Low Tryptophan (TRP) Chronic Intake Present Consequences

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Abstract

Essential amino acids (EAA) deficiency will have important consequences for health upkeep, especially in Low- and Middle-income Countries (LMIC). By mixing different plant derived products complete proteins can be obtained but this is not achieved at present.

Tryptophan (TRP) has three main metabolic routes leading to the synthesis of proteins, serotonin and nicotinic acid (niacin or vitamin B3). Its deficiency will lead to micronutrient undernutrition especially in LMIC. The specific requirements throughout the lifespan are now well known due to new techniques such as indicator amino acid oxidation. Also, the EAA role in food intake (amount and quality) regulation through the protein kinases is a recent discovery action. The consequences of the TRP lack have been evident since pellagra times. Knowledge of undernutrition has improved since accurate body measurements, the new unified growth standards and metabolomics that allow us to measure not only the micronutrients but also the intermediate compounds along their metabolic pathways.

Nutrition in the first 1000 days of life in some LMIC is the cornerstone period which can be extended up to the 5th anniversary. Hopefully evidence based study conclusions could be applied to all young children of the world. New agricultural technologies allow to enrich different cereals with the deficient amino acids, the best results come from lysine enriched maize, but unfortunately this has not been attempted in chickpeas. Until this moment arrives little results come from lysine enriched maize, but unfortunately this has not been attempted in chickpeas. Until this moment arrives little

Introduction

Tryptophan (TRP) is an essential amino acid (humans cannot synthesize it) belonging to the neutral amino acid group that once absorbed may follow three routes: protein synthesis, serotonin (or 5-hydroxytryptamine) synthesis and nicotinic acid (niacin or vitamin B3) synthesis. This proves its importance not only in nutrition but also in human health. As is frequent in nutrition the first induced deficiency came from the animal experience, demonstrating that TRP protracted deficiency led to a delayed growth among other consequences [1]. Again as in other micronutrient deficiencies unethically induced in humans the inherited diseases of amino acid metabolism give us the possibility to analyze some of the consequences of TRP deficiency. This is the case of Hartnup disease, an autosomal recessive defect affecting the transport of mono amino-mono carboxylic, ie all ten neutral amino acids, across the intestinal mucosa and renal tubules. It is not a severe disease but has red and rough skin after sun exposure, ataxia (wide-base gait), short stature, glossitis, diarrhea and important emotional instability although no mental deficiency. This picture is very close to that of niacin deficient classical pellagra. We should remember at this point that 60 mg of TRP are equivalent to 1 mg of niacin and how nicotinic acid and nicotinamide form part of the coenzymes NAD and NADP. The first has a prime redox function (NAD+/NADH), non-redox functions (DNA repair, telomere maintenance) and also extracellular functions. NADP has very similar functions but it is used in lipid synthesis and in the elongation of free fatty acids. It would not be exaggerated to assume that even a modest deficit of these coenzymes present in all body cells would carry a growth and development impairment. The mitochondrial oxidation of NADH which is coupled with ATP synthesis is metabolic evidence supporting the energetic component of micronutrient undernutrition.

Tryptophan is also a precursor of serotonin (TRP-> 5-OH TRP-> 5-OH tryptamine), a neurotransmitter located in the gastrointestinal tract, platelets and central nervous system. The serotonin functions in the CNS have contributed to the wide workforce towards the end of the last century applied to its mechanisms and eventual applications to different human alterations of sleep, food intake or depression [2]. In 1980's studies showed the administration of TRP to varied neurological conditions such as depression, autism, La Tourette syndrome, sexual dysfunctions, Down syndrome, Epilepsy…that overuse moved the FDA to prevent the tryptophan importation. Research from this period was very valuable i
tryptophan transport across the blood-brain barrier [3,4] or on clinical grounds [5,6]. Nowadays this enthusiasm has been replaced by more accurate results but some information on food intake regulation will be considered below.

**Nutritional Aspects**

Amino acid metabolism as the building blocks of proteins is influenced by the amount, quality of food proteins and the sources of preformed α-amino nitrogen. The classical division into three groups: essential, conditionally essential and non-essential amino acids are valid. The conditional group being those with a rate of synthesis that is not able to meet the cellular needs in certain physiological conditions, such as cysteine in the newborn period or first year of life [7]. TRP is an essential amino acid (EAA) and its age requirements after the initial Estimated Average Requirements (EAR), followed by Recommended Daily Allowances (RDA) are finally settled by the widely accepted norm of the Dietary Reference Intakes DRIs [8], these can be summarized as follows (mg/kg/day): 0-6 months 25; 7-12 months 10; > 1 year 7; and adult 4. These figures varied a little according to the mentioned sources of Requirements and were not far from those obtained by the Indicator Amino Acid Oxidation (IAAO) a most reliable technique. Incomplete proteins are those that one (or more) essential amino acids are in insufficient quantity for growth, development and repair of body tissues. Unfortunately in wide areas of the world plant proteins do not contain all essential amino acids and they are the main food base for children and adolescents. The role of tryptophan in undernutrition has been studied through its metabolites of indicant, 5-HT, and nicotinic acid...since long ago specially in countries where ‘protein-energy malnutrition’ were common [9]. It was the era end of triple D, dermatitis, diarrhea and dementia. Most recently the new approach comes when more precise clinical (somatology) and biochemical (metabolomics and mass spectrometry) methods are widely used, even in large epidemiological studies. It was possible to ascribe the micronutrient alterations to the clinical findings. One of the most representative paradigms is the protein kinase CGN2 gene [10] which has a central role maintaining amino acid homeostasis in yeast and animals. If the essential amino acids uncharged tRNA reaches the anterior piriform cortex of the rats, then the animal restricts the intake of diets lacking essential amino acids. Animals on tryptophan deficient diet also showed an aversive response [11]. Similar regulating mechanisms are related to the protein kinase mTOR (mechanistic TOR) of mammals when nutrient availability is variable [12] with a therapeutic prospect that is that mTOR activity can be modified by rapamycin. These induced modifications can explain better some of the long-term effects of obesity- insulin resistance, but they also play a role in undernutrition (low levels of EAA) as we will see below. These new breakthroughs affecting the biological response, in this case by the essential amino acids require further research [13].

Nutritional consequences of tryptophan deficiency have been approached from the beginning of the micronutrient undernutrition measurements. In a healthy adult the synthesis of protein is around 300 g per day, that implies an uptake and release of 150 g of essential amino acids, if dietary supply is roughly of 6 g per day, a recycling amino acid from protein breakdown is probably the major source for maintaining the dynamic essential pool. In pediatric ages with steady and peak growth periods the balance must be positive although its timing and the extent is unknown accurately. From the animal experience it is well known that plasma tryptophan increases when the intake exceeds normal requirements and when there is a high carbohydrate content in the diet, in this last situation due to the increased TRP bound to albumin [14]. Another important issue is that undernutrition has not a specific pattern of plasma essential amino acids. In a study on severe undernourished children [15] the levels of tyrosine, tryptophan and leucine were lower than in the control group. This pattern is heavily affected by the protein intake amount and quality (ratio of EAA closer to that of egg protein). The plant quality protein is very varied as has been demonstrated after the study through stable isotopes in 116 LMIC [16,17]. Furthermore the habitual and accessible plasmatic window is also affected by the fact that amino acids cannot be stored and

<table>
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<tr>
<th>ESSENTIAL AMINO ACIDS</th>
<th>COMPLETE PROTEIN mg/g prot</th>
<th>CHICKPEA FLOUR mg/g prot</th>
<th>SOYBEAN FLOUR mg/g prot</th>
<th>SORGHUM GRAIN mg/g prot</th>
<th>MAIZE WHOLE mg/g prot</th>
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<tr>
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<td>10.2</td>
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<td>Thr</td>
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<td>76</td>
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<td>36</td>
<td>10.4</td>
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TABLE. Essential amino acids and protein content in chickpea flour and different cereal products in comparison to the complete protein pattern.
must be incorporated to proteins or oxidized.

Undernutrition is more precisely assessed nowadays when the classic definitions are giving way to not only an accurate somometry but also uniform references (Cole, CDC, WHO) have been established and made comparable different samples. Conceptually it is important to determine first the context of Undernutrition and make clear that Malnutrition (bad nutrition) includes both Undernutrition and Obesity [18]. Undernutrition can be defined as inadequate nutrition resulting from lack of food (plus other factors) or failure of the body to absorb or assimilate nutrients properly. At present it is evaluated through the widely recognized Z-score system [19], being undernutrition and underweight respectively when Zs is <-2 SD and between <-1SD to <-2 SD. These limits are important due to the vast majority of underweights being frequently overlooked. Other terms such as stunting (<-2SD from median height) are less informative if the target height is not known. Classical terms are less used probably because they are largely arbitrary. Due to the advances in the biochemical measurements, the undernutrition concept includes the micronutrient deficiencies also known as Hidden hunger and it is pertinent to say that the classical ’big four’ (vitamin A, zinc, iodine and iron) have been expanded although not evenly. Also the concept of Food security (access to food for all) although with a more epidemiological background has proved quite useful because its application has allowed us to discover populations with moderate undernutrition even in high income countries.

In this frame it is difficult to identify the specific clinical consequences of TRP deficiency, whether by deprivation or by a kynurenine pathway block, the development of a weak pellagra syndrome [20,21] points out the relation to inadequate nutrition. Another aspect derived from TRP deficiency came from the mouse chronic granulomatous disease. When this condition appears in young children it is serious due to the incapacity to cope with bacterial infections, granulomas and eventual death is not infrequent despite the adequate antimicrobial therapy. The cause lies in the incapacity of phagocytes to produce NADPH and consequently reactive oxygen species so lethal for bacteria [22]. Finally the nutritional studies on the Autism spectrum disorders show that the prevalence of low levels of tryptophan (and tyrosine) was related to the frequent un

dernutrition and not as a possible cause of the disease [23]. A wider involvement of all lower circulating essential amino acids has recently been approached as coexistent with stunting (24). Even this group has raised a suggestive hypothesis [25] according to which the mentioned mechanistic TORC1 pathway, which is exquisitely sensitive to EAA availability, will repress protein and lipid synthesis and consequently growth velocity. All this should be considered in the context of other macro and micronutrients and further adverse conditions frequent in LMIC.

Tryptophan fortified foods have been used for improving clinical conditions of undernourished children using hydrolyzed collagen [26] and egg white [27] with acceptable results, although not generalized. Conversely more recent mixtures of available cereals and legumes still have tryptophan as limiting amino acid [28]. The Dietary Guidelines for Americans [29] have taken into account the importance of adequate nutrition of infant and young children, even the expanded concept of first 1000 days of child existence and subsequent health status, has started the ’B-24 Project’. The conundrum of references existence for nutrition in this period will be sieved through a systematic review procedure in the first path steps.

In these aspects of nutritional effects of TRP its relation to obesity should be mentioned. It is accepted that high brain levels of serotonin contribute to satiety and this was the origin of the now retired drugs that activate postsynaptic 5-HT receptors. Furthermore it has been described [30] how obese individuals have low levels of TRP that could originate serotonin deficiency in the brain thus diminishing the satiety response to food intake. The balance hunger/ satiety as a primarily survival function is widely preserved and regulated (more than 10 peptides) in hypothalamic nuclei of mammals. To consider only an isolated factor will probably lead to picturesque diets doomed to failure.

**Nutritional and Food Resources in Low- and Middle-Income Countries**

Good nutrition is not evenly adequate in the world because malnutrition is increasing at the expense of overweight and obesity and although its other component undernutrition is receding it is a quanti and qualitatively reality still too important in Low- and Middle-Income Countries (LMIC). The absolute figures and trends are a matter of concern at individual, national and international level.

Another relevant aspect is the specific prevalence of undernutrition in children under-five years, a period of special health relevance according to the WHO Global Health Observatory in which infections are a real threat with a higher mortality rate especially in LMIC. The good news is that this pediatric health problem is receding all over the world: In 1960 there were 300 million that in 2015 had gone down to 113 million (-33%), but the problem is still important especially in South Asia with 28.7 million and Sub-Saharan Africa (> 50 countries) with 51.3 million [31]. These worrying figures will continue because the world population will be of 9.1 billion by 2050, whereas the developed countries will increase by 6%, South Asia will do so by 48% and Sub-Saharan Africa by 130%. Consequently malnutrition as the present double burden that is the coexistence of underweight and overweight will go on according to the United Nations Population Division [32].

As mentioned essential amino acids are not completely present in plants or crops that are important if not the only food available for people living in certain wide areas such as Sub-Saharan Africa or South-Central Asia. Basic genetic and genetic engineering technologies initiated in the middle of the past century have evolved at a fast rate allowing the improvement of this lacking problem. Genetic modification has been successful in maize and increasing lysine content is possible in sorghum but no transformation has been settled at large scale. In the case of chickpeas the only genetic transformation has been related to agronomic traits (resistance to pests, drought…) but not for increasing TRP content [33], this despite the high concern of the Indian Council of Agriculture...
This could be the solution at mid or long term but in the meantime mixes of plant foods adapted to local climate arid conditions can be a feasible solution.

Chickpea seeds [34] or flours [35,36] are a reasonable food resource but their low content in the essential amino acid tryptophan (Table) is a nutritional risk especially for weaned infants and under-fives [37]. Preformed nicotinic acid is widely present in foods derived from plants or animals. The last one, meat, meat products, dairy products and eggs is the normal source in high income countries. In cereals nicotinic acid is esterified to macromolecules complexes that make it unavailable for intestinal absorption. The animal sources are normally scanty or nearly absent in LMIC. Human milk contains a higher concentration of preformed nicotinic acid than cow’s milk. Therefore once weaning is established the lack of TRP in chickpea flour and the unabsorbable nicotinic acid generates a situation of nutritional risk especially for under-fives. On the other hand sorghum flour a cereal with similar nutritional capacities, has also the very low content of another essential amino acid (lysine) with well established consequences in neurodevelopment and growth, but with acceptable amounts of TRP [38,39]. The mixture of both flours (~20/80 %) will provide a complete protein, the fact of small losses of essential amino acids as consequence of food processing (even by microwaves) is an added advantage. Due to the pragmatic idea of the FAO Forum digest about pulses the possibility of having both flours or preferably crops, both being resilient to dry conditions, in these vast rural areas of LMIC could help to improve nutrition in general and especially in this crucial age which affects the rest of life for the survivors. UN Inter-agency 2015 report child mortality in under-fives still at 5.9 million all over the world; 60 percent occur in ten countries of the mentioned areas of Africa and Asia.

References


32. (2016) GMO Compass.


