# METHODS AND COMPOSITIONS FOR THE TREATMENT OF METABOLIC DISORDERS

## CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation of U.S. patent application Ser. No. 11/143,887, now U.S. Pat. No. 7,566,714 filed Jun. 1, 2005 which in turn is a continuation of U.S. patent application Ser. No. 10/991,573, which was filed on Nov. 17, 2004 now abandoned and which claimed the benefit under 35 U.S.C. §119(e) of U.S. Provisional Patent Application Ser. No. 60/520,767, which was filed Nov. 17, 2003. The entire disclosure of each of these priority applications is hereby incorporated herein by reference.

## BACKGROUND

1. Field

The present invention is generally directed to the therapeutic intervention of metabolic disorders, particularly those involving amino acid metabolism. More particularly, the present invention is directed to methods and compositions for the treatment of phenylketonuria, vascular diseases, ischemic or inflammatory diseases, or insulin resistance, or conditions and patients that would benefit from enhancement of nitric oxide synthase activity.

2. Background of the Related Technology

Phenylketonuria (PKU) is an inherited metabolic disorder that was first identified in the 1930s. In most cases, and until the mid-1990s, it was thought that this is a disorder of amino acid metabolism resulting from a deficiency in the liver enzyme phenylalanine hydroxylase (PAH). Deficiencies in PAH in turn result in an excess of phenylalanine (Phe) in the brain and plasma. The deficiency in PAH ultimately manifests in a lack of tyrosine, which is a precursor for the neurotransmitters.

Left undetected and untreated early in the life of an infant, PKU leads to irreversible damage of the nervous system, severe mental retardation and poor brain development. Features other than mental retardation in untreated patients include brain calcification, light pigmentation, peculiarities of gait, stance, and sitting posture, eczema, and epilepsy. It has been reported that an infant suffers a loss of 50 IQ points within the first year of infancy and PKU is invariably accompanied by at least some loss of IQ. Once detected, the condition is treated by providing the infant, and later the child, with a low Phe diet. In adults, the protein supplements routinely taken by classic PKU patients may be Phe-free with the assumption that such adults will receive sufficient quantities of Phe through the remaining diet, controlled under a strict regimen, so that the overall diet is a low Phe diet. Also, pregnant women who suffer from the condition are recommended a diet that is low in Phe to avoid the risk of impairment of the development of the fetus and congenital malformation (maternal PKU syndrome).

In more recent years it has been shown that pathological symptoms which manifest from the condition of excess of Phe, collectively termed hyperphenylalaninemia (HPA), may be divided into multiple discrete disorders, which are diagnosed according to plasma Phe concentrations and responsiveness to a co-factor for PAH. At an initial level, HPAs may be divided into HPA caused as a result of a deficiency in the cofactor 6R-L-erythro-5, 6, 7, 8, tetrahydrobiopterin (BH4; malignant PKU) and HPA resulting from a deficiency in PAH. The latter category is further subdivided into at least three categories depending on the plasma concentration of Phe in



the absence of dietary or other therapeutic intervention (referred to herein as "unrestricted plasma Phe concentration").

Normal plasma Phe homeostasis is tightly controlled resulting in a plasma Phe concentration of 60 μmol/L±15 μmol/L. Classical PKU (OMIM No. 261600) is the most severe form of PKU and it results from null or severe mutations in PAH, which lead to unrestricted plasma Phe concentrations greater than 1200 μmol/L when left untreated. Individuals with classical (or severe) PKU must be treated with a strict dietary regimen that is based on a very low Phe diet in order to reduce their Phe concentrations to a safe range. Milder forms of HPA also have been characterized. A less severe form of PKU is one which manifests in plasma Phe concentrations of 10-20 mg/dL (600-1200 μmol/L), and is generally termed "mild PKU". This moderate form of PKU is managed through the use of moderate dietary restrictions, e.g., a low total protein diet, but otherwise not necessarily Phe-free. Finally, mild HPA, also referred to as benign or non-PKU HPA is characterized by plasma Phe concentrations of between 180-600 μmol/L. The individuals with non-PKU HPA are not routinely treated as they are considered to have plasma Phe levels that are within the "safe" range. Nevertheless, as mentioned above, these Phe levels are still significantly elevated in these individuals as compared to normal, non-PKU subjects and may present detrimental sequelae in at least pregnant women and very young patients. For a more detailed review of HPA resulting from PAH deficiency, those of skill in the art are referred to Scriver et al., 2001 (*Hyperphenylalaninemia: Phenylalanine Hydroxylase Deficiency*, In: Scriver C R, Beaudet A L, Sly W S, Valle D, Childs B, Vogelstein B, eds. The Metabolic and Molecular Bases of Inherited Disease. 8th ed. New York: McGraw-Hill, 2001: 1667-1724). NIH Guidelines indicate that for children with PKU, it is preferable reduce the plasma Phe to be 360-420 μmol/L.

HPA also results from defects in BH4 metabolism. BH4 is an essential cofactor of both tyrosine and tryptophan hydroxylase, the rate limiting enzymes in the biosynthesis of the neurotransmitters dopamine and serotonin. The effects of deficiencies in dopamine and serotonin are collectively known as "atypical" or "malignant" HPA. Thus, traditional diagnoses of HPA have involved a determination of whether the HPA is a result of BH4 deficiency or PAH deficiency. Typically, diagnosis of PKU is established on the basis of a persistently elevated blood Phe concentration. Following a positive screen for elevated blood Phe (plasma Phe>120 μmol/L; Weglage et al., *J. Inherit. Metab. Dis.,* 25:321-322, 2002), a differential screen is performed in which it is determined whether the elevated Phe is a result of BH4 deficiency or PAH deficiency. The differential diagnosis involves determining whether the elevated Phe concentration is decreased as a result of BH4 administration (BH4 loading test). The BH4 loading test typically involves a one-time load of BH4 e.g., 5-20 mg/kg being administered to the subject who is on a normal (i.e., unrestricted) diet and determining whether the subject experiences a decrease in Phe levels (see e.g., Ponzone et al., *Eur. J. Pediatr.* 152:655-661, 1993; Weglage et al., *J. Inherit. Metab. Dis.,* 25:321-322, 2002.)

Typically, individuals that respond to a BH4 loading test by a decrease in plasma Phe levels are diagnosed as having a defect in BH4 homeostasis. However, there have been various reports of patients with a BH4 responsive type of PAH deficiency (Kure et al., *J. Pediatr.* 135:375-378, 1999; Lassker et al., *J. Inherit. Metabol. Dis.* 25:65-70, 2002; Linder et al., *Mol. Genet. Metab.* 73:104-106, 2001; Spaapen et al., *Mol. Genet. and Metabolism,* 78:93-99, 2003; Trefz et al., 2001). These subjects have plasma Phe levels that are typical of