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THE FUTURE OF TRYPTOPHAN—UNCOVERING METABOLIC FUNCTIONS AND POSSIBLE THERAPEUTIC APPLICATIONS

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Contents

Résumé

Le stress et l'épuisement (burnout) au travail constituent une réponse physique et mentale à un débordement face à un challenge professionnel. Ses conséquences économiques sont estimées à 7 milliards d'euros en 2018. L'un des possibles candidats permettant d'alléger une partie de coût est le L-tryptophane (tryptophane). L'état actuel de la recherche sur le stress et l'épuisement au travail est néanmoins relativement naissant et encore loin de converger vers une compréhension complète des bénéfices apportés par le tryptophane. Cet article examine le rôle du tryptophane dans la synthèse des protéines et d'autres fonctions métaboliques majeures connues pour affecter directement ou indirectement l'état de santé de ses utilisateurs, à partir de la littérature scientifique existante. En particulier, une revue du rôle du tryptophane sur la synthèse de la sérotonine dans le cerveau et d'autres métabolites tels que la kynurénine et la mélatonine sera fournie. Cette étude de cas examinera également d'autres connaissances basées sur les dernières recherches et essais cliniques sur l'utilisation du tryptophane comme complément alimentaire permettant de répondre à un manque de tryptophane et de produire d'autres métabolites connus pour avoir un impact significatif sur les niveaux de stress et d'épuisement, la dépression, les troubles du sommeil tels que l'insomnie et d'autres conditions liées.

Abstract

Workplace stress and burnout is a physical and mental response to being overwhelmed by a work-related challenge and the health economic burden of its consequences in France was more than € 7.0 billion in 2018, including over € 100 million in benzodiazepine drug sales. However, the current state of research as it relates to work place stress and burnout and its complexity is far from converging toward a more-certain understanding of the best set of treatments available to manage the consequences of stress and burnout. One possible candidate ingredient that may help alleviate some of this burden is L-tryptophan (tryptophan). This article reviews the role of tryptophan in protein synthesis and other major metabolic functions from the scientific literature that are known to directly and indirectly impact the user's health state. Specifically, a review of the role of tryptophan on the synthesis of beneficial serotonin in the brain and other metabolites including kynurenine and melatonin will be provided. Also, insights on the latest research and clinical trials on the use of tryptophan supplements in order to address to tryptophan depletion and the production of other metabolites that are known to be related to significant health conditions including stress and burnout, depression and related conditions, and sleep disorders like insomnia is investigated in detailed in this case study.

Introduction

It is becoming increasingly apparent that workplace stress and burnout is becoming a major health and economic problem in France. In 2014, a survey concluded that 3 million French workers were close to workplace burnout and another recent study that surveyed 32,137 employees working across 39 companies over a period of four years between 2013 and 2017, 52% of French workers suffer from a high level of anxiety at work that could be putting their physical and mental health at risk and 16% of workers likely have an anxiety disorder [69,70]. 29% have a high depressive level (show symptoms of depression) and 6% "probably have depression", according to the study. 24% of French workers reported that they are hyperstressed which is defined as the psychological strain people often feel when they perceive themselves as being overwhelmed by work [69]. Hyperstress makes a person feel pushed beyond the limits of what they can handle, whether it's caused by an excessively high workload, unreasonable deadlines or working too long and too hard. Hyperstress can lead to a short temper, as well as induced or increased anxiety and/or depression [69].

Workplace stress and burnout is a physical and mental response to being overwhelmed by a work-related challenge. The perceived challenge causes a worker's neuroendocrine system to respond accordingly. The neuroendocrine system is the process by which the brain regulates the hormonal activity (endocrine system) which in turn (and in combination with the nervous system) regulates the physiological processes of the human body. A classic example of this is the flight versus fight response a person experiences when confronted a scary situation. Workplace stress and burnout has been shown to release cortisol levels in the body which can provide a short-term burst of energy and focus in order to address the latest workplace challenge but in the long run, high cortisol levels lead to higher risk physical and mental disorders including increased risk of CVD, sleeping disorders, immunoallergic issues, functional colitis and hormonal disorders [69]. Interestingly, while just over half reported elevated levels of stress at work, the remaining workers did not report being under stress despite being in similar work environments [69].

The burden of workplace stress and burnout on the French healthcare system is substantial. Conservative estimates of the total burden of workplace stress and burnout based on a literature review of the key health burden drivers attributed to workplace stress is € 7.0 billion in 2018 and is expected to grow to € 8.3 billion by 2025. Measuring the economic burden bore by each individual sufferer of workplace stress and burnout includes a mix of both direct medical costs and indirect non-medical costs related to supporting the individual sufferer's quality of life. A recent set of research exploring the direct and indirect economic burden of diseases attributed to workplace stress in France found that 8.8 to 10.2% of coronary heart disease (CHD) morbidity was attributed to workplace stress. 15.2 to 19.8% and 14.3 and 27.1% of mental disorders (MD) was attributable to job strain for men and women, respectively [71, 72, 73]. The authors of the aforementioned study consistently reported that the total costs of CHD and MD attributable to job strain exposure ranged from 1.1 to 1.3 billion euros per year from 2000 to 2007. This is equivalent to 1.9 billion euros in

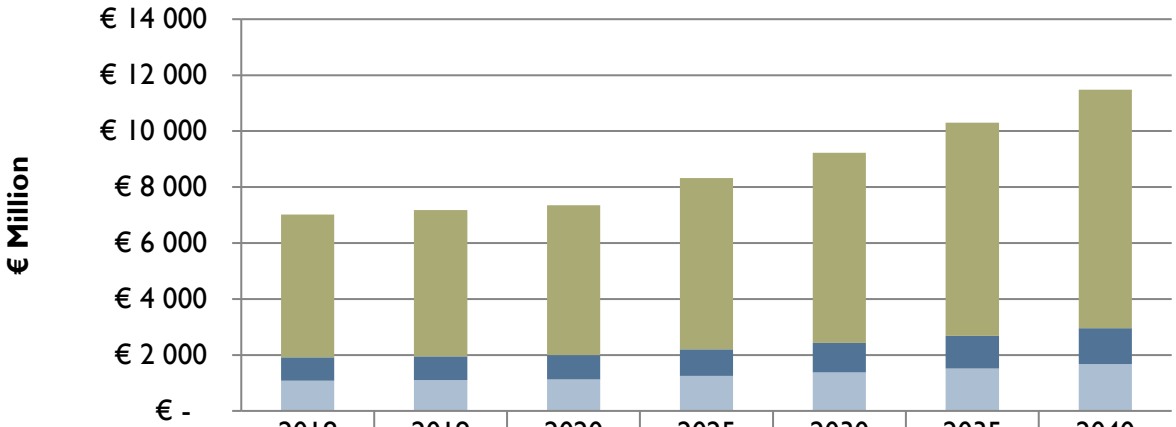
2018 euros. Eleven percent of attributable costs were direct medical costs, 13-15% was attributed to the value of loss life and the remaining three-quarters are attributed to productivity losses due to sick leave [71, 72, 73].

Figure 1. Cost of Workplace Stress and Burnout in France per Case and by Cost Type, France, 2018

Morbidity	Direct Medical Cost, € Million	Indirect Cost*, € Million	Total Cost, € Million	# of Cases, '000	Cost per Case, €
CVD	91.6	992.9	1,084.5	152	7,114
Depression	361.7	466.4	828.1	158	5,238
Sleep Disorders	1,224.2	3,877.0	5,101.2	2,002	2,549
Total	1,677.6	5,336.3	7,013.9	2,312	3,034

Source: Godet-Cayré V et al. 2006, Béjean, S. & Sultan-Taïeb, H. 2005., Sultan-Taïeb, H., Chastang, J. F., Mansouri, M., & Niedhammer, I. 2013 and Frost & Sullivan analysis

Figure 2. Total Cost of Workplace Stress and Burnout in France by Selected Morbidities, France, 2018-2040



	2018	2019	2020	2025	2030	2035	2040
Sleep Disorders	€ 5 102	€ 5 230	€ 5 365	€ 6 116	€ 6 797	€ 7 618	€ 8 524
Depression	€ 828	€ 845	€ 862	€ 953	€ 1 052	€ 1 160	€ 1 279
CVD	€ 1 085	€ 1 107	€ 1 129	€ 1 248	€ 1 377	€ 1 520	€ 1 676
Total	€ 7 015	€ 7 182	€ 7 356	€ 8 317	€ 9 225	€ 10 298	€ 11 479

Source: Godet-Cayré V et al. 2006, Béjean, S. & Sultan-Taïeb, H. 2005., Sultan-Taïeb, H., Chastang, J. F., Mansouri, M., & Niedhammer, I. 2013 and Frost & Sullivan analysis

Workplace stress and burnout can also lead to increased incidence of sleeping disorders. In 2018, it is expected that the extra healthcare cost due to insomnia per sufferer in France is €

611.7 more per individual per year after adjusting for the rate of monetary inflation. Also, it is expected that the extra indirect cost due to insomnia-associated loss productivity in France is € 1,937 per patient per year after adjusting for the rate of monetary inflation in 2018 [76]. Consequently, it is expected that the total direct and indirect cost of insomnia in France attributed to increased healthcare cost, loss productivity and extra costs bore by employers was valued at € 5,101.0 million in 2018 and based on population growth will surpass € 6,7 billion in economic burden attributed to insomnia-attributed absenteeism and additional healthcare costs by 2030 [76].

Benzodiazepines are sometimes prescribed to treat anxiety disorders, panic, insomnia, and agitation caused by hyperstress. Benzodiazepines are a class of psychoactive drugs that affect the neurotransmitter gamma-aminobutyric acid (GABA), resulting in sedation anti-anxiety properties [83]. However, the side effects of benzodiazepines use often outweigh the short term relive these drugs provide. For example, nervous system disorders account for about 23% of serious benzodiazepines anxiolytics and hypnotics with cases of drowsiness, comas, convulsions and amnesia [10] Psychiatric conditions account for 12% of serious adverse reactions for benzodiazepines anxiolytics and 17% for hypnotic benzodiazepines [10]. Also, accidents and falls are also commonly reported with benzodiazepines anxiolytics and hypnotics and especially in the elderly [10]. Finally, the use of these drugs illegally, whether by the user, or in order to enable criminal submission (such as in cases of rape, pedophilia or delictual acts have been reported [10]. Thus, the use of benzodiazepines and related molecules is seen as an inferior solution for addressing the rising epidemic of hyperstress in the workplace due to the great social costs these types of psychoactive drugs bear on French society.

Nonetheless, France ranked second in the consumption of benzodiazepines, behind Spain in 2015 [10]. France is ranked 3rd in the consumption of hypnotics and the 2nd rank of the consumption of anxiolytics [10]. Total sales of benzodiazepines were 118 million euros (in manufacturer price excluding taxes) in 2015, or about 0.6% of total medicine sales to pharmacies. [10]. Sales data in the city indicate that 111.6 million boxes sold in the city and 117 million including the hospital, which represents almost 4% of total drug consumption in France in 2015 [10]. Consequently, about 13.4% of the French population consumed a benzodiazepine at least once whatever the indication and the prevalence of use of benzodiazepines anxiolytic or hypnotic is higher among women (16.6%) than among men (9.7%) regardless of age [10]. This prevalence increases with age and is highest in women aged 80 and over (38.3%).

As the above case shows, stress, anxiety, and burnout has a wide array of consequences and often times the perceived "treatments" leads to additional unintended consequences and social burden. Thus, there is an unmet need for a smarter approach to helping people manage stress, anxiety, and burnout. A low-technology, yet smart, approach that could be more extensively adopted might feature certain nutritional ingredients that have been scientifically shown to help reduce the direct and indirect burdens of stress, anxiety, and burnout. Nutritional ingredients that may support in the alleviation of consequences of hyperstress, anxiety, and burnout include valerian, omega-3, niacin, lemon balm, l-theanine, and magnesium [2]. But quite possibly the most scientifically reviewed nutritional ingredient is L-tryptophan (tryptophan). This essential amino acid is instrumental in a range of metabolic functions related to the neuroendocrine system and thus has been the subject of many research studies and clinical trials over the last several decades..

Accordingly, this article reviews the role of tryptophan and other major metabolic functions that are known to directly and indirectly impact the user's physical and mental health state in both states of rest and high stress. Specifically, a review of the role of tryptophan on the synthesis of beneficial serotonin in the brain and other metabolites including kynurenine and melatonin will be provided. Also, insights on the latest research and clinical trials on the use of tryptophan supplements and the production of other metabolites that are known to be related to significant health conditions including stress and burnout, depression and related conditions, and sleep disorders like insomnia will be explored in detailed in this case study.

The Metabolic Functions of Tryptophan

The main role of the amino acid tryptophan is, as almost every amino acid, that it is an input in protein synthesis and a wide range of other metabolic functions. Tryptophan is actually less prevalent in the body when compared to other amino acids despite its major role of protein synthesis in the brain and most tryptophan must be obtained through the diet, thus making it an essential nutrient (6,7). Specifically, tryptophan is the antecedent input of two other important metabolic pathways: kynurenine synthesis and serotonin synthesis. The first level of the regulation of tryptophan metabolism occurs in the complex ecosystem of the intestine through the gut microbiota. Indeed, diet including tryptophan supplementation is first digested in the gut and metabolized by the gut microbiota. Directly or indirectly, the gut microbiota controls the serotonin, kynurenine and indole derivatives tryptophan metabolism pathways, therefore suggesting that the quality and composition of the gut microbiota is the first modulator of the tryptophan metabolism upon diet supplementation.

Apart from host enzymes that metabolize tryptophan, intestinal bacteria can directly transform tryptophan into several molecules including indole-3-aldehyde (IAld), indole-3-acid-acetic (IAA), indole-3-propionic acid (IPA), indole-3-acetaldehyde (IAAld) and indoleacrylic acid, all these molecules being ligand for aryl hydrocarbon receptor (AhR). AhR pathway is a crucial modulator of the immune response (77).

Kynurenine synthesis is the second most prevalent metabolic pathway of tryptophan after protein synthesis and accounts for approximately 90% of tryptophan metabolism (7,8). Above all, kynurenine is the precursor of kynurenic and quinolinic acids (7,8). Kynurenic acid is a glutamate receptor antagonist, meaning it blocks neurotransmitted signals in the brain and quinolinic acid is a glutamate receptor agonist (9).

Considering the kynurenine pathway, IDO1 (Indoleamine 2,3-Dioxygenase 1) is the key enzyme whose activity is stimulated by the gut microbiota and that produces kynurenine and derivatives molecules involved in inflammation, immune response and neurotransmission (77). Finally, the gut microbiota plays a key role in the production of 5-HT from tryptophan. Indeed, 5-HT is an important gastrointestinal signalling molecule (77). Altered gut microbiota in inflammatory diseases including inflammatory bowel diseases (IBDs, including Crohn disease, ulcerative colitis, irritable bowel syndrome and metabolic syndrome) affects Tryptophan metabolism and is associated with intestinal immunity dysfunction (77). Therefore the gut microbiota can influence the brain through the tryptophan metabolism and subsequent derivatives molecules in the three major pathways: kynurerine metabolism, serotonin metabolism and indoles – Ahr ligands metabolism.

Apart from tryptophan-metabolizing microorganisms that are far from being fully elucidated, opportunities of using tryptophan metabolism through tryptophan supplementation in diet may be considered in association with the gut microbiota quality and composition**.** In metabolic syndrome and gut dysbiosis, increased vitamin C intakes (100mg/d) restore gut function, modulate vitamin E levels, reduce endotoxemia, decrease pro-inflammatory biomarkers and restore antioxidant status to control oxidative stress and in particular reactive oxygen species (ROS) (78). Thus, it can be suggested that considering that stressed microorganisms in the gut can interfere with digestion and metabolism, diet supplementation with tryptophan in addition to bioactive molecules such as vitamin C that will facilitate or help the gut microbiota activity may be seriously considered for future research

Oxidative stress meaning ROS and probably more largely reactive species has to be considered. Since 2017, the so-called oxidative stress is strongly debated considering the novel integrative concept of Reactive Species Interactome (RSI) for ROS, reactive nitrogen species (RNS), reactive sulphur species (RSS) and reactive carbonyl species (RCS) (79). Related to kynurerine pathway from tryptophan metabolism, KYNA or kynurenic acid is an irreversible product from kynurenine and ROS (80). KYNA not only influences the immune system but also the inflammation and carcinogenesis. Moreover KYNA is an agonist of AhR, linking indole pathway and kynurenine pathway from tryptophan metabolism to AhR modulation and subsequent physiological and pathological consequences. Thus, it is suggested that future research focus not only investigate the role and relationship of ROS with tryptophan metabolism but the complete RSI (ROS, RNS, RSS, RCS) role and relationship with tryptophan metabolism. Moreover, considering vitamin C as antioxidant, the combination of tryptophan and vitamin C in diet may be of interest to maintain antioxidant status and to modulate RSI status, status that will need to be defined and characterized as actually it remains unknown.

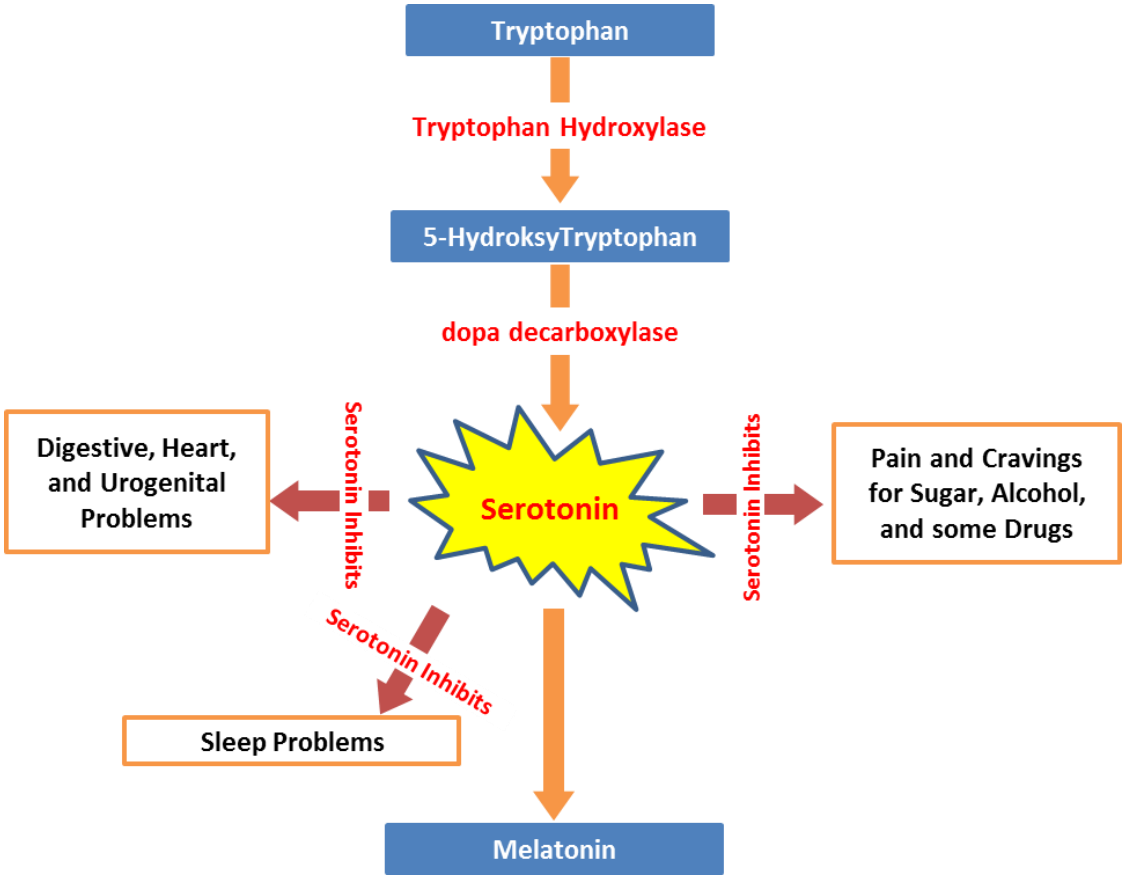
Regarding specific diseases and in particular cancer and brain tumors (namelly glioma including glioblastoma), it has been recently shown that the grade of the tumor is associated with the AhR activation and with altered tryptophan metabolism (82). AhR antagonism efficiently reduced tumor cell viability *in vitro* in the same cited paper. In addition, tryptophan metabolism and more specifically L-tryptophan metabolism is of great interest in ageing and against at least cancer, neurodegenerative diseases, neuropsychiatric diseases, autoimmune diseases and through the gut microbiota, the liver, the immune system and the brain (81).

In addition to kynurenine, tryptophan also plays an important role in serotonin synthesis (11). Serotonin is a specific neurotransmitter that relays signals to nerve cells and is mostly known for its role as a major provider of feelings of well-being and happiness, yet serotonin's actual biological function is highly complex and multifaceted making it difficult to monitor in clinical research). Serotonin is expected to have a major metabolic role in modulating cognition, reward, learning, memory, and numerous physiological processes. Furthermore, serotonin deficiency in the brain can result in states of 'emotional dysfunction' such as anxiety, depression and insomnia which in turn can lead to fibromyalgia, chronic headaches and weight gain, among other physical consequences (12).

Overall, only 3% of tryptophan obtained from the diet is used in serotonin synthesis throughout the entire body and only 1% tryptophan obtained from the diet is used for serotonin synthesis in the brain and furthermore, it is estimated that only 5% of serotonin found in body resides in the brain (13). However, despite the relatively low concentration of brain serotonin compared to that in the rest of the body, it has a wide-ranging role as a neurotransmitter and neuromodulator (12,13).

Serotonin synthesis is a two-step process. First, the tryptophan hydroxylase enzyme biologically-catalyzes tryptophan in the brain in order to make 5-HTP. Then, the resultant 5-HTP is again catalyzed with the aromatic amino acid decarboxylase enzyme to make serotonin (2). Some research does suggest that direct supplementation with 5-HTP could be beneficial for serotonin production, but observed clinical outcomes highly varies owing to a number of uncontrollable environmental factors such as genetics, the subject's typical stress and activity levels, and their diets which are difficult to control for in a research setting (2). See Figure 3 for a visual representation of the tryptophan-serotonin-melatonin pathway and links to the burden of health problems.

Figure 3. The Tryptophan-Serotonin-Melatonin Pathway and Links to the Burden of Health Problems



Melatonin is a key hormone that is produced in the tryptophan-serotonin pathway (14,15). Melatonin regulates diurnal rhythms of the body and is expected to influence the reproductive system, the immune system and digestive and gastrointestinal processes (14). Melatonin production is regulated by the blue light spectrum in either natural (sun) or artificial light and it is actively secreted at night (in darkness) from the pineal gland in order to induce hormonal and neural impacts including the regulation of behavioural circadian rhythms and sleep patterns (16).

Besides kynurenine, serotonin and melatonin, other metabolic functions are induced by tryptophan, which complicates the ability to zero-in on a useful mechanism of action from tryptophan supplementation. One of these active compounds is tryptamine. Tryptamine is an active compound that is an important neuromodulator of serotonin (17). Specifically, a number of animal studies have shown that tryptamine may act as an emotional balancing control between the excitation and inhibition functions of serotonin (17) Tryptamine has also shown to act as a neurotransmitter with specific receptors that are independent of the function of serotonin (17). More human-based clinical research is required to understand the role of tryptamine in cognitive health.

In addition, tryptophan acts as a substrate for coenzyme nicotinamide adenine dinucleotide (NAD) synthesis and NAD phosphate (NADP) synthesis. Specifically, NAD and NADP are

essential for redox reactions or electron transfer reactions in all living cells. These enzymes can be synthesized from ingested tryptophan or from ingested niacin (vitamin B3) (7,18,19). And complicating the matter, niacin can be directly synthesized from tryptophan through the kynurenine/quinolinic acid pathway though this is a less efficient use of tryptophan since approximately 60 mg of tryptophan are necessary to generate a single milligram of niacin. This is especially true since the median intake of niacin from food in France is sufficient to fill nutritional needs making the need for additional niacin from tryptophan synthesis low.

Finally, tryptophan also affects other neurotransmitters and nervous system compounds. For example, dopamine, norepinephrine, and beta-endorphin have all been shown to increase following oral dosing of tryptophan (11,20-23). Cortisol is also shown to be directly modulated from tryptophan ingestion through serotonin synthesis, though the exact meaning of this effect is still up for debate based on the mixed results from the clinical literature (50-59).

Review of Tryptophan's Mechanisms of Action

The mechanism of act of tryptophan and how this will directly or indirectly manifest itself as a health benefit is still an active question of inquiry within the scientific community. However, and despite over four decades of active research into this topic, there is still a high degree of heterogeneity of research designs, tested hypotheses, and protocols when it comes to understanding tryptophan's mechanism of action. What is known is that tryptophan is the single precursor of serotonin produced in the brain. Also, tryptophan is distributed in the body in the circulatory system. More specifically, tryptophan is that is available for passage across the blood-brain barrier, especially the non-albumin bound free tryptophan, though most blood-circulating tryptophan found in the blood is bound to albumin (6,7,24-27). Tryptophan does have a higher affinity for the blood-brain barrier transporter compared to albumin, meaning that up to three-quarters of albumin-bound tryptophan may be available to cross the blood-brain barrier (28).

Tryptophan contends with other amino acids such as histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tyrosine, and valine for the blood-brain barrier transporter and researchers have found that tryptophan's bioavailability for transport across the blood-brain barrier can be described as a ratio of tryptophan to the sum of its competing amino acids (25,29-31). Consequently, a change in the ratio of tryptophan compared to the other amino acids can impact the amount tryptophan available for serotonin synthesis in the brain (32-35). This means that the bioavailability of tryptophan in the brain can increase by either increasing the plasma concentration of tryptophan in the blood or by decreasing concentrations of the other amino acids since either approach will increase tryptophan's relative availability for serotonin synthesis. Thus, the tryptophan-competing amino acid ratio and total availability of tryptophan are the two primary factors most likely to affect serotonin production.

This suggests that diet is a major contributing factor in the bioavailability of tryptophan for serotonin production. Specifically, it is hypothesized that tryptophan availability can increase by eating more carbohydrate-rich foods and less protein-rich food (7,25,24,36). This is because protein-rich foods would significantly increase the bioavailability of all types of amino acids, including tryptophan but at a lesser extent compared to the competing amino acids (7,25,24,36,37). Thus, the tryptophan-competing amino acid ratio decreases. Furthermore, eating carbohydrate-rich food increases insulin in the blood which in turn decreases plasma levels of competing amino acids, thus, the tryptophan-competing amino acid ratio increases. Time of ingestion is also a factor that impacts the tryptophan-competing amino acid ratio where earlier in the day the meal is consumed, the greater the observed change in the ratio.

In summary, dietary considers, especially with respect to the ratio of carbohydrate to protein in meal, is expected to change the bioavailability of tryptophan for synthesis of brain serotonin which in turn increases the difficulty of quantifying an effective dosage size for tryptophan unless the diet is strictly controlled for. But despite this, it is expected that controlling the tryptophan-competing amino acid ratio and the absolute amount of free tryptophan (non-albumin bound) will most likely to affect serotonin production levels in the brain.

Review of Tryptophan's Therapeutic Applications

Tryptophan is required for the production of niacin (vitamin B3) (1,2). It is predominantly used in animal and human health applications including dietary supplements, animal feed additives, and in pharmaceutical applications (1,2). With respect to dietary supplements, tryptophan was originally marketed as a means to support appetite suppression, help to alleviate the symptoms of menopause, and as a weight loss aid (1). In 1990, the U.S. Food and Drug Administration (FDA) banned the sales of tryptophan stating that it caused a deadly disease called Eosinophillia-mylagia Syndrome (EMS) though it was later found to be actually related to specific batches of tryptophan supplied by a single manufacturer and it was shown that this batch was actually contaminated by trace impurities (3,4). The ban on the use of tryptophan in dietary supplements was eventually lifted in 2005. According to the Tryptophan in Food Regulation (England) 2005, tryptophan can be added to dietary supplements, provided it met the purity criteria specified in the European Pharmacopoeia (1).

Tryptophan also aids in the digestion of food and regulates appetite in animals, which has driven its increased use in animal feed formulations (1). It enhances the appetite of young animals, which in turn helps in improving their growth and meat quality. Corn and corn-soy diets are low in this amino acid. Tryptophan is therefore used to supplement animal feed, especially pig and poultry feed, though other amino acids have grown more common in recent years including valine and thereonine. In addition, the use of tryptophan in animal feed reduces

the requirement of natural protein sources, which in turn minimizes nitrogen excretion levels and helps to minimize environmental pollution.

Tryptophan is necessary for the production of a neurotransmitter serotonin, which is a mood regulator and has found some useful applications in the production of some pharmaceutical products. Tryptophan is widely used in antidepressant pharmaceutical formulations. Alti-Tryptophan is an example of one of the drugs containing tryptophan, which is used in antidepressant therapy (1).

The use of tryptophan supplements has been the focus of numerous clinical research and homeopathic applications for over four decades, though the therapeutic targets are varied. Today, tryptophan is commonly used as a natural remedy for managing depression, pain, insomnia, hyperactivity, and eating disorders though the breadth and depth of clinical research supporting efficacy is still comparably small (2). However, it has been found that tryptophan has revealed positive results in the treatment of seasonal affective disorder (SAD) and may be just as effective as the more common light therapy (55). Also, improvements in sleep latency time have been supported by the clinical research due to its link to melatonin mechanisms (38,39). Finally, the use of tryptophan supplements have been found to have positive therapeutic effects on brain health including the use of tryptophan combined with iproniazid, a monoamine oxidase inhibitor (MAOI) to manage schizophrenia, the use of tryptophan as tricyclic antidepressants, and as a regimen to support the nicotine withdrawal symptoms when one is quitting smoking (40).

While direct consumption of tryptophan supplements has shown promise based on a review of the clinical research, there has been a noticeable lack of convergence on defining the link between ingested tryptophan takes serotonin and/or other metabolite production. Consequently, many researchers have looked for other related ingredients that may help to better define this link. One of these ingredients is 5-HTP, or 5-Hydroxytryptophan. 5-HTP is an intermediate metabolite derived from the amino acid tryptophan that is produced during the melatonin and serotonin production process (2). The idea behind the direct supplementation and use of 5-HTP instead of tryptophan is that it skips a production step in the metabolic process and thus may enhance the ability to directly control melatonin and serotonin production levels and in turn ensure a more effective certain health benefit (2).

Supplementation with 5-HTP has been explored by researchers with regards to possible therapeutic links to managing anxiety, depression, fibromyalgia, insomnia and even obesity (41-49). However, the state of the science of 5-HTP supplementation and its link to specific health benefits is still in nascent stage. In human trials, most of the research on 5-HTP supplementation involves trials with small sample sizes that yield mixed results (2). A relatively small number of studies suggest that 5-HTP may reduce anxiety though additional research is needed to confirm effectiveness and safety (45,48). In summary, more work is needed to further identify the mechanisms of action and situations under which 5-HTP may impart positive effects or pose safety concerns when used, especially when used in conjunction with other medications due to 5-HTP's modulatory effects on neurotransmitters.

One area of intense research of the impact on the use of tryptophan and 5-HTP has been on trying to identify an observable and measureable biomarker as a means to support the link of supplementation to the aforementioned therapeutic areas. One such biomarker thought to promising is saliva cortisol levels given the environmental conditions of the user base and each user's self-reported wellness.

Researchers began exploring the link between serum cortisol levels given the use of 200 mg of 5-HTP in the 1980s with explorations of the effect of 5-HTP supplementation on seasonal affective disorder (SAD) (55). The authors found that an enhanced melatonin secretory response to 5-HTP in a single hypermelatonemic patient with SAD (55). In 1997, 83 subjects were administered 5-HTP or a placebo and found that increased plasma cortisol among medicated and unmedicated patients with major depression or OCD (56). The 5-HTP–induced cortisol levels were significantly higher in fluoxetine-treated patients compared to unmedicated major depressed patients. The same group of researchers found similar results in 1984 (57-59).

In 2002, researchers found that use of 200 mg of 5-HTP among 30 patients show that 5-HTP stimulated salivary cortisol could be a useful probe of serotonin function in healthy volunteers as well as panic disorder patients, and they provide some evidence against a serotonin receptor hypersensitivity in panic disorder (52) Gijsman et al 2002 discovered that an increase of cortisol after 5-HTP plus carbidopa is comparable with the effect of other drugs used in challenge tests that were expected to temporarily raise stress levels among participants (53). The researchers found that a further increase of the dose of 5-HTP might improve the size of the effect on endpoints as long as the tolerability remains good (53)

Cerit et al 2013 found from a sample of 46 patients that 2.8 grams of tryptophan attenuates the cortisol response to acute social stress depending on 5-HTTLPR genotype. S'/S' carriers may indeed be more reactive to 5-HT manipulations (50). Markus et al 2009 found that 5-HTTLPR differentially mediates the effect of tryptophan on mood and performance among a group of 30 patients tested given use of a 800 mg of tryptophan daily (51). Only in S'/S' genotypes, tryptophan improved mood and backward counting. This suggests pronounced 5-HT vulnerability to tryptophan challenge in healthy S'/S' as compared with L'/L' carriers. Effects of tryptophan administration in S'/S' genotypes were not influenced by stress exposure, probably because stress exposure was too mild to be of any relevance. Brain 5-HT manipulation might still influence mood under stress in S'/S' when including a more severe stressful event.

In 2014, Capello et al tested saliva cortisol levels among a group of 118 patients that either used a 3000 mg of tryptophan or a placebo and it was revealed that tryptophan treatment caused a clear reduction in stress-induced cortisol levels in S/S-allele carriers exclusively, and prevented a stress-induced increase in appetite only in S/S-allele carriers with high trait neuroticism (54). The findings reveal an advantageous effect of sub chronic tryptophan treatment on stress experience and appetite depending on stress and genetic serotonergic vulnerability (54).

In summary, the research suggests that for those individuals that carry the S'/S' genotype, the use of tryptophan has an expected reduction in cortisol levels of 50 to 200 µg/dL or 1300 to 5500 nmol/L. These changes in cortisol levels in the response to stress, if frequently occurred such as in a workplace environment, could lead to reduced CAC progression over time which in turn can lead to decreased risk of CHD in the future given the use of tryptophan. See Figure 4 for the summary of selected studies that demonstrate the impact of tryptophan use on salivia cortisol levels under conditions of induced stress.

Figure 4. The Impact of Tryptophan Use on Salivia Cortisol Levels under Conditions of Induced Stress, Selected Clinical Research

Author	Sample Size	Geno-type	Person Type	Dose Size	Stress?	Baseline Cortizol Levels, µg/dl	Reported Effect Size[1], µg/dl	Description of Change/Result
Cerit H 2013	25	S'/S'	Healthy	2.8 g/d for 6 days	Yes	143.3	(194.7)	Significant reduction of Cortisol Stress and tryptophan intake
Cerit H 2013	21	L'/L'	Healthy	2.8 g/d for 6 days	Yes	166.6	(8.3)	Minor reduction of Cortisol Stress and tryptophan intake
Markus CR 2009	16	S'/S'	Healthy	0.8 g/d	Yes	362.5	(50.0)	Significant reduction of Cortisol Stress and tryptophan intake
Markus CR 2009	14	L'/L'	Healthy	0.8 g/d	Yes	362.5	25.0	Increase of Cortisol Stress and tryptophan intake
Capello AEM 2014	60	S'/S'	Healthy	3.0 g/d	Yes	184.6	(57.9)	Significant reduction of Cortisol Stress and tryptophan intake
Capello AEM 2014	58	L'/L'	Healthy	3.0 g/d	Yes	199.7	27.5	Increase of Cortisol Stress and tryptophan intake

1. Change in saliva cortisol levels from baseline after tryptophan intake

The Potential Economic Benefits of Tryptophan Supplementation

In terms of possible economic benefits from the use a tryptophan supplement on relieving the burden of workplace-related stress in France, the first task is to determine the proportion of the working age population that carries the S'/S' genotype. Based on a review of the scientific literature, this data point is unknown, but it is expected to be approximately 20 to 30% of the total population, or approximately 11 million French citizens of working age based on a review of the few studies that explore this relationship [51,52,53] In addition and as shown in Figure 5, those individuals that carry the S'/S' genotype is expected to have an approximate -45% reduction in the mean size of the change in cortisol levels when user is stressed. This means that approximately 11 million working age individuals in France could minimize the accumulation of cortisol by as much as 45% compared to non-users of tryptophan if they were use a tryptophan supplement prior to being introduced into a stressful environment.

In 2012, researchers assessed the correlation between cortisol responses to laboratory-induced mental stress and the progression of coronary artery calcification (CAC) [74]. The researchers found that there was an association between cortisol stress reactivity and CAC progression (odds ratio = 1.27, 95% CI, 1.02–1.60) all else being equal. Approximately 40% of the sample responding to the stress tasks had an increase in cortisol levels of at least 1 mmol/l [74]. CAC progression is defined as an increase >10 Agatston units between baseline and follow up [74]. CAC levels have been shown to be a statistically significant risk factor for CHD. One study found that the hazard ratios associated with a 1 standard deviation CAC increase were 1.40 (95% CI, 1.16-1.69; P < .001) for cardiovascular disease, 1.44 (95% CI, 1.02-2.02; P = .04) for myocardial infarction, 1.39 (95% CI, 1.10-1.76; P = .006) for heart failure, and 1.19 (95% CI, 0.94-1.51; P = .15) for all-cause mortality [74]. Thus, this suggests that controlling cortisol reactivity, such as through a nutrition-based regimen may influence the risk of CHD though more research is needed to test for this expectation.

Thus, the expression of changes in cortisol in the response to stress is a product of the interaction of nervous system and hormones including kynurenine, melatonin and serotonin. These changes in cortisol levels in the response to stress, if frequently occurred such as in a workplace environment, can lead to elevated CAC progression which in turn can lead to increased risk of CHD in the future. But this is just one of many examples of possible metabolic functions that are a part of workplace-related stress and burnout and that could be modified through nutrition-based intervention.

As stated, CAC levels have been shown to be a statistically significant risk factor for CHD. One study found that the hazard ratios associated with a 1 standard deviation CAC increase were 1.40 (95% CI, 1.16-1.69; P < .001) for cardiovascular disease, 1.44 (95% CI, 1.02-2.02; P = .04) for myocardial infarction, 1.39 (95% CI, 1.10-1.76; P = .006) for heart failure, and 1.19 (95% CI, 0.94-1.51; P = .15) for all-cause mortality [74]. Assuming that the inverse still holds and that the hazard ratio is broadly equivalent to relative risk of CVD, then the use of tryptophan to reduce the accumulation of cortisol during stressful events that the user faces and in turn reduce CAC progression by 1 standard deviation, then the estimated relative risk reduction of getting cardiovascular disease is 28%, the relative risk reduction of a myocardial infarction event is 30%, the relative risk reduction of a heart failure event is 28%, and the relative risk reduction of all-cause mortality event is approximately 18% among users.

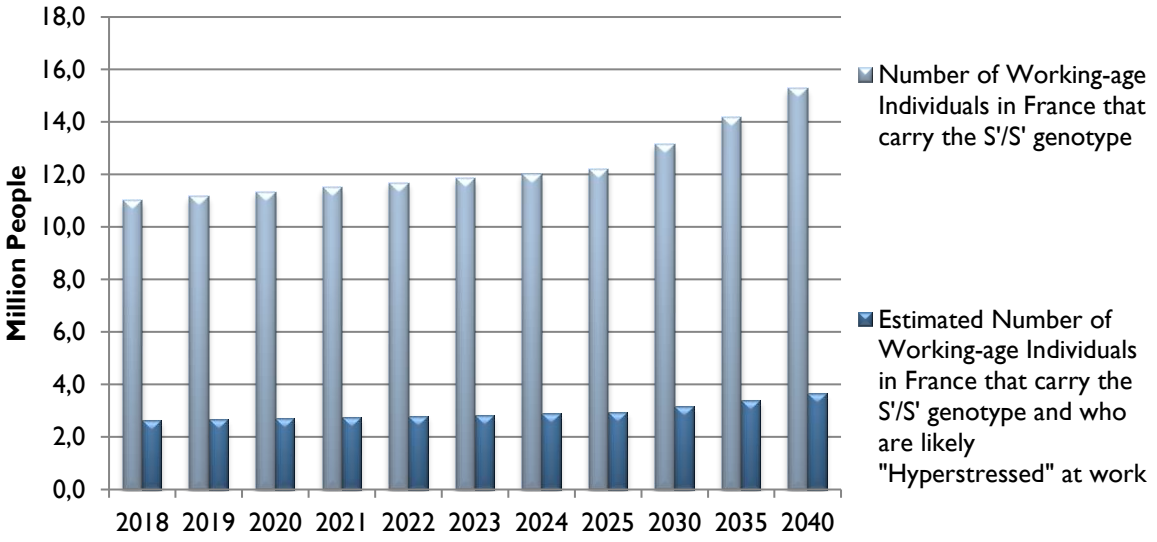
Figure 5: Total Population of France by Age Cohort - All Genders, Million People, 2015-2040

Age Cohort	2018	2019	2020	2021	2022	2023	2024	2025	2030	2035	2040
Number of Working-age Individuals in France that carry the S'/S' genotype, Million	11.0	11.2	11.3	11.5	11.7	11.9	12.0	12.2	13.2	14.2	15.3
Estimated Number of Working-age Individuals in France that carry the S'/S' genotype and who are likely "Hyperstressed" at work, Million*	2.6	2.7	2.7	2.8	2.8	2.8	2.9	2.9	3.2	3.4	3.7

* Estimate based on calculating the product of the number of working-age individuals in france that carry the S'/S' genotype by 24% expected to "hyperstressed".

Source: Godet-Cayré V et al. 2006, Béjean, S. & Sultan-Taïeb, H. 2005., Sultan-Taïeb, H., Chastang, J. F., Mansouri, M., & Niedhammer, I. 2013 and Frost & Sullivan analysis

Figure 6: Total Population of France by Age Cohort - All Genders, Millions of People, 2015-2040



Source: Godet-Cayré V et al. 2006, Béjean, S. & Sultan-Taïeb, H. 2005., Sultan-Taïeb, H., Chastang, J. F., Mansouri, M., & Niedhammer, I. 2013 and Frost & Sullivan analysis

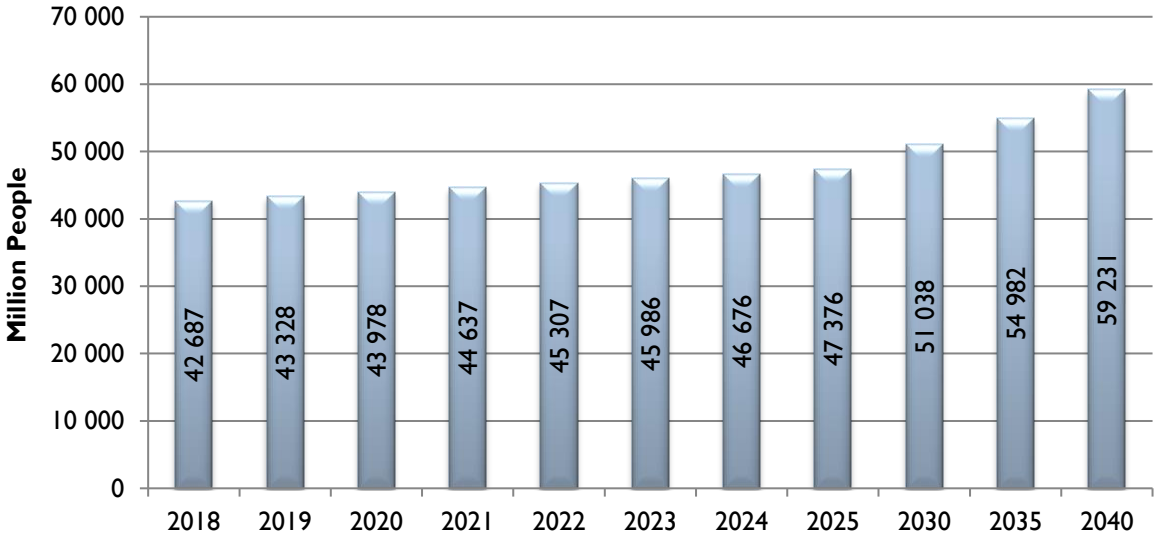
Figure 7: From Health Benefits to Economic Benefits, Author Calculations, 2015-2040

Measurements	2018
Number of Working-age Individuals in France that carry the S'/S' genotype, Million People	11.0
Estimated Number of Working-age Individuals in France that carry the S'/S' genotype and who are likely "Hyperstressed" at work, Million People	2.6
Risk of CVD in the Working Age Population in France, %	6%
Expected Reduction in Cortisol Accumulation given use of a Tryptophan Supplement, %	-45%
Reduction in the Odds of CAC progression given the Reduction in Cortisol Accumulation from Tryptophan Supplement, %	20%
Estimated Relative Risk due to the Reduction in the Odds of CAC progression given the Reduction in Cortisol Accumulation from Tryptophan Supplement, %	72%
Estimated Relative Risk Reduction due to the Reduction in the Odds of CAC progression given the Reduction in Cortisol Accumulation from Tryptophan Supplement, %	28%
Estimated Number of Avoidable CVD events given Use Tryptophan to Realize the Reduction in the Odds of CAC progression given the Reduction in Cortisol Accumulation from Tryptophan Supplement, People Cases	42,687
Estimated Cost of Avoidable CVD events given Use Tryptophan to Realize the Reduction in the Odds of CAC progression given the Reduction in Cortisol Accumulation from Tryptophan Supplement, € Million	309.7

Source: Godet-Cayré V et al. 2006, Béjean, S. & Sultan-Taïeb, H. 2005., Sultan-Taïeb, H., Chastang, J. F., Mansouri, M., & Niedhammer, I. 2013 and Frost & Sullivan analysis

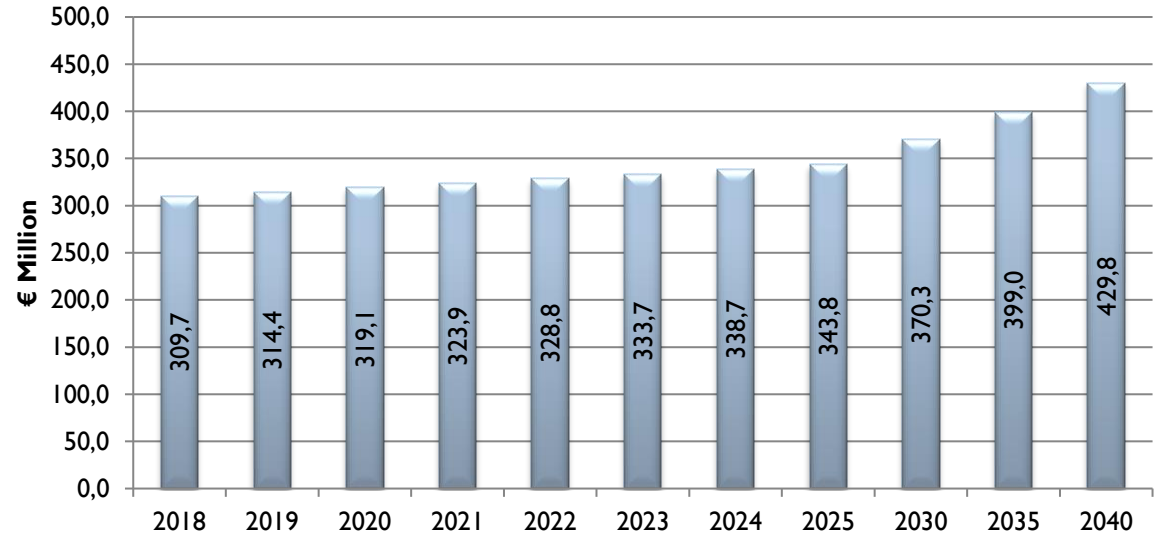
In 2018, it is estimated that up to 2.6 million working-age people in France are hyperstressed and carry the S'/S' genotype. These individuals and are most at risk of developing CVD and could benefit from using a tryptophan. These individuals that use a tryptophan supplement is expected to yield 42.7 thousand potential benefactors. See Figures 7 and 8 for detailed results number needed to use tryptophan to realize a beneficial effect to one individual given the use of tryptophan at supportive intake levels and total number of possible benefactors in France.

Figure 8: Estimated Number of Avoidable CVD events given Use Tryptophan to Realize the Reduction in the Odds of CAC progression given the Reduction in Cortisol Accumulation from Tryptophan Supplement, 2015-2040



Source: Frost & Sullivan

Figure 9: Estimated Cost of Avoidable CVD events given Use Tryptophan to Realize the Reduction in the Odds of CAC progression given the Reduction in Cortisol Accumulation from Tryptophan Supplement, € Million, 2015-2040



Source: Frost & Sullivan

Regarding total potential economic benefits that are possible, if all adults utilized tryptophan at supportive intake levels, € 309.7 million in health care system and opportunity cost savings could have been realized in 2018 given the average cost of a CVD treatment of € 7,114. By 2025, € 344 million in health care system cost savings could be realized and by 2040, up to € 8.4 billion in cumulative cost savings could be obtained. Figure 7 reports the total potential benefits that could be realized from the use of tryptophan supplements at supportive intake levels. Of course, achieving 100% utilization among the entire target end user base is a hypothetical best case and the purchase and utilization of tryptophan supplements is required to capture the aforementioned healthcare cost savings. For the daily use of a tryptophan supplement as a means to support the adverse effects (increasing cortisol levels leading to increased progression of CAC) to be deemed cost effective, then the annual cost of tryptophan use per capita cannot exceed € 115 per capita.[1] See Figure 9 for the estimated cost of avoidable CVD events given use tryptophan to realize the reduction in the odds of CAC progression given the reduction in cortisol accumulation from tryptophan supplement.

Concluding Remarks

To increase the utility of tryptophan research for understanding the relationship of serotonin dysregulation as an underlying mechanism in psychiatric disorders, as well as behavioral, cognitive, and physical problems, it will be important to understand the factors that have contributed to the inconsistent results from previous studies. Furthermore to advance the efficacy and utility of tryptophan for therapeutic purposes, future clinical studies must improve and standardize research protocols and avoid the methodological mistakes that have occurs in past research. These protocols include standardizing dosing, research and treatment methodologies, and improved selection of treatment and control groups being tested.

There is a biomarker to be used in priority to demonstrate the positive relationship between tryptophan and the couple serotonin and melatonin. This biomarker is the 5-HT, standing for 5-Hydrotryptophan. Its levels could be measured in the human body thanks to conventional methods of measuring serotonin synthesis and methods using positron emission tomography (PET) tracers. PET is a non-invasive technique which can trace metabolic processes, like serotonin synthesis. One tracer developed for this purpose is 5-hydroxy-l-[β-11C]tryptophan ([11C]5-HTP). 5-HTP is difficult to produce, but trapping this compound may represent well serotonin synthesis. PET with radiolabelled substrates for the serotonergic pathway is the only direct way to detect changes of serotonin synthesis in the living brain (60). Thus, research should be oriented towards proving the efficiency of tryptophan and identifying how its positive effects must be leveraged.

[1] To determine the maximum cost of supplementation given that its use is still deemed cost effective, then the annual cost cannot be greater than the average product of Cost of CVD treatment (€ 7,114 per person case), the risk of CVD for the target population (5.8%) and the expected relative risk reduction (28%)

In addition, a common and well-known deficiency in Vitamin B1 is very often observed in healthy populations. This issue is particularly predominant in developing geographies such as Africa or Southern Asia. (62, 63) This can also be problematic amongst alcoholic or obese populations (64). Vitamin B1 depletion – also called thiamine depletion – is at the root of a disease called Beriberi leading to tiredness in its weak form, and more generally affects negatively the nervous and cardiac systems (65). The fact that thiamine depletion is a widespread problem throughout the world could jeopardize the assessment of Tryptophan as a good supplement to fight against stress through the pathway activation and the already described mechanism of action. Indeed, thiamine deficiency has been demonstrated to inhibit the effect of a Tryptophan increase on the pathway activation (61). Therefore, Tryptophan would be relevant to be assessed in clinical trials as a stand-alone with the use of two different groups - one control and one nitrogen-neutral placebo group, that is to say which will not have received any amino-acids supplementation - as it will ensure the Tryptophan's effect would not be erased. When it comes to actual supplementation, an assessment of the local population Vitamin B1 status might be relevant in order to decide whether the best solution would be a Tryptophan stand-alone supplementation or a couple Tryptophan / Vitamin B1 to ensure the targeted effect will not be reduced because of thiamine deficient populations.

Finally, there are some important considerations to for researchers when it comes to the future of tryptophan supplement research. First, cortisol cannot be used as the only biomarker to illustrate tryptophan's efficiency. It is neither the best option nor is it the only option. Cortisol excretion is a brain response that is modulated by a wide variety of factors including tryptophan. More specifically, stress is positively correlated to cortisol excretion based on the review of the scientific literature, but in non-stress situations the opposite was found to be true. (66). Even the time of the day impacts cortisol levels. Thus, cortisol cannot be used as the only biomarker showing the positive influence of Tryptophan on stress as Cortisol levels reduction will be an indirect factor as studied already, a response of the serotonin / melatonin secretion.

Also, a general consideration needs to be reinforced about Tryptophan. The diet, and more specifically the amino acids intake, plays a crucial role in the positive or limited effect of Tryptophan. Indeed, in the case of Arginine for instance, as this amino acid is known for activating the RNS pathway which stands for the Reactive Nitrogen Species (67). This RNS pathway has a negative impact on the Tryptophan pathway as it would favor kynurenic acid creation – this still needs to be demonstrated clinically however (68). As a conclusion, tryptophan would fully release its benefits as long as no other amino acids are interfering in the subject's diet.

In summary, controlling the tryptophan-competing amino acid ratio and the absolute amount of free tryptophan levels available for synthesis directly affects serotonin production levels in the brain. Consequently, this should be the future researcher's priority moving forward. However, it should be remembered that dietary considers, especially with respect to the ratio of carbohydrate to protein in the diet, impacts the bioavailability of tryptophan for brain

serotonin synthesis but this also increases the difficulty of quantifying an effective dose size for tryptophan.

References

[1] European Amino Acids Market. B521-01 Frost & Sullivan. 2006

[2] Memorial Sloan-Kettering Cancer Center. About Herbs, Botanicals & Other Products - Intergrative Medicine. Retrieved June 2019, http://www.mskcc.org/cancer-care/integrative-medicine/about-herbs-botanicals-other-products

[3] Kilbourne EM. Eosinophilia-Myalgia Syndrome: Coming to grips with a new illness. Epidemiol Rev. 1992;14:16–36.

[4] Takagi H, Ochoa MS, Zhou L, et al. Enhanced collagen synthesis and transcription by Peak E, a contaminant of L-tryptophan preparations associated with Eosinophilia-Myalgia Syndrome epidemic. J Clin Invest. 1995;96:2120–5. [PMC free article] [

[6] Wurtman RJ, Hefti F, Melamed E. Precursor control of neurotransmitter synthesis. Pharmacol Rev. 1981;32:315–35.

[7] Sainio EL, Pulkki K, Young SN. L-tryptophan: Biochemical, nutritional and pharmacological agents. Amino Acids. 1996;10:21–47.

[8] Harper AE, Yoshimura NN. Protein quality, amino acid balance, utilization, and evaluation of diets containing amino acids as therapeutic agents. Nutrition. 1993;9:460–9.

[9] Moroni F. Tryptophan metabolism and brain function: Focus on kynurenine and other indole metabolites (Review) Eur J Pharmacol. 1999;375:87–100.

[10] Agence nationale de securite du medicament et des produits de sante. (2017) État des lieux de la consommation des benzodiazépines en France . ansm.sante.fr

[11] Sanger GJ. 5-Hydroxytryptamine and the gastrointestinal tract: Where next. Trends in Pharmacological Sciences. 2008;29:465–71.

[12] Sandyk R. L-tryptophan in neuropsychiatric disorders: A review. Int J Neurosci. 1992;67(14):127–44.

[13] van Praag HM, Lemus C. Monoamine precursors in the treatment of psychiatric disorders. In: Wurtman RJ, Wurtman JJ, editors. Nutrition and the Brain. New York: Raven Press; 1986. pp. 89–139.

[14] Szczepanik M. Melatonin and its influence on immune system. J Physiol Pharmacol. 2007;58S:115–24.

[15] Marz RB. Medical Nutrition from Marz. 2nd ed. Portland, OR: Omni-Press; 1999. pp. 200–5.

[16] Kayumov L, Casper RF, Hawa RJ, et al. Blocking low-wavelength light prevents nocturnal melatonin suppression with no adverse effect on performance during simulated shift work. J Clin Endocrinol Metab. 2005;90:2755–61. [Google Scholar

[17] Jones RSG. Tryptamine: A neuromodulator or neurotransmitter in mammalian brain. Prog Neurobiol. 1982;19:117–39.

[18] Rambali B, Van Andel E, Schenk G, et al. The contribution of cocoa additive to cigarette smoking addiction. RIVM report 650270002/2002. The National Institute for Public Health and the Environment; Netherlands: 2002.

[19] Mattevi A. A close look at NAD biosynthesis. Nat Struct Mol Biol. 2006;(7):563–4.

[20] Guilleminault C, Tharp BR, Cousin D. HVA and 5HIAA CSF measurements and 5HTP trials in some patients with involuntary movements. J Neurol Sci. 1973;18:435–41.

[21] Maes M, Vandevelde R, Suy E. Influences on cortisol and noradrenergic turnover of healthy controls and depressed patients during L-tryptophan loading. J Affect Disord. 1989;17:173–82.

[22] Traskman-Bendz L, Haskett RF, Zis AP. Neuroendocrine effects of L-tryptophan and dexamethasone. Psychopharmacol (Berl) 1986;89:85–8.

[23] Winokur A, Lindberg ND, Lucki I, et al. Hormonal and behavioral effects associated with intravenous L-tryptophan administration. Psychopharmacol (Berl) 1986;88:213–9.

[24] Fernstrom JD. Role of precursor availability in control of monoamine biosynthesis in brain. Physiol Rev. 1983;63:484–546.

[25] Hood SD, Bell CJ, Nutt DJ. Acute tryptophan depletion. Part I: Rationale and methodology. Aust N Z J Psychiatry. 2005;39:558–64.

[26] Etienne P, Young SN, Sourkes TL. Inhibition by albumin of tryptophan uptake by rat brain. Nature. 1976;262:144–5.

[27] Yuwiler A, Oldendorf WH, Geller E, et al. Effect of albumin binding and amino acid competition on tryptophan uptake into brain. J Neurochem. 1977;28:1015–23.

[28] Pardridge WM. The role of blood-brain barrier transport of tryptophan and other neutral amino acids in the regulation of substrate-limited pathways of brain amino acid metabolism. J Neural Transm. 1979;15(Supplement):43–54. [Google S

[29] Reilly JG, McTavish SFB, Young AH. Rapid depletion of plasma tryptophan: A review of studies and experimental methodology. J Psychopharmacol. 1997;11:381–92.

[30] Marsh DM, Dougherty DM, Moeller FG, et al. Laboratory-measured aggressive behavior of women: acute tryptophan depletion and augmentation. Neuropsychopharmacol. 2002;26:660–71.

[31] Lucini V, Lucca A, Catalano M, et al. Predictive value of tryptophan/large neutral amino acid ratio to antidepressant response. J Affect Disodr. 1996;36:129–33.

[32] Biggio G, Fadda F, Fanni P, et al. Rapid depletion of serum tryptophan, brain tryptophan, serotonin and 5-hydroxyindoleacetic acid by a tryptophan-free diet. Life Sci. 1974;14:1321–9.

[33] Gessa GL, Biggio G, Fadda F, et al. Effect of the oral administration of tryptophan-free amino acid mixtures on serum tryptophan, brain tryptophan and serotonin metabolism. J Neurochem. 1974;22:869–70.

[34] Young SN, Ervin FR, Pihl RO, et al. Biochemical aspects of tryptophan depletion in primates. Psychopharmacol (Berl) 1989;98:508–11.

[35] Nishizawa S, Benkelfat C, Young SN, et al. Differences between males and females in rates of serotonin synthesis in human brain. Proc Natl Acad Sci USA. 1997;94:5308–13. [PMC free article]

[36] Caballero B, Finer N, Wurtman RJ. Plasma amino acids and insulin levels in obesity: Response to carbohydrate intake and tryptophan supplements. Metabolism. 1988;37:672–6.

[37] Lieberman HR, Caballero B, Finer N. The composition of lunch determines afternoon plasma tryptophan ratios in humans. J Neural Transm. 1986;65:211–17.

[38] Korneer E, Bertha G, Flooh E, et al. Sleep-inducing effect of L-tryptophan. Eur Neurol. 1986;25:75–81.

[39] Schneider-Helmert D, Spinweber CL. Evaluation of tryptophan for treatment of insomnia: A review. Psychopharmacol (Ber) 1986;89:1–7.

[40] Lauer JW, Inskip WM, Bemsohn J, et al. Observations on schizophrenic patients after iproniazid and tryptophan. AMA Arch Neurol Psychiatry. 1958;80:122–30.

[41] Caruso I, Sarzi Puttini P, Cazzola M, et al. Double-blind study of 5-hydroxytryptophan versus placebo in the treatment of primary fibromyalgia syndrome. J Int Med Res. May-Jun 1990;18(3):201-209.

[42] Ceci F, Cangiano C, Cairella M, et al. The effects of oral 5-hydroxytryptophan administration on feeding behavior in obese adult female subjects. J Neural Transm. 1989;76(2):109-117.

[43] Iovieno N, Dalton ED, Fava M, et al. Second-tier natural antidepressants: review and critique. J Affect Disord. May 2011;130(3):343-357.

[44] Jangid P, Malik P, Singh P, et al. Comparative study of efficacy of l-5-hydroxytryptophan and fluoxetine in patients presenting with first depressive episode. Asian J Psychiatr. Feb 2013;6(1):29-34.

[45] Kahn RS, Westenberg HG, Verhoeven WM, et al. Effect of a serotonin precursor and uptake inhibitor in anxiety disorders; a double-blind comparison of 5-hydroxytryptophan, clomipramine and placebo. Int Clin Psychopharmacol. Jan 1987;2(1):33-45.

[46] Kious BM, Sabic H, Sung YH, et al. An Open-Label Pilot Study of Combined Augmentation With Creatine Monohydrate and 5-Hydroxytryptophan for Selective Serotonin Reuptake Inhibitor- or Serotonin-Norepinephrine Reuptake Inhibitor-Resistant Depression in Adult Women. J Clin Psychopharmacol. Oct 2017;37(5):578-583.

[47] Nolen WA, van de Putte JJ, Dijken WA, et al. Treatment strategy in depression. II. MAO inhibitors in depression resistant to cyclic antidepressants: two controlled crossover studies with tranylcypromine versus L-5-hydroxytryptophan and nomifensine. Acta Psychiatr Scand. Dec 1988;78(6):676-683.

[48] Schruers K, van Diest R, Overbeek T, et al. Acute L-5-hydroxytryptophan administration inhibits carbon dioxide-induced panic in panic disorder patients. Psychiatry Res. Dec 30 2002;113(3):237-243

[49] Turner EH, Loftis JM, Blackwell AD. Serotonin a la carte: supplementation with the serotonin precursor 5-hydroxytryptophan. Pharmacol Ther. Mar 2006;109(3):325-338.

[50] Cerit H (2013) The effect of tryptophan on the cortisol response to social stress is modulated by the 5-HTTLPR genotype.

[51] Markus CR, (2009) Effects of carbohydrates on brain tryptophan availability and stress performance.

[52] Schruers K, (2002) L-5-hydroxytryptophan induced increase in salivary cortisol in panic disorder patients and healthy volunteers.

[53] Gijsman HJ (2002) Placebo-controlled comparison of three dose-regimens of 5-hydroxytryptophan challenge test in healthy volunteers.

[54] Capello AE (2014) Effect of sub chronic tryptophan supplementation on stress-induced cortisol and appetite in subjects differing in 5-HTTLPR genotype and trait neuroticism.

[55] Jacobsen FM (1987) Neuroendocrine response to 5-hydroxytryptophan in seasonal affective disorder.

[56] Meltzer HY (1997) Fluoxetine, but not tricyclic antidepressants, potentiates the 5-hydroxytryptophan-mediated increase in plasma cortisol and prolactin secretion in subjects with major depression or with obsessive compulsive disorder.

[57] Meltzer HY (1984) Effect of 5-hydroxytryptophan on serum cortisol levels in major affective disorders. II. Relation to suicide, psychosis, and depressive symptoms.

[58] Meltzer HY (1984) Effect of 5-hydroxytryptophan on serum cortisol levels in major affective disorders. III. Effect of antidepressants and lithium carbonate.

[59] Meltzer HY (1984): Effect of pindolol on the L-5-HTP-induced increase in plasma prolactin and cortisol concentrations in man.

[60] Visser, A.K., Waarde, A.V., Willemsen, A.T., Bosker, F.J., Luiten, P.G., Boer, J.A., Kema, I.P., & Dierckx, R.A. (2010). Measuring serotonin synthesis: from conventional methods to PET tracers and their (pre)clinical implications. European Journal of Nuclear Medicine and Molecular Imaging.

[61] Katsumi Shibata, Ryoko Kobayashi & Tsutomu Fukuwatari (2015) Vitamin B1 deficiency inhibits the increased conversion of tryptophan to nicotinamide in severe food-restricted rats, Bioscience, Biotechnology, and Biochemistry, 79:1, 103-108, DOI: 10.1080/09168451.2014.962473

[62] Whitfield, Kyly C et al. "High prevalence of thiamine (vitamin B1) deficiency in early childhood among a nationally representative sample of Cambodian women of childbearing age and their children." PLoS neglected tropical diseases vol. 11,9 e0005814. 5 Sep. 2017, doi:10.1371/journal.pntd.0005814

[63] Whitfield, Kyly C et al. "Thiamine deficiency disorders: diagnosis, prevalence, and a roadmap for global control programs." Annals of the New York Academy of Sciences vol. 1430,1 (2018): 3-43. doi:10.1111/nyas.13919

[64] Costello E and Kerns J. Thiamine Deficiency in People with Obesity (P18-060-19) Curr Dev Nutr. 2019 Jun; 3(Suppl 1): nzz039.P18-060-19.65.

[65] Adamolekun, B. and Hiffler, L. (2017), A diagnosis and treatment gap for thiamine deficiency disorders in sub-Saharan Africa?. Ann. N.Y. Acad. Sci., 1408: 15-19. doi:10.1111/nyas.13509

[66] J. González-Cabrera, M. Fernández-Prada, C. Iribar-Ibabe & J. M. Peinado (2014) Acute and chronic stress increase salivary cortisol: a study in the real-life setting of a national examination undertaken by medical graduates, Stress, 17:2, 149-156, DOI: 10.3109/10253890.2013.876405

[67] Zsombor Lacza, Andrey V. Kozlov, Eszter Pankotai, Attila Csordás, Gerald Wolf, Heinz Redl, Márk Kollai, Csaba Szabó, David W. Busija & Thomas F. W. Horn (2006) Mitochondria produce reactive nitrogen species via an arginine-independent pathway, Free Radical Research, 40:4, 369-378, DOI: 10.1080/10715760500539139

[68] Mangas, A., Heredia, M., Riolobos, A., De la Fuente, A., Criado, J. M., Yajeya, J., … Coveñas, R. (2018). Overexpression of kynurenic acid and 3-hydroxyanthranilic acid after rat traumatic brain injury. European journal of histochemistry : EJH, 62(4), 2985. doi:10.4081/ejh.2018.2985

[69] Observatoire de la Sante Psychologique au Travail Evaluation du Stress. Stimulus. Retrieved at https://www.stimulus-conseil.com/wp-content/uploads/2017/11/Observatoire-Stress-novembre-2017.pdf

[70] "More than 3 million Frenchmen on the verge of burnout" Le Monde. 22 January 2014 at 13h29 - Updated 22 January 2014 at 16h48. Retrieved at https://www.lemonde.fr/economie/article/2014/01/22/plus-de-3-millions-de-francais-au-bord-du-burn-out_4352438_3234.html

[71] Béjean, S. & Sultan-Taïeb, H. (2005). Modelling the economic burden of diseases imputable to stress at work. European Journal of Health Economics, 6(1), 16–23.

[72] Sultan-Taïeb, H., Chastang, J. F., Mansouri, M., & Niedhammer, I. (2013). The annual costs of cardiovascular diseases and mental disorders attributable to job strain in France. BMC Public Health, 13, 748. doi: 10.1186/1471-2458-13-748.

[73] Trontin, C., Lassagne, M., Boini, S.,& Rinal, S. (2010). Le coût du stress professionnel en France en 2007. Institut National de Recherche et de Sécurité: Paris, 2010. Available at: http://amsndev.circum.net/iso_album/coutstressprofessionnel2007.pdf

[74] Hamer M, Endrighi R, Venuraju SM, Lahiri A, Steptoe A (2012) Cortisol Responses to Mental Stress and the Progression of Coronary Artery Calcification in Healthy Men and Women. PLoS ONE 7(2): e31356. doi:10.1371/journal.pone.0031356

[75] OECD

[76] Godet-Cayré V, Pelletier-Fleury N, Le Vaillant M, Dinet J, Massuel MA, Léger D. Insomnia and absenteeism at work. Who pays the cost? Sleep. 2006 Feb;29(2):179-84.

[77] Allison Agus,Julien Planchais,Harry Sokol (2018). Gut Microbiota Regulation of Tryptophan Metabolism in Health and Disease. Cell Host & Microbe. VOLUME 23, ISSUE 6, P716-724, JUNE 13, 2018

[78] Traber, M. G., Buettner, G. R., & Bruno, R. S. (2019). The relationship between vitamin C status, the gut-liver axis, and metabolic syndrome. Redox biology, 21, 101091. doi:10.1016/j.redox.2018.101091

[79] Cortese-Krott, M. M., Koning, A., Kuhnle, G., Nagy, P., Bianco, C. L., Pasch, A., … Feelisch, M. (2017). The Reactive Species Interactome: Evolutionary Emergence, Biological Significance, and Opportunities for Redox Metabolomics and Personalized Medicine. Antioxidants & redox signaling, 27(10), 684–712. doi:10.1089/ars.2017.7083

[80] Wirthgen, E., Hoeflich, A., Rebl, A., & Günther, J. (2018). Kynurenic Acid: The Janus-Faced Role of an Immunomodulatory Tryptophan Metabolite and Its Link to Pathological Conditions. Frontiers in immunology, 8, 1957. doi:10.3389/fimmu.2017.01957

[81] Michael Platten, Ellen A. A. Nollen, Ute F. Röhrig, Francesca Fallarino & Christiane A. Opitz (2019). Tryptophan metabolism as a common therapeutic target in cancer, neurodegeneration and beyond. Nature Reviews Drug Discoveryvolume 18, pages379–401

[82] Guastella AR, Michelhaugh SK, Klinger NV, Fadel HA, Kiousis S, Ali-Fehmi R, Kupsky W, Juhász C, Mittal S. (2018) Investigation of the aryl hydrocarbon receptor and the intrinsic tumoral component of the kynurenine pathway of tryptophan metabolism in primary brain tumors. J Neurooncol. 2018 Sep;139(2):239-249. doi: 10.1007/s11060-018-2869-6. Epub 2018 Apr 17.

[83] Page C, Michael C, Sutter M, Walker M, Hoffman BB (2002). Integrated Pharmacology (2nd ed.). C.V. Mosby. ISBN 978-0-7234-3221-0.

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