HPPD), which is responsible for converting 4-hydroxyphenylpyruvate
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(HPPA) into HGA (Figure 5), effectively blocking HGA formation. The block in the tyrosine metabolic pathways at the HPPD step leads to a cumulation of HPPA, which is readily converted back to tyrosine. Therefore, the nitisinone treatment induces hyper-tyrosinemia within days after initiation of the treatment, and in the long term, leads to progressive adaptations by decreasing phenylalanine conversion to tyrosine (Ranganath et al. 2022a).

Based on SONIA 2 results, where no dietetic management to enforce low-protein diet was used, approximately 15% of AKU patients on nitisinone develop keratopathy as an expected side effect (Ranganath et al. 2020b). Therefore, monitoring of tyrosine plasma levels and low-protein diet has been recommended to maintain tyrosine plasma levels between 500 and 800 µmol/l, the range considered relatively safe in terms of the keratopathy risk (Ranganath et al. 2022b). In the case of the keratopathy development, nitisinone treatment must be stopped and reintroduced only after symptoms have disappeared.

**Alkaptonuria dietary measures and nutritional therapy**

The life-long, low-protein diet has been a basic dietary intervention in the pre-nitisinone era in AKU. The rationale was that low-protein intake could delay clinical progression by lowering of HGA concentration. Currently, with the treatment available, the low-protein diet aims to minimize nitisinone-induced hyper-tyrosinemia to minimize the risk of keratopathy; however, the low-protein diet is poorly tolerated by majority of AKU patients (Ranganath et al. 2022b). The low compliance with low-protein diet in AKU patients often leads to increased tyrosine concentrations above 800 µmol/l increasing the risk of

![Figure 5. Metabolism of phenylalanine and tyrosine. Nitisinone inhibits the enzyme 4-hydroxyphenylpyruvic acid dioxygenase, which is responsible for converting 4-hydroxyphenylpyruvate into homogentisic acid.](image-url)
keratopathy development and therapy interruptions (Ranganath et al. 2022c). Furthermore, in patients, who comply with low-protein diet, severe muscle loss occurs (Ranganath et al. 2013). Results from SONIA 2 also clearly showed that protein-restricted diet had led to increase in body weight (Olsson et al. 2022). The latter observations combined with the fact that AKU patients have increased energy intake from carbohydrates and fats significantly increases metabolic risk including development of type 2 diabetes in the long-term. General recommendations of the World Health Organization of minimum protein intake for adults are 0.80 g per kg of body weight per day. A recent study analysed basic nutritional data using dietary journals in 74 adult AKU patients with an average age of 55 years (Judd et al. 2020). Compared to controls, AKU patients had significantly lower than expected upper arm circumference, fist grip strength, BMI, total energy intake and protein intake, and higher than the expected percentage of body fat. Therefore, they meet the ESPEN criteria for evaluation as clinically malnourished. Judd and co-workers (2020) have noted that AKU patients are at risk of protein deficiency associated with other clinical complications such as decrease in muscle strength. Furthermore, due to frequent hospitalizations for major joint-replacement surgeries the protein deficiency can lead to suboptimal recovery with major impact on the disease itself (Judd et al. 2020). In a recent study, tyrosine/phenylalanine dietary restriction significantly reduced nitisinone-induced tyrosinemia in AKU mice model with phenylalanine restriction alone proving ineffective (Hughes et al. 2020). The latter study also showed that protein restriction significantly reduced circulating tyrosine in 10 AKU patients; however, only 4 out of 10 patients achieved tyrosine <700 μmol/l (Hughes et al. 2020).

**Low protein diet and taste preferences**

The protein content of food can be predicted based on amino acids composition, which are often present in free form in protein-containing foods. Thus, most amino acids have a taste, which makes some of them important as taste-active components in food. In addition to taste, post-ingestive mechanisms can also generate signals for satiety and appetite. Dietary amino acid deficiency may activate a specific hunger for missing amino acids (Bachmanov et al. 2016). It can be assumed that the missing amino acids during low-protein diet can drive craving for meat food in AKU patients, which would explain poor compliance with the strict, life-long, and low-protein diet in these patients. As far as we know, food preferences in AKU patients and their relationships to low-protein diet adherence have not been studied yet. Better designed dietary and nutritional plans may substantially improve compliance and adherence to the low tyrosine and phenylalanine diet in AKU patients.

**Conclusion**

Currently, the nutritional regime in nitisinone-treated alkaptonuria patients is aimed solely at the overall reduction of protein intake as a prevention of keratopathy leading to increased risk of muscle loss and weight gain associated with metabolic complications, including obesity and type 2 diabetes. Therefore, the well-balanced combination of low-protein diet with protein supplements without phenylalanine/tyrosine may prevent the muscle loss and weight gain while limiting the nitisinone-induced hypertyrosinemia. In addition, the nutritional intervention for adult alkaptonuria patients treated with nitisinone should also improve the intervention compliance by alterations of the taste preferences. Specific nutritional programs should also be accompanied with complementary targeted physiotherapy to increase the muscle strength and prevent the obesity and insulin resistance. Currently, development of the novel nutritional approaches represents a high priority research area for the clinical management of alkaptonuria.

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