1. Introduction

Amino acids are the building blocks of proteins and play an important role in metabolism. Amino acids are composed of amine (-NH$_2$) and carboxylic acid (-COOH) functional groups along with side chain specific to the particular amino acid. Amino acids are primarily composed of Carbon, Nitrogen, Oxygen and Hydrogen though few other elements are found in the side chain of certain amino acids. Proteins are large molecules and made up of hundreds to thousands of amino acids and protein synthesis in the body requires the availability of all amino acids in sufficient amounts. Both, indispensable amino acid (IAA) and dispensable amino acids are required in proper proportions and adequate quantity to allow the synthesis of tissue proteins in the body. The 20 amino acids are combined variably to make up the 100,000 different proteins that maintains the dynamic structural and functional system of the human body (WHO/FAO/UNU, 2007).

If a diet is limiting in any IAA, protein synthesis cannot proceed beyond the rate at which that amino acid is required. The requirement for lysine, which is in limited concentration in cereal based diets such as rice and wheat has received attention (Young VR & Pellet PL, 1994). Lysine cannot be synthesized by mammalians and it does not participate in transamination reactions, hence is consequently a strictly indispensable amino acid as it plays an important role in protein synthesis. Furthermore, it also acts as a precursor for the biosynthesis of carnitine, which plays an important role in β-oxidation (Broquist, 1973). Majority of the world’s children live in developing countries (including India) and are predominantly fed cereal-based diets lacking in lysine. Determination of lysine requirement is particularly relevant in such populations, because there is evidence to show that protein quality influences linear growth in children (Allen, 1994; Golden, 1994).

According to WHO estimates, malnutrition, as measured by stunting, affects 32.5 percent of children in developing countries (de Onis et al., 2000). The prevalence of underweight children in India is the highest in the world, approximately 47% of the children are malnourished (World Bank Report on Malnutrition in India, 2009) and about 25% of them suffer from chronic undernourishment as mostly reflected by stunting or low height-for-age. In addition, infections have an adverse effect on metabolism of most nutrients, including protein and amino acids, and the actual requirement of indispensable amino acids may be increased due to catch up growth, sub-clinical infections due to chronic immunostimulation, parasitic infestations and poor hygiene.
Among all infestations, intestinal parasitic infection is commonly found in developing countries. It has been reported that rural South Indians have very high rates of intestinal parasitic infestations (Kurpad et al., 2003; 2003c & Kang et al., 1998).

The prevalence rate of single and/or multiple intestinal parasitic infestations among Indian school aged children ranges 12 to 70% (Wani et al., 2008; Bansal et al., 2004; Fernandez, et al., 2002; Sethi, et al., 2000; Das, et al., 1981; Awasthi & Pande, 1997 & Vidyarthi, 1969). Particularly, long term studies (5 and 10 years) found that the overall prevalence of different intestinal parasitic infestations was 21.8% and 21.4% respectively (Yogyata & Singh, 2011 & Patel, 1986). Intestinal parasites alter net nutrient absorption mainly by diverting nutrients for their own need and therefore less is available for absorption by the host (Huang, 2006). Furthermore, parasite induced mucosal inflammation and villous atrophy over time and impairs nutrient absorption (Huang, 2006). Previous studies have shown that the intestinal parasites increased lysine requirement by about 50% in chronically undernourished adult men (Kurpad et al., 2003a&c). However, this phenomenon has not been demonstrated in chronically undernourished growing children. Furthermore, the lysine requirement in the undernourished otherwise apparently normal Indian adults is identical to the healthy Indian adult requirement of 30mg/kg/day (Kurpad et al., 2001a & b, 2003a).

Majority of the children living in regions like India are depend predominantly on cereals based foods, thus the direct measurement of IAA requirement in healthy and undernourished Indian children with intestinal infestation is essential. Furthermore, so far there is no data generated on the lysine requirements in these vulnerable groups. Traditionally, nitrogen balance was the primary method used to determine amino acid and protein requirements. However, the nitrogen balance technique has many limitations, due to overestimation of nitrogen intake and an underestimation of nitrogen excretion which leads to an overly positive balance and an underestimation of the requirement (Rand et al., 1976; WHO/FAO/UNU, 2007). The current WHO/FAO/UNU (2007) estimate of the daily requirement of lysine in children (35 mg/kg/day) is based on a factorial approach and derived from nitrogen balance studies in 10 to 12 year old children (Nakagawa et al., 1961). The factorial approach also assumes that children have the same maintenance value as adults to which it is added the requirements for growth. For the WHO estimate, the maintenance value used is 30 mg/kg/day.
Methods based on carbon oxidation, which is measured using stable isotopes have proven to be more rapid and sensitive to the changes in amino acid intakes and have resulted in identification of requirement values much higher than those based on nitrogen balance. These isotope tracer techniques are based on the measurement of the oxidation of a labelled amino acid or tracer, with $^{14}$C tracers used for animal and $^{13}$C stable isotopes used in human studies. Amino acid oxidation studies are based on the principle that any amino acid, provided in excess of the needs of protein synthesis, is preferentially oxidized (Zello et al., 1995). Commonly, carbon balance methods measure Direct Amino Acid Oxidation (DAAO), Direct Amino Acid Balance (DAAB), Indicator Amino Acid Oxidation (IAAO) and Indicator Amino Acid Balance (IAAB) methods are used to measure the amino acid requirements.

The IAAO method was initially developed by Bayley and colleagues for the determination of amino acid requirements in young growing pigs (Kim et al. 1983a; Kim & Bayley, 1983, & 1984; Kim et al., 1983b & Ball and Bayley, 1986) had validated this concept. The IAAO method is, therefore, based on the concept that when one IAA is deficient for protein synthesis, then the excess of all other IAA, including the indicator amino acid, will be oxidized. With increasing intakes of the limiting amino acid, IAAO rate will decrease, reflecting increasing incorporation into protein. The requirement is taken as the intake of amino acid that provides for an inflection (or breakpoint) in the pattern of the indicator amino acid oxidation or balance response to different test amino acid intakes. The IAAO estimates are comparable to the values obtained using the more elaborate 24h-indicator amino acid oxidation and balance (24h-IAAO/IAAB) model.

Followed by the short term minimally invasive IAAO method was developed and has been used with lysine or phenylalanine as an indicator amino acid (Zello et al., 1993; Roberts et al., 2001), where the appearance of the $^{13}$C label (from the labelled amino acid) in the breath is measured in the fed state over a few hours. Importantly, these studies were conducted with a short (48h) dietary adaptation period. Therefore, the relative ease, the short dietary adaptation and the relatively non-invasive nature of experimentation, is a strength that allows for many experiments at different levels of test amino acid intake, to be carried out on the same subject which in turn allows the measurement of the variance of the requirement estimate for each amino acid. Due to its non-invasive nature, the IAAO method has also been used to determine requirements for amino acids in neonates and children. This method was successfully applied in ill children, first in children with phenyl-ketonurea (PKU) to measure the phenylalanine and tyrosine requirements.
(Bross et al., 2000). Recently the minimally invasive techniques, has been applied successfully to healthy, school-aged children to determine branched-chain amino acid (Mager et al., 2003), total sulfur amino acid (Turner et al., 2006), minimum methionine (Humayun et al., 2006), and lysine requirements (Elango et al., 2006).

Using this techniques, the lysine requirement in healthy, school-aged Canadian children was determined to be 35 mg/kg/day (Elango et al., 2006), a value that is identical to the maintenance values found in adults using the same minimally invasive IAAO technique (Kriengsinyos et al., 2002) but ~16% higher than that obtained by a 24h IAAO and balance method in Indian adults (Kurpad et al., 2001 & Kurpad et al., 2002a). Studies on the requirement of several IAA such as lysine, leucine, threonine, and methionine have shown that their daily requirements in Indian adults are similar to those in Western populations (Kurpad et al., 2001; 2001a; 2002b & Kurpad et al., 2003b). Although it is likely that the same may apply to growing children, this is not known with any certainty. The IAA requirements may also be affected by subclinical infection or by catch-up growth or may even be reduced by adaptive mechanisms. For example, the daily requirement of lysine has been shown to be higher than normal in undernourished men with parasite infestations presented with optimal diets (Kurpad et al., 2003). Therefore, we aimed to measure the lysine requirement of otherwise healthy children, chronically undernourished children and in children with intestinal parasite infestations from a developing country using a similar methodology to earlier Western studies, to allow us for comparisons between environments.
Hypothesis

Based on the above concerns, the hypothesis of this study were:

1. Lysine requirement may be lower in school-aged (6–10 years) Indian children compared to western population

   Lysine requirements of well-nourished school-aged (6–10 years) Canadian children (Elango et al., 2007) have been found to be 35 mg/kg/day, which is identical to the current recommendation (WHO/FAO/UNU, 2007) of 33.5 mg/kg/day. Western population primarily consist of mixed diet, hence receive high lysine, and while Indian population depends on cereal-based diet in which lysine content is negligible.

2. Influence of adaptation of low lysine intake/habitual cereal based dietary pattern on the lysine requirement

   The adult’s lysine requirement in western population of 30 mg/kg/day (Zello et al., 1993) is similar to that of healthy Indian adult requirement of 30 mg/kg/day measured by both IAAO and IAAB methods (Kurpad et al., 1998; 2001; 2001a; 2002a and 2003a & c).

3. Lysine requirement in undernourished children may be similar as healthy children

   The lysine requirement of chronically undernourished Indian adult was (30 mg/kg/day) (Kurpad et al., 2003a) found to be similar to that of the healthy Indian adult (Kurpad et al., 2001, 2002a) and western population (Zello et al., 1993) as well. Unlike adults, the amino acid requirement in children has to be accounted for the growth and maintenance also (WHO/FAO/UNU, 2007).

4. Intestinal parasitic infestations may increase the lysine requirement

   Kurpad et al have shown that the daily lysine requirement increased by ~50% due to intestinal parasitic infestations (Kurpad et al., 2003c) in chronically undernourished men who lived in unhygienic conditions (Kurapd et al, 2003a). However, it is not known if a similar phenomenon occurs in undernourished children who live in poor and unsanitary environments.
5. The expected outcomes of these investigations

The protein and amino acids are key components of the diet. Recently, isotope-based methods have been developed, which permits the determination of essential amino acid requirements. The result from this study will be of great importance in determining the essential amino acid requirements for children in India. Thus, with above hypothesis, the objectives of this research framed are as follows.

Objectives

1. To determine the lysine requirements of healthy and malnourished (chronically undernourished) school-age Indian children using IAAO method.
2. To determine the effects of intestinal parasites infestations on the lysine requirement of the children who are from low socio-economic and poor sanitary environments.
3. To determine the effects of living with cereal-based diets (adaptation) on the lysine requirement of school-aged Indian children.