



Water-soluble Vitamins in the Prophylaxis and Treatment of Metabolic Diseases and Related Disorders

Stefano Agostini

Research and Development and Scientific Direction MèDISIN Srl, Lissone (MB), Italy

ABSTRACT

This article aims to define the complex relationships between cellular biochemistry, metabolic diseases, and the effects of some drugs. Water-soluble vitamins (WSVs) are indispensable cofactors in the reactions of the Krebs cycle, folate cycles, methionine cycles, numerous biochemical reactions related to these cycles, and the electron transport chain. The function of each vitamin is carried out in concert with the functions of others in a complex network of biochemical reactions that require the presence of all WSVs simultaneously to maintain cellular homeostasis. The chemical reactions in which WSVs are involved occur mainly, but not exclusively, in the mitochondria and are essential for cellular catabolism and anabolism. Restricting the availability of just one of them hampers the functions of all others, contributes to mitochondrial dysfunction and hinders cellular metabolism. This should be a fundamental consideration in assessing the action of these vitamins and their potential therapeutic use. Deficiencies, whether severe, marginal, functional, or increased demand for WSVs, are widespread in populations of developed countries, despite seemingly well-nourished populations. Obesogenic diets provide an excess of energy substrates associated with inadequate intake of WSVs. This contributes to mitochondrial dysfunction and represents a contributing factor in the development of metabolic diseases and related disorders. The treatment of metabolic diseases includes the administration of drugs that can induce depletion of WSVs. Some drugs used in the treatment of patients suffering from metabolic diseases increase the risk of type 2 diabetes mellitus and peripheral neuropathies, disorders associated with WSVs deficiency. Based on these premises, this article highlights the importance of proper cellular biochemical balance and the use of WSVs as an inseparable unit for the prophylaxis and treatment of metabolic diseases, even in relation to the drugs commonly used.

Keywords: water-soluble vitamins, obesity, type 2 diabetes mellitus, metabolic syndrome, mitochondrial dysfunction.

INTRODUCTION

Diet, lifestyle, and regular drug intake often contributes to disturbances in the body's biochemical balance, making it difficult to maintain homeostasis and exposing individuals to the risk of various disorders that can lead to chronic and degenerative diseases. A growing body of scientific evidence demonstrates that the pathogenesis of these disorders is often linked to inadequate intake of water-soluble vitamins (WSVs), which must be introduced through daily nutrition in adequate amounts since the human body cannot synthesize or store them. WSVs include thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), biotin (B8), folate (B9), cobalamin (B12), and ascorbic acid (C).

When addressing the involvement of WSVs in metabolic diseases, two common attitudes can be encountered. The first is related to the fact that major metabolic diseases are considered diseases of affluence, leading to the belief that WSVs are adequately present in diets and therefore in the bodies of well-nourished individuals in developed countries, while inadequate intake of these vitamins is attributed exclusively to populations in underdeveloped or developing countries. This attitude leads to the assumption that all actions involving WSVs are optimally performed by the body, attributing metabolic diseases to causes other than vitamins. The second common attitude considers each individual vitamin as a separate entity. This approach investigates the status and assesses the potential therapeutic action of one vitamin at a time, as if it were the active ingredient of a drug, neglecting the fact that a single vitamin can do nothing if not placed in a biochemical context where all other WSVs are adequately present. This is due to the complex biochemical relationships that exist among them in cellular metabolism. To encourage reflection on the inadequacy of these attitudes, it is considered highly useful to delve briefly into the field of biochemistry and provide a summary of research demonstrating the frequent occurrence of severe, marginal, functional deficiency, or increased demand for WSVs in individuals from seemingly well-nourished populations in developed countries and how inadequate intake of these vitamins can affect health.

In addition to the well-established evidence of some biochemical functions of WSVs, the latest scientific literature reveals complex biochemical interconnections shedding new light on the involvement of WSVs in previously unclear aspects of cellular functioning, urging consideration of all WSVs as an inseparable unit. Highlighting the crucial role played by all WSVs and the close biochemical relationships they maintain with each other, it becomes clear that paying significant attention to some while neglecting others is not a scientifically correct approach. Therefore, the best approach is to adopt a more comprehensive and global perspective. This work is an expansion of a previous study that considered only some of the B complex vitamins [1].

This article provides a brief overview of current knowledge regarding the relationships between WSVs, metabolic diseases, and related disorders. The aim is to encourage consideration of all WSVs as an indivisible prophylactic and therapeutic unit to be used for maintaining or restoring cellular homeostasis, health, and to optimize the action and reduce the risk of adverse events associated with the often-necessary use of drugs.

WATER-SOLUBLE VITAMINS AND CELLULAR METABOLISM

Living organisms are dynamic flow systems in which all vital manifestations are marked and conditioned by chemical reactions. Although this may seem obvious, the biochemical needs of the body are often neglected, with inadequate attention given to the role played by vitamins, crucial substances for human body biochemistry. They deserve special attention because human cells lack the biochemical apparatus necessary to synthesize them.

WSVs act as coenzymes and are required, in synergy, for energy metabolism, catabolic and anabolic processes, replication, and cellular function. WSVs have pro-metabolic and pro-energetic actions, play a primary role in mitochondrial functions, are involved in the synthesis of neurotransmitters, hormones, neuropeptides, and substances involved in iron metabolism, and have antioxidant, neuroprotective, cardioprotective, and osteoprotective actions.

WSVs are involved in numerous chemical reactions that occur in the cytosol and mitochondria of cells. These include reactions that transform carbohydrates, fats, and amino acids into energy substrates, reactions in the Krebs cycle, and reactions in the electron transport chain. All these reactions have a central role in cellular energy metabolism. Furthermore, through cataplerotic reactions, intermediates of the Krebs cycle provide the cell with substances necessary for gluconeogenesis, the synthesis of fatty acids, cholesterol, porphyrins (e.g., the EME group), and other metabolic pathways, such as the urea cycle and purine synthesis. WSVs are also involved in cataplerotic and anaplerotic reactions. Observing a schematic representation of the main reactions of cellular energy metabolism (Figure 1), all WSVs are involved, except for folate.

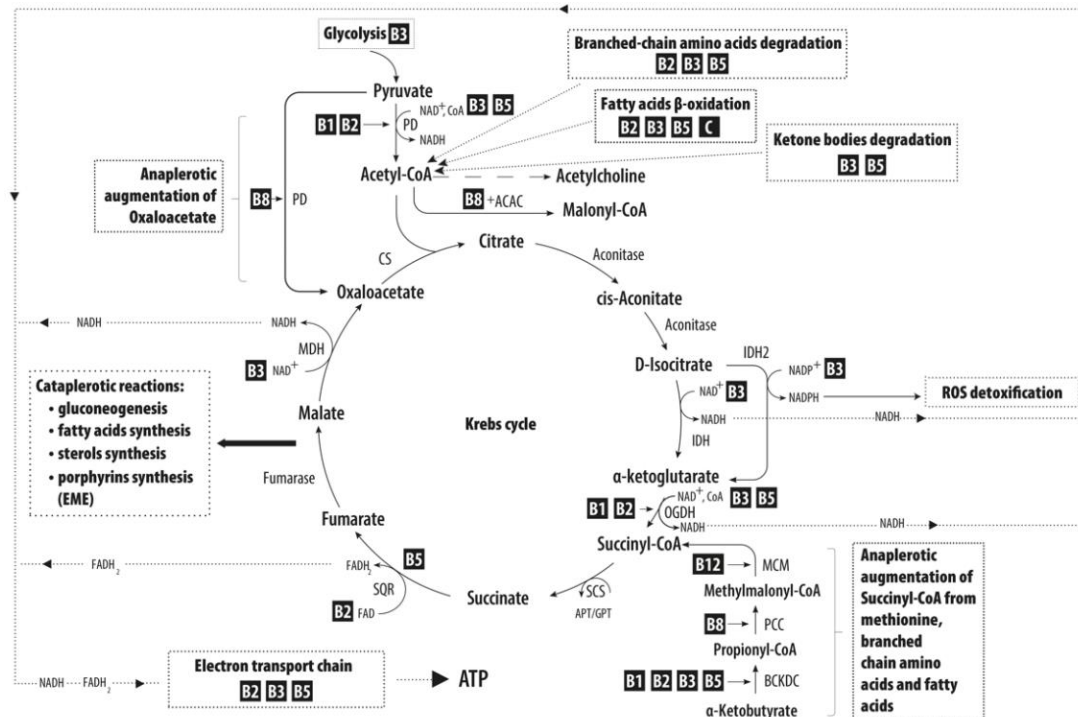


Figure 1: Schematic representation of Krebs cycle, other cellular energy metabolism reactions, anaplerotic and cataplerotic reactions. Abbreviations: ACAC, Acetyl-CoA carboxylase; BCKDC, branched-chain α -ketoacid dehydrogenase complex; CS, citrate synthase; CoA, coenzyme A; FAD/FADH₂, flavin adenine dinucleotide (oxidized/reduced); IDH, isocitrate dehydrogenase; NAD, nicotinamide adenine dinucleotide (+/H = oxidized/reduced); NADP, nicotinamide adenine dinucleotide phosphate (+/H = oxidized/reduced); MDH, malate dehydrogenase; MCM, methylmalonyl-CoA mutase; OGDH, α -ketoglutarate dehydrogenase; PCC, propionyl-CoA Carboxylase; PC, pyruvate carboxylase; PD, pyruvate dehydrogenase; SCS, succinyl-CoA synthetase; SQR, succinate-coenzyme Q reductase.

The Krebs cycle, other enzymatic reactions of energy metabolism, and numerous other cellular biochemical reactions require precise gene regulation, which in turn depends on the efficient functioning of the folate cycle and the methionine cycle. In the methionine cycle, S-adenosylmethionine is generated, serving as a universal methyl donor and enabling DNA and RNA methylation. The folate cycle allows for the regeneration of methionine and is essential for DNA and RNA synthesis.

The folate and methionine cycles are closely linked to numerous other cellular chemical reactions through which homocysteine is recycled and transformed, nitric oxide is produced, neurotransmitters, amines, catecholamines, proteins, and antioxidants are generated. Observing a schematic representation of the folate cycle, the methionine cycle, and the other biochemical reactions stemming from them (Figure 2), it becomes apparent that WSVs are also essential for these complex biochemical reactions.

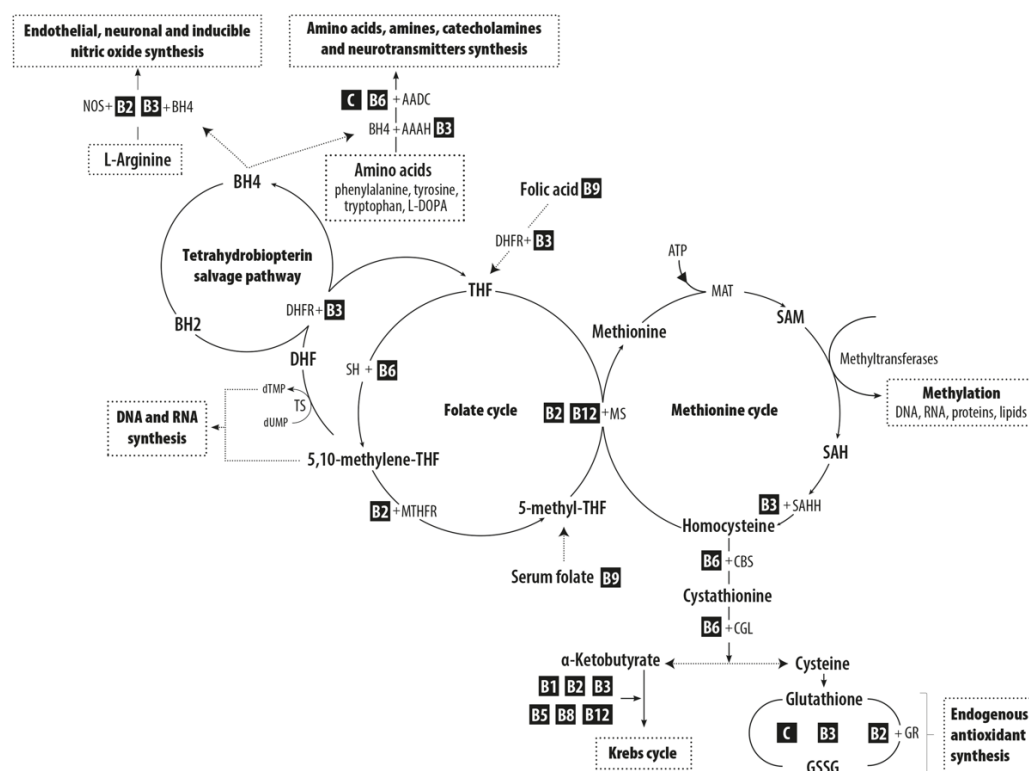


Figure 2: Schematic representation of the tetrahydrobiopterin salvage pathway, the folate cycle, the methionine cycle, and the connection of the latter with the Krebs cycle and the endogenous antioxidant synthesis. Abbreviations: AADC, aromatic L-amino acid decarboxylase; AAAH, aromatic amino acid hydroxylases; ATP, adenosine triphosphate; BH2, dihydrobiopterin; BH4, tetrahydrobiopterin; CBS, cystathionine beta synthase; CGL, cystathionine gamma-lyase; DHFR, dihydrofolate reductase; dTMP, thymidine monophosphate; dUMP, deoxyuridine monophosphate; GR, glutathione reductase; GSSG, glutathione disulphide; MAT, methionine adenosyltransferase; MS, methionine synthase; MTHFR, methylenetetrahydrofolate reductase; NOS, nitric oxide synthase; SAH, S-adenosylhomocysteine; SAHH, S-adenosylhomocysteine hydrolase; SAM, S-adenosyl methionine; SH, serine hydroxymethyltransferase; THF, tetrahydrofolate; TS, thymidylate synthase.

The crucial evidence that we intend to emphasize through Figures 1 and 2 is that, as there is a close correlation between them, all WSVs must be simultaneously available in cells in adequate quantities. The insufficient intake of even one of these vitamins compromises the biochemical processes that occur in cells, regardless of the more or less abundant availability of the other vitamins.

THE REQUIREMENT FOR WATER-SOLUBLE VITAMINS

After the necessary biochemical background, to introduce some considerations regarding the genuine need for WSVs for the performance of cellular metabolic functions, it can be useful to provide a significant example provided by the brain, an organ that represents only 2% of the human body's weight but is responsible for more than 20% of the body's total energy expenditure [2]. It is evidently an organ that is particularly demanding from an energy perspective and, therefore, has a high requirement for all vitamins involved in energy metabolism. In support of this, interesting clinical studies show that the intake of high doses of WSVs by healthy individuals before intense brain activity can increase energy metabolism, electrophysiological measurements, and blood flow in the brain, thereby improving intellectual performance [3,4]. Faced with the results of these studies, one may wonder if apparently healthy individuals are actually suffering from a deficiency of WSVs or if healthy individuals, despite having adequate levels of WSVs, can further improve their performance with additional intake of these vitamins, or if healthy individuals, during particularly strenuous brain activity, are exposed to an increased need for WSVs. Based on the evidence presented in the following paragraphs, it can probably be hypothesized that all these conditions occur simultaneously, but first, it is necessary to consider what the right daily dose of WSVs should be. For each WSV, a Recommended Dietary Allowance (RDA) has been defined, which are the doses to be introduced daily through food to meet the nutritional needs of the majority of the healthy population. By meeting this requirement, it is believed that the onset of diseases related to the deficiency of the specific WSV considered can be prevented. RDAs and RNIs are based on population statistics and are therefore largely approximate estimates. In fact, for most WSVs, the necessary information is not available to calculate the daily requirement precisely for each individual, and therefore RDAs and RNIs are the result of hypotheses and considerations in which a wide margin of uncertainty is inherent [5-7]. Furthermore, thanks to multiple studies, numerous lines of evidence have emerged regarding individual differences in the absorption and excretion of WSVs, as a result of numerous factors, including genetic polymorphisms, gender, ethnicity, endocrine dysfunction, thyroid function, habitual drug use, smoking, illegal drug use, alcohol or certain foods consumption, obesity, energy consumption, intense physical exercise, and age [8-14]. Scientific studies, therefore, cast doubt on the existence of a *normal* reference population [15], and the view emerges that RDAs and RNIs are simply arbitrarily calculated doses that cannot reflect the real needs of individuals [5]. There is evidence that the benefits of consuming WSVs can increase well above the reference nutritional values. Scientific data show that even patients with normal WSVs status can benefit from their intake. Furthermore, the serum level of vitamins does not always reflect the actual vitamin status: there are documented cases where symptoms of vitamin deficiency occur without such deficiency being detected by blood tests; there are also documented cases where blood tests show a deficiency condition before signs and symptoms of deficiency manifest. Therefore, other considerations should guide the prescription of WSVs, especially the benefit that the patient experiences once the therapy has been initiated. This is the so-called use according to the *ex adiuvantibus* criterion (i.e., confirming the accuracy of a diagnostic hypothesis based on the beneficial effect of therapy).

FRANK DEFICIENCY, MARGINAL DEFICIENCY, FUNCTIONAL DEFICIENCY, AND INCREASED REQUIREMENTS FOR WATER-SOLUBLE VITAMINS

According to the triage theory, during the evolution of the human species, selective pressure favored micronutrient allocation mechanisms that preserve short-term survival at the expense

of long-term health [16]. An insufficient intake of micronutrients causes the body to allocate them in a way that preserves some essential metabolic functions for immediate survival. If insufficient micronutrient intake persists over time, this allocation leads to DNA damage, mitochondrial dysfunction, aging, cellular decay, and the initiation and acceleration of chronic and degenerative diseases that undermine long-term survival [16,17].

As will be seen below, scientific literature defines various types of insufficient WSVs intake, and it is demonstrated that such conditions are common even in apparently well-nourished populations in developed countries, where the most striking vitamin deficiency diseases, such as pellagra, beriberi, and scurvy, have long been eradicated and are simply considered medical curiosities. It is this situation of apparent well-being, contradicted by epidemiological statistics, that often leads to doubts about the therapeutic usefulness of administering WSVs. In reality, insufficient WSVs intake is common in developed countries as a result of unbalanced diets, often rich in calories but poor in micronutrients [18], and as a result of the instability of many WSVs, which are destroyed by cooking, preservation, contact with acids and alkalis, exposure to sunlight, and humidity. Based on what has been stated and the fact that the same foods are often potential sources of various WSVs, it can be concluded that insufficient WSVs intake often involves multiple vitamins rather than a single vitamin.

In the general population, various consequences of insufficient WSVs intake are evident. The less frequent but easily detectable consequence, through blood tests, is frank deficiency. Other consequences of insufficient intake are more difficult to detect, but their timely identification is crucial for the preservation or restoration of patient health. These include marginal deficiencies, functional deficiencies, and increased requirements. In the case of marginal deficiency, blood tests do not show levels below normal but slightly above the lower limits of the reference range. Nevertheless, many individuals begin to manifest a condition of distress related to the deficiency of one or more WSVs, demonstrating that their body's need for these micronutrients is not met. In the case of functional deficiency, blood tests show normal or even elevated levels of WSVs. Nevertheless, characteristic signs and symptoms of deficiency can occur because the body can only partially utilize the vitamins, often due to the presence of one or more gene polymorphisms for proteins involved in their absorption, transport, distribution, and metabolism, or due to autoimmune diseases. Finally, there may be cases where individuals are exposed to conditions that increase their need for WSVs, which, if not met, can pose health risks. A common condition that increases the need for WSVs is the response to acute or chronic stress, which involves increased consumption of WSVs [19]. In such cases, serum levels of these vitamins cannot serve as a guide to detect the condition of distress that occurs in the body and can lead to the onset of pathological conditions.

Whether it is one of the described deficiency forms or increased need, the failure to meet the WSVs requirement is the premise for compromising the state of health, and consequently, the only rational strategy for restoring or preserving the state of health is vitamin supplementation.

INSUFFICIENT INTAKE OF VITAMINS IN THE GENERAL POPULATION

Insufficient WSVs intake has been the subject of some interesting investigations that allow a better understanding of its scope. Population-based cross-sectional studies have shown that 10-20% of the Western population may be diagnosed with vitamin C deficiency, and insufficient vitamin C status is associated with increased all-cause mortality [20-24]. An investigation

conducted in Italy on the population over 65 years of age found a malnutrition prevalence of 25% for thiamine, 20% for riboflavin, 15% for folates, and 14% for vitamin C [25].

An investigation conducted in the United States, Germany, the United Kingdom, and the Netherlands found that a significant portion of the population consumes a dose below the RDAs for each of the WSVs considered, namely thiamine, riboflavin, niacin, vitamin B6, folate, vitamin B12, and vitamin C. The situation is most critical for folate and least critical for niacin [26].

Data published by the British government show deficiency levels of vitamin B12 and folate in 3% and 5% of the adult population, respectively [27], while data published by the US government show that 10.5% of the entire US population is deficient in vitamin B6 [28]. A subsequent analysis, excluding subjects who regularly consume dietary supplements, revealed even higher deficiency rates, ranging from 23% to 27% of the adult population [29].

In the United States, more than 30% of a nationally representative sample of individuals over 60 years of age shows vitamin B12 deficiency, which is believed to be the result of a progressive reduction in the ability to absorb the vitamin [30]. Similar data are found in populations following a vegetarian or vegan diet [31]. In elderly people also is common the thiamine deficiency, reaching 16-18% of the population in New Zealand [32].

Although rarely investigated, riboflavin deficiency is believed to be extremely widespread, also due to the high prevalence of a hereditary restriction in riboflavin absorption/utilization that affects 10-15% of the world's population [33]. This hereditary disorder causes reduced intestinal absorption, increased urinary loss, and disruption of riboflavin metabolite homeostasis. Some researchers hypothesize that it may be the primary risk factor for various diseases, also because it alters the metabolism of various biomolecules such as vitamin B6, folate, vitamin B12, vitamin D, nitric oxide, lipids, amino acids, proteins, DNA, homocysteine, and more [33]. The onset of oxidative stress and apoptotic and necrotic phenomena are consequences of riboflavin deficiency and are related to disorders such as migraine, cerebral ischemia, neurodegenerative disorders (primarily Parkinson's disease and Alzheimer's disease), mitochondrial myopathies, and more [33,34]. In an investigation conducted in the United Kingdom, 66% of the non-elderly adult population was found to be at least marginally deficient in riboflavin [27]. The widespread marginal deficiency of WSVs also predisposes to an increased risk of diseases. Some scientific studies suggest that neurological/psychological manifestations due to vitamin B12 insufficiency can be evident even at plasma levels higher than those indicating deficiency [35,36]. If a blood level of 220 pmol/L (approximately 298 pg/mL) were used as the lower limit of the reference range, 20% of the U.S. population over 50 years of age would be marginally deficient in vitamin B12 [37]. In fact, according to some authors, based on the available evidence, the lower limit of the blood level of vitamin B12 is too low, as negative health effects can be observed even when the value falls within the reference range and is seemingly normal [35].

INSUFFICIENT INTAKE OF WATER-SOLUBLE VITAMINS AND MITOCHONDRIAL DYSFUNCTION

When insufficient WSVs intake becomes chronic, it leads to chronic impairment of metabolic functions, resulting in a variety of repercussions on metabolism, including the onset and acceleration of chronic and degenerative diseases. One of the most significant contributors to

this condition is mitochondrial dysfunction. It is easily conceivable and also confirmed by basic research and clinical studies that WSVs deficiency causes mitochondrial dysfunction [38,39], as WSVs have antioxidant action and are indispensable coenzymes for the numerous biochemical activities that take place in the mitochondria [40,41]. Mitochondrial dysfunction consists of maladaptive physiological responses, including irregularities in catabolizing substrates and the inability to generate sufficient ATP [42], mutations in mitochondrial DNA, changes in functional dynamics, ROS production, mitochondrial size, and morphology [43,44]. Some scientific articles further strengthen the case for the relationship between WSVs and mitochondrial dysfunction, emphasizing both the deficiency of these vitamins in patients with diseases involving mitochondrial dysfunction and the importance of these vitamins in the treatment of mitochondrial dysfunction, in association with adequate caloric intake and regular physical exercise [45,46]. Since the activity of mitochondrial enzymes is regulated by WSVs levels, maintaining a balanced pool of these vitamins in the mitochondria is essential to support metabolic reactions and other biochemical reactions orchestrated by these mitochondrial enzymes [41].

Evidence has emerged that the wide range of metabolites produced in the mitochondria not only supports mitochondrial respiration and ATP production but also communicates with other parts of the cell, including the nucleus [47]. This retrograde signaling pathway from the mitochondria to the nucleus is called mito-nuclear communication [47] and allows mitochondria to influence numerous cellular processes, including the cell cycle, cell signaling, and epigenetic regulation [48]. It has been established that the failure to regulate the interaction between mitochondrial metabolites and the nucleus plays a direct role in the onset of many diseases [49].

Insufficient intake of WSVs contributes to mitochondrial dysfunction and the associated health consequences. Conversely, adequate intake of WSVs is essential for maintaining mitochondrial homeostasis and preventing diseases.

INSUFFICIENT INTAKE OF WATER-SOLUBLE VITAMINS AND OBESITY

Since 1980, the prevalence of overweight and obesity worldwide has doubled, with approximately one-third of the global population now classified as overweight or obese. Obesity rates have increased in both sexes and across all age groups, regardless of geographical location, ethnicity, or socioeconomic status, although higher obesity rates are observed in older individuals and women [50].

Obesity increases the risk of various diseases associated with increased mortality, including type 2 diabetes mellitus (T2DM), cardiovascular diseases, metabolic syndrome (MetS), chronic kidney diseases, hyperlipidemia, hypertension, non-alcoholic fatty liver disease, certain types of cancer, obstructive sleep apnea, osteoarthritis, and depression [51].

Some interesting scientific articles have closely linked obesity to secondary mitochondrial dysfunction [52,53]. Obesogenic diets are typically composed of foods rich in fats and simple sugars with low micronutrient content, resulting in insufficient intake of a wide range of vitamins and minerals [18,54]. Additionally, WSVs are labile and can be affected by cooking and preservation processes, exposure to acids and alkalis, and exposure to light and moisture. Therefore, insufficient intake of micronutrients is often associated with excessive caloric intake,

is common among overweight or obese individuals, and often goes unnoticed as suboptimal micronutrient intake is challenging to define for each individual and does not result in immediate symptoms.

The scientific literature has already highlighted the correlation between increased adipose tissue and reduced serum vitamin levels. The main WSVs evaluated in obese individuals are thiamine, vitamin B6, folate, vitamin B12, and vitamin C. Studies show that deficiencies or lower levels of WSVs are more prevalent in obese individuals [55,56].

The situation appears to be more serious for vitamins directly involved in the metabolism of high-fat, high-sugar, and high-protein foods. For example, thiamine plays a crucial role in glucose metabolism and is deficient in 15.5-29% of obese patients examined before bariatric surgery and in 17-79% of diabetic patients [8,54].

Another interesting example is represented by vitamin C. Numerous studies have shown an inverse association between body weight or BMI and vitamin C status, with markedly obese individuals having lower status [57]. A difference of up to 15 $\mu\text{mol/L}$ in serum vitamin C levels has been reported between obese women and normal-weight women (45 and 60 $\mu\text{mol/L}$, respectively) [58]. Some researchers have found that individuals with hypovitaminosis C (levels below 23 $\mu\text{mol/L}$) exhibit significantly higher weight, body mass index, and waist circumference [59]. The lower status of vitamin C observed in individuals with higher weight may be partly due to lower dietary intake [60,61]. Supporting this, lower vitamin C status has been found in individuals with higher fat intake [62].

Multiple lines of evidence support the association between obesity and inadequate intake of WSVs and suggest that inadequate intake of these vitamins contributes to the onset and development of diseases associated with overweight and obesity. Further research in the literature provides valuable insights into this situation. For example, adipocytes contribute to maintaining the balance between energy storage and expenditure, which requires normal mitochondrial function [52]. Mitochondria in adipocytes play a crucial role in regulating metabolism, ATP production, energy expenditure regulation, disposal of reactive oxygen species (ROS), and adipogenesis. When mitochondrial function is compromised, mitochondria play a central role in the pathophysiology of obesity and insulin resistance (IR) [52]. The excessive supply of energy substrates and inadequate intake of micronutrients in obese individuals lead to mitochondrial dysfunction with consequent effects on lipid and glucose metabolism [52]. Furthermore, it is believed that impaired one-carbon metabolism caused by WSVs deficiency may contribute to obesity and IR by increasing homocysteine levels, which disrupt the insulin signalling pathway [63].

Another aggravating factor is chronic stress, which, as mentioned earlier, increases the need for WSVs and is associated with weight gain and obesity [64]. Chronic elevation of stress mediators such as cortisol, norepinephrine, and corticotropin-releasing hormone induces metabolic disturbances and inflammatory process control disorders, changes in eating behaviors, and can contribute to the development of visceral obesity [64]. The endocrine consequences of increased cortisol activity include inhibition of the growth hormone axis, thyroid axis, and gonadal axis, contributing to muscle and bone loss and visceral fat accumulation, typically observed with higher exposure to glucocorticoid hormones [64]. The

onset of visceral obesity and muscle loss is associated with clinical parameters that characterize MetS, leading to an increased risk of cardiovascular diseases, dyslipidemia, hypertension, and T2DM [64].

INADEQUATE INTAKE OF WATER-SOLUBLE VITAMINS, INSULIN RESISTANCE, TYPE 2 DIABETES MELLITUS, NON-ALCOHOLIC FATTY LIVER DISEASE, AND METABOLIC SYNDROME

According to data published by the World Health Organization, the prevalence of T2DM has dramatically increased in both developed and developing countries over the last three decades. It is estimated that 422 million people worldwide have diabetes, with more than 95% of cases being T2DM. Over 85% of diabetic patients are overweight or obese [65]. Like obesity, T2DM is closely related to mitochondrial dysfunction [66].

MetS is a highly prevalent issue, and its prevalence continues to rise. Two studies found that the prevalence of MetS in the Italian adult population progressively increases with age, ranging from 3-5% in individuals aged 20-29 to 25-30% in individuals over 65 [67,68]. In the United States, among individuals aged 20 and older, the overall prevalence of MetS increased from 37.6% in 2011-12 to 41.8% in 2017-18 [69]. The development of MetS depends on both the amount and distribution of fat, with abdominal fat excess, known as the apple shape, posing a higher risk, reflecting a relatively low ratio of muscle mass to fat mass [70]. IR develops along with compensatory hyperinsulinemia, compromising glucose metabolism and potentially leading to T2DM. Due to lipid metabolism disturbances, dyslipidemias, including hypertriglyceridemia, hypercholesterolemia, and low levels of HDL cholesterol, manifest, along with hypertension. Serum uric acid levels are typically elevated, and a prothrombotic state with increased fibrinogen levels and plasminogen activator inhibitor-1 is established [70]. Excess abdominal fat leads to increased free fatty acids in circulation, contributing to lipid accumulation in the liver and triggering non-alcoholic fatty liver disease, which can progress to non-alcoholic steatohepatitis [71]. This represents additional harm to the body and a condition that further disrupts lipid and carbohydrate metabolism, as the liver is responsible for regulating these two metabolisms.

MetS and its consequences are also associated with mitochondrial dysfunction [72,73].

In recent years, as already noted for obesity, scientific evidence linking T2DM and MetS to inadequate intake of WSVs has multiplied. Below is an overview including only some of the numerous published studies.

In a cross-sectional study, inadequate serum vitamin C levels (from 0.6 to 0.3 mg/dL) were found in 25.68% of 292 adult patients with T2DM, and hypovitaminosis C (serum vitamin level < 0.3 mg/dL) was found in 55.13% of them [74]. A low vitamin C level was associated with increased blood pressure, levels of glycated haemoglobin, malondialdehyde, and markers of oxidative stress. The study also found a reverse relationship between fasting blood glucose, total cholesterol, and vitamin C levels [74].

In a study conducted in Poland and published in 2016, 182 patients with MetS aged between 30 and 65 years were compared to 91 healthy subjects aged between 41 and 65 years [75].

Through the measurement of plasma concentration, it was found that 79.12% of the patients with MetS exhibited a deficiency in vitamin C, in contrast to 8.79% of the healthy subjects [75]. A study published in 2018 found that euthyroid subjects with elevated serum vitamin B12 levels had a lower prevalence of MetS compared to those with low vitamin levels [76], while a study published in 2019 found that vitamin B12 deficiency led to metabolic changes related to obesity and T2DM [77].

A 2020 article presents preclinical and clinical evidence regarding the association between lower vitamin B12 levels and alterations in lipid and carbohydrate metabolism [78]. In preclinical studies, vitamin B12 deficiency was shown to contribute to obesity, IR, dyslipidemia, and cardiovascular disorders. In clinical studies conducted on pregnant women, an association was found between low levels of vitamin B12 and folate and higher BMI and the risk of adiposity and IR in neonates [78]. Furthermore, lower serum levels of vitamin B12 were observed in obese, insulin-resistant, and dyslipidemic children, adolescents, and adults compared to normal-weight individuals [78]. A high prevalence of low vitamin B12 levels was found in European patients (27%) and in patients from southern India (32%) with T2DM [78]. In addition, a prospective study in pregnant women found that low vitamin B12 levels could independently cause the onset of T2DM in children five years after birth. Children born to mothers with low vitamin B12 levels, given the importance of this vitamin in lipid metabolism, may accumulate excess fat, which in turn can cause increased IR and an increased risk of T2DM and cardiovascular disease in adulthood [78].

A clinical study published in 2020 assessed the relationship between serum vitamin B12 levels and lipid profiles in 341 healthy women aged 19 to 30. The study found that serum vitamin B12 levels were inversely associated with total cholesterol, LDL cholesterol, and triglyceride levels. This result provides further evidence that low serum vitamin B12 levels are independently associated with abnormal lipid profiles [79].

Vitamin C and vitamin B12 are not the only WSVs that have been investigated. Great importance is also attributed to vitamin B6, which is a cofactor in at least 150 enzymatic reactions involved in regulating glucose, lipid, amino acid, DNA, and neurotransmitter metabolism. Studies indicate an inverse association between serum vitamin B6 levels and T2DM and also demonstrate the protective effect of vitamin B6 against diabetes complications [80]. This vitamin plays an important role in antioxidant defense and counteracts the formation of reactive oxygen species and advanced glycation end products [80].

Inadequate folate intake is implicated in the development of IR. The National Health and Nutrition Examination Survey (NHANES), involving 1530 non-diabetic adults between 2011 and 2012, found a significant inverse relationship between serum folate levels and IR. Moreover, a 25% increase in serum folate levels was associated with a 3.06% decrease in the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index, a 2.77% decrease in insulin levels, and a 2.55% increase in the glucose-to-insulin ratio [81]. This is not surprising, as recent studies have suggested that epigenetic changes due to altered DNA methylation play a crucial role in the pathogenesis of IR [81], and folate, riboflavin, niacin, vitamin B6, and vitamin B12 play key roles in providing the cell with methyl groups.

Further confirmation comes from a significant prospective cohort study published in JAMA in 2023 [82]. The study, initiated in 1985 in the United States, included 4414 participants (50.4% people of color and 52.8% women) with a mean age at baseline of 24.9 years. Over 30 years of follow-up, 1240 incident cases of MetS occurred, and higher serum levels of homocysteine were associated with an increased incidence of MetS. After adjusting for potential confounding factors, participants in the highest quintile of total folate intake had a 61% lower incidence of MetS than those in the lowest quintile ($P < 0.001$). Similarly, the incidence of MetS was 39% lower in the highest quintile of vitamin B6 intake ($P = 0.002$) and 26% lower in the highest quintile of B12 intake ($P = 0.008$). Serum concentrations of these three WSVs were inversely associated with MetS and inversely correlated with homocysteine levels. It emerged that the intake of folates is inversely associated with all individual components of MetS. The inverse association between vitamin B6 and MetS can be primarily explained by its favorable effects on adiposity, hypertriglyceridemia, HDL cholesterol level, and blood pressure. As for vitamin B12, its inverse association with MetS can be mainly explained by its favorable impacts on adiposity, hypertriglyceridemia, and HDL cholesterol level. It is interesting to note that the inverse association between vitamin B12 intake and the risk of MetS was observed only among supplement users, presumably because these participants consumed higher quantities of vitamin B12. The study's authors conclude that lower serum concentrations of these vitamins may be indicators of a higher risk of MetS, and adequate intake of these vitamins should be recommended for MetS prevention [82].

FACTORS AFFECTING THE ABSORPTION AND METABOLISM OF WATER-SOLUBLE VITAMINS

Numerous factors can interfere with the absorption and metabolism of WSVs. Malabsorption of WSVs can lead to a condition of frank or marginal deficiency. Several factors are responsible for the significant prevalence of malabsorption of WSVs in the population. Among these, aging plays a significant role, as well as various pathological conditions, including celiac disease, chronic inflammatory bowel diseases, and gastric atrophy. The consumption of alcoholic beverages, coffee, tea, and certain foods also interferes with the absorption and metabolism of WSVs [83-86].

Furthermore, there are other substances regularly taken that can interfere with the absorption and metabolism of WSVs: drugs. According to statistics published by the Centers for Disease Control and Prevention, 48.6% of adults in the United States regularly take at least one prescription drug, 24.0% take three or more, and 12.8% take five or more [87]. In 2019, in Italy, approximately 7 out of 10 citizens received at least one prescription for drug [88], and among the population over the age of sixty-five, 98% received at least one prescription for drug in 2019 [88]. On average, 7.7 different substances were prescribed per patient, with the lowest average value, 6.2, recorded in the age group between 65 and 69 years and the highest, 8.8, for users over the age of 84 [88].

Some of the most commonly prescribed drugs inhibit the absorption and metabolism of WSVs and can therefore cause depletion. Among the most prescribed drugs for metabolic disease, metformin causes depletion of WSVs, interferes with the absorption of thiamine and vitamin B12, and exhibits antifolate activity through a mechanism that is not yet fully understood [89-91]. Metformin is the most prescribed oral antidiabetic drug in the world, accounting for 45-

50% of all antidiabetic drug prescriptions and being taken by more than 150 million patients annually [92,93].

It has been proposed that metformin and other biguanide molecules inhibit the absorption of thiamine by inhibiting the thiamine transporter (THTR-2) in the small intestine [94]. Recently, the clinical relevance of thiamine absorption inhibition by metformin has been confirmed, especially in populations already at risk of thiamine deficiency [95]. The study's authors emphasize that metformin's inhibition of THTR-2 may contribute to adverse events associated with its use [95]. For example, a common and potentially fatal adverse effect associated with metformin is lactic acidosis, which can also occur due to thiamine deficiency [96].

Since 1969, it has been known that metformin causes vitamin B12 depletion, and deficiency manifests in 2-3 months, affecting between 5.6% and 36% of treated patients [65]. Patients treated with metformin have lower vitamin B12 levels and higher levels of homocysteine and methylmalonic acid, and the combination of these factors is related to more severe forms of diabetic neuropathy [65]. In a study conducted in Italy, 21% of patients treated with metformin were found to be deficient in vitamin B12 [97]. Deficiency worsens with increasing dose and duration of therapy [97]. A randomized controlled study involving patients with T2DM who received metformin for 16 weeks demonstrated that the drug led to a reduction in folate and vitamin B12 levels, resulting in an increase in homocysteine [98]. This result was confirmed by a subsequent study [99].

The depletion of WSVs induced by metformin and other biguanides should be a cause for concern. These are drugs designed to promote glycemic regulation and are systematically prescribed to patients suffering from obesity, IR, T2DM, fatty liver disease and MetS. As highlighted in the previous paragraphs, these pathological conditions are associated with low serum levels of WSVs, which are believed to either arise or worsen due to insufficient intake of these vitamins. Therefore, the initiation of a potential vicious cycle between drug use to treat the disease and the induction of a deficient state that can exacerbate the disease itself or facilitate the onset of related disorders becomes evident.

METABOLIC DISEASE AND GASTROESOPHAGEAL REFLUX DISEASE

Adiposity, obesity, and T2DM play a causative role in the onset of gastroesophageal reflux disease (GERD) [100]. T2DM is a risk factor for GERD, and 40.7% of T2DM patients suffer from symptomatic GERD [65]. Among T2DM patients, peripheral neuropathy is an independent risk factor for the development of erosive esophagitis, and 60-70% of diabetics suffer from damage to the nervous system, which partially explains why low-grade esophageal dysplasia is twice as common in diabetics compared to non-diabetic individuals [65]. There is also a well-established association between MetS and GERD [101].

Therefore, overweight, obese, and T2DM patients frequently experience GERD. As a result, they are chronically prescribed proton pump inhibitors (PPIs) and histamine H2 receptor antagonists (H2RAs). It is well-established that the use of PPIs and H2RAs reduces the absorption of vitamin B12 [102,103] and folate [104], and chronic use of these drugs, especially by individuals with diets low in vitamin B12 and folate, can lead to marginal or frank deficiencies. This is because gastric acid is necessary for releasing vitamin B12 from dietary proteins and allowing its absorption in the terminal ileum.

As early as 1980, it was found that patients on H2RAs could absorb unbound vitamin B12 from supplements but not protein-bound vitamin B12 from food sources [105]. When the absorption of protein-bound vitamin B12 was evaluated before and after therapy with H2RAs, a 53% decrease in absorption was observed [106]. When the absorption of protein-bound vitamin B12 was evaluated before and after ranitidine therapy, an 89% decrease in absorption was observed [107].

In a recent systematic review of 65 clinical studies on PPIs conducted between 1988 and 2022, it was found that 23.4% of the world's adult population uses PPIs. About 98% of patients take the highest dose, a quarter of patients take these products for a year or more, and 27.8% of them take them for more than 3 years [108].

A study compared 25,956 patients with vitamin B12 deficiency to 184,199 patients without deficiency to assess whether there was an association between vitamin B12 deficiency and PPI and H2RA therapy [102]. Among patients with deficiency, 12.0% took PPIs for 2 or more years, 4.2% took H2RAs for 2 or more years (without taking PPIs), and 83.8% did not take either PPIs or H2RAs. Among non-deficient subjects, 7.2% took PPIs for 2 or more years, 3.2% took H2RAs for 2 or more years, and 89.6% did not take either PPIs or H2RAs. The study concluded that past or current therapy with gastric acid inhibitors is significantly associated with vitamin B12 deficiency [102]. Patients who took PPIs or H2RAs for 2 or more years showed a 65% increased risk of vitamin B12 deficiency compared to those not undergoing such therapy [109].

Vitamin C is found in gastric juice as ascorbic acid, its biologically active and antioxidant form. To neutralize potentially carcinogenic nitrosamine species resulting from the reaction between saliva and gastric juice, ascorbic acid is partially converted into dehydroascorbic acid, its oxidized form [110]. Dehydroascorbic acid, once absorbed, can be easily reconverted to ascorbic acid through the action of glutathione. The entire process requires an acidic intragastric environment. Any increase in pH, especially to levels above 4.0, affects vitamin C absorption because as pH increases, dehydroascorbic acid is irreversibly hydrolyzed to 2,3-diketogulonic acid, which cannot be absorbed [111]. PPIs reduce the bioavailability of vitamin C by lowering its concentration in gastric juice and the proportion of vitamin in its reduced form. In one study, the effect of the administration of 40 mg/day of omeprazole for 4 weeks on the concentration of vitamin C in the gastric juice of healthy individuals was evaluated: the median concentration of ascorbic acid in the fasting gastric juice was reduced from 3.8 to 0.7 µg/ml ($P < 0.001$) and the total concentration of vitamin C in gastric juice was reduced by approximately 50% [112]. In another study, the administration of 40 mg/day of omeprazole for 4 weeks induced a significant increase in intragastric pH and a reduction in mean serum vitamin C concentration by 12.3% ($P = 0.04$) [113]. This indicates a reduction in the bioavailability of vitamin C [113].

Further clinical studies have shown that *H. pylori*-infected patients taking PPIs significantly decrease serum vitamin C concentrations, as early as 1 month after starting PPI therapy [114]. This may be due to altered absorption due to an even higher gastric pH in the presence of both factors [115] or because *H. pylori*-positive patients have a lower vitamin C intake compared to the general population due to lower socioeconomic status, as indicated by observational studies [113]. Therefore, patients with hypochlorhydria/achlorhydria due to any cause, including

treatment with PPIs, especially in combination with *H. pylori* infection, are at risk of vitamin C depletion.

As mentioned in previous paragraphs, patients with obesity, T2DM, and MetS are predisposed to inadequate intake of WSVs. The intake of metformin by these patients exacerbates the situation as it leads to the depletion of WSVs. These same patients also have a high prevalence of GERD, which is treated with PPIs and H2RAs. These drugs, in turn, deplete WSVs. Conditions that cause insufficient fulfillment of the WSVs requirements accumulate, and this deficiency is related to the same metabolic diseases these patients suffer from and the onset of related disorders, creating an evident vicious cycle.

Additional support for this conclusion can be derived from the following reflection. It is established that obesity, T2DM, and MetS are associated with the onset of peripheral neuropathies [116-118]. The signs of vitamin B12 deficiency neuropathy can be easily confused with those of diabetic neuropathy [65]. Clinical studies demonstrate that the administration of WSVs to diabetic patients provides benefits for the signs and symptoms of neuropathy [119-121]. Given that the administration of WSVs to diabetics improves signs and symptoms of neuropathy, it can be suspected that at least some of the neuropathies presented by patients with metabolic diseases, which respond to treatment with these vitamins, are actually vitamin deficiency neuropathies. These neuropathies are caused by the initial predisposition of these patients to suffer from vitamin deficiencies and the chronic intake of drugs such as metformin and PPIs, which have been proven to induce the depletion of these micronutrients [65].

DRUGS THAT INCREASE THE RISK OF METABOLIC DISEASE

The results of clinical studies allow us to state that the use of PPIs increases the risk of developing T2DM. In 2020, an article was published presenting data collected from three prospective cohort studies [122]. Over 2,127,471 person-years of follow-up, 10,105 cases of T2DM were documented. It emerged that patients who regularly take PPIs have a 24% higher risk of developing T2DM compared to individuals who do not take PPIs. Furthermore, the risk of developing T2DM increases with the duration of PPI therapy [122]. More recent studies have extensively confirmed these findings [123,124].

Some researchers have focused on identifying the mechanism through which PPIs increase the risk of T2DM, and notable elements have emerged, but it is not the objective of this article to review the formulated hypotheses. Instead, attention is drawn to some interesting details: there is accumulating evidence that the use of PPIs, in addition to increasing the risk of T2DM, can also lead to weight gain [125], chronic liver disease [126], and MetS [127], conditions that, as seen, are associated with mitochondrial dysfunction, which is related to the restriction of the availability of WSVs.

Statins are among the most widely used cholesterol-lowering drugs in the world. In 2018, they were taken by over 145 million people worldwide, with significant differences in consumption between developed and developing countries [128]. Despite the relative safety and tolerability of statins, observational studies, clinical trials, and meta-analyses indicate an increased risk of developing T2DM after prolonged treatment with statins [129]. Lipophilic statins (atorvastatin, simvastatin, lovastatin, fluvastatin, and pitavastatin) may be more diabetogenic compared to hydrophilic statins (pravastatin and rosuvastatin) [129]. One of the most significant results is

that in a study of 8,749 non-diabetic subjects aged between 45 and 73, followed for 5.9 years, the onset of T2DM was diagnosed in 625 subjects [130]. In the 2,142 patients treated with statins, there was a 46% increase in the risk of T2DM compared to the rest of the study population. Statin treatment significantly increased fasting blood glucose levels, decreased insulin sensitivity by 24%, and insulin secretion by 12% compared to subjects not receiving statin therapy. The increased risk of T2DM, reduced insulin sensitivity, and insulin secretion were dose-dependent for simvastatin and atorvastatin [130].

The mechanism by which statins can induce T2DM has not yet been clarified, but in the context of this article, attention is drawn to the fact that chronic statin use is also related to the onset of peripheral neuropathy, at a rate of 1 case per 2200 per year [131]. In cases where statins are administered together with WSVs, in particular niacin has been extensively experimented with in combination, neuropathy does not occur. This observation was made possible because statins were associated with niacin as the latter is believed to have beneficial effects on the lipid profile. The fact that the concomitant intake of WSVs negates the risk of neuropathy in cases of chronic statin treatment raises the question of whether chronic statin use may contribute to restricting the availability of WSVs and whether this may contribute to the onset of statin-induced neuropathy. It is interesting to note that since the results regarding the contribution of niacin to improving the action of statins have not proven to be sufficiently significant, the statin-niacin combination has been abandoned, completely neglecting the beneficial effect regarding the prevention of peripheral neuropathies [131].

Drugs that interfere with the absorption and metabolism of WSVs can induce the onset of metabolic diseases and related disorders. This implies that such drugs can worsen the condition of patients already suffering from these diseases. These assumptions further reinforce the hypothesis that this article aims to support: WSVs is necessary for the proper functioning of metabolism, and therefore, its insufficient intake, even iatrogenic, can lead to the development of metabolic diseases, exacerbate existing ones, and induce related disorders such as neuropathies.

THE TREATMENT OF METABOLIC DISEASES WITH WATER-SOLUBLE VITAMINS

Several lines of evidence demonstrate the effectiveness of treating metabolic diseases with WSVs in animal models. However, this article does not aim to review the literature on this topic. Instead, a brief mention is made of some clinical studies that have shown the effectiveness of individual WSV in the treatment of metabolic diseases, emphasizing once again that the intention of this article is not to prove the effectiveness of individual WSV in the treatment of metabolic diseases but to contribute to the demonstration that all WSVs must be considered an inseparable therapeutic unit for the treatment of metabolic diseases.

A systematic review with meta-analysis published in 2021 demonstrates that folate supplementation plays an important role in reducing IR. Glycemic markers significantly decreased with doses of folate alone exceeding 5 mg/day [132]. Furthermore, it is believed that dietary fortification with B complex vitamins can prevent the cognitive impairment associated with metformin intake in T2DM patients [133].

Some randomized clinical trials have shown that supplementation with WSVs alleviates the disturbances associated with MetS. Folic acid, alone or in combination with vitamin B6 and/or

vitamin B12, reduces blood pressure [134,135], improves IR [136], and has favorable effects on the lipid profile [137,138].

A study published in 2019 demonstrates that vitamin C supplementation for 4 months, compared to a placebo, reduces blood glucose and blood pressure in patients with T2DM [139]. In a placebo-controlled study, high doses of biotin (15 mg/day) decreased glucose and insulin concentrations in oral glucose tolerance tests in patients with fasting hyperinsulinemia and increased the insulin response to a glucose load. Biotin increased insulin sensitivity by $51.1 \pm 23\%$ and $61.3 \pm 22\%$ after 14 and 28 days of treatment, respectively. These findings suggest that biotin treatment may be useful in treating insulin resistance [140]. The results of a randomized double-blind placebo-controlled clinical trial demonstrate that the administration, for three months, of biotin (40 $\mu\text{g/kg}$ of body weight) as an adjuvant to the insulin regimen can improve glycemic management and decrease plasma concentrations of total cholesterol, low density lipoprotein and triglycerides in poorly controlled type 1 diabetes young patients [141]. A further placebo-controlled clinical study demonstrated that the administration of 15 mg/day of biotin for 28 days significantly reduces the level of triglycerides and VLDL both in patients with T2DM and in non-diabetic patients affected from hypertriglyceridemia [142].

CONCLUSION

In Figure 3, an attempt has been made to schematically summarize the relationships highlighted in the article.

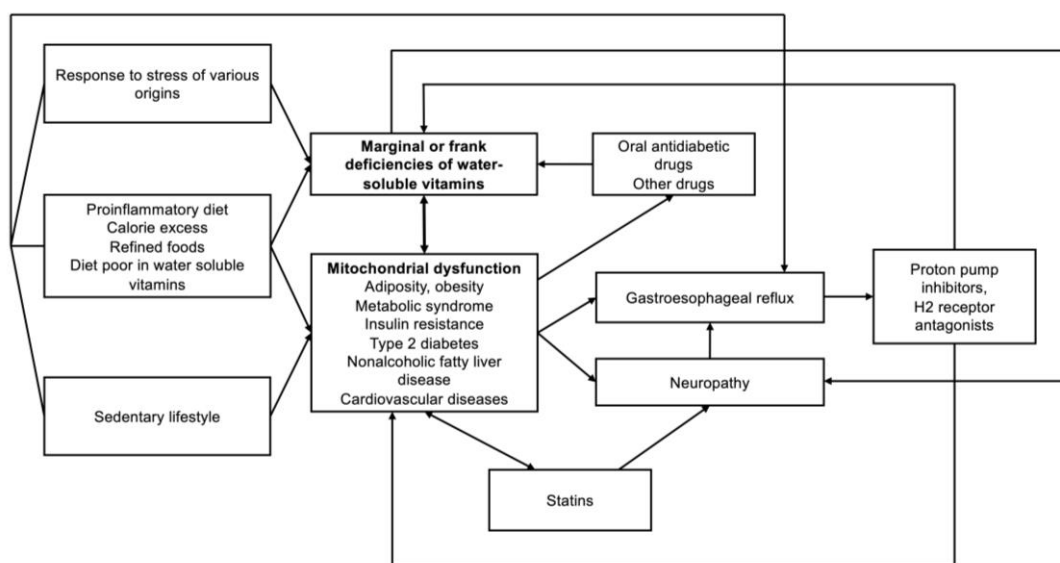


Figure 3: Schematic representation of the relationships between metabolic diseases, associated disorders, drug intake, and insufficient intake of WSVs.

When addressing any state of organism suffering, one should inquire about the cellular biochemical balance, as its imbalance, often caused by inadequate intake of WSVs, could be the main cause or an important contributing factor to the state of suffering and could represent an insurmountable obstacle to the success of any therapy.

WSVs play a crucial role in the functioning of the organism. They are cofactors of enzymes involved in the Krebs cycle, folate and methionine cycles, reactions related to these cycles, and the electron transport chain.

There is growing evidence that WSVs deficiencies are widespread in populations of developed countries, apparently well-nourished. Researchers investigating the serum levels of WSVs in developed countries' populations have found that often dietary intake is lower than recommended, and frank deficiencies, marginal deficiencies, functional deficiencies, and increased requirements are common. According to a fascinating and rational theory, an inadequate intake of essential nutrients does not immediately result in signs and symptoms of suffering because the body has evolved to prioritize these nutrients for functions that ensure immediate survival. However, this comes at the expense of long-term health, favoring the onset of chronic and degenerative diseases.

Based on this premise, considering the functions performed by WSVs and the widespread deficiencies in the population, one would expect that deficiencies would lead to metabolic disorders, influenced by worsening mitochondrial dysfunction. Mitochondria play a central role in cellular metabolism and have a high demand for WSVs. This expectation is met by the evidence emerging in the scientific literature. Adiposity, obesity, IR, T2DM, hepatic steatosis, dyslipidemia, and MetS are related to mitochondrial dysfunction, and studies suggest that a low status of WSVs can lead to adiposity, dyslipidemia, endothelial dysfunction, glucose intolerance, and IR, all of which are implicated in the pathogenesis of MetS. The high prevalence of various forms of WSVs deficiency in the population corresponds to the high prevalence of metabolic diseases.

It has already been demonstrated that chronic intake of certain drugs, such as metformin, proton pump inhibitors (PPIs), and H₂ receptor antagonists (H₂RAs), which represent the cornerstone of pharmacological treatment for obesity, IR, T2DM, hepatic steatosis, dyslipidemia, MetS, or associated disorders, contributes to WSVs deficiency, while suspicions grow for other drugs, for example statins.

Chronic intake of some of the mentioned drugs also increases the risk of developing T2DM, further prompting consideration of the importance of WSVs for proper cellular metabolism. These unwanted effects of drugs are potentially capable of triggering a dramatic vicious cycle, which should be considered in the therapeutic plan.

One would expect that the many lines of evidence accumulated in the literature would have prompted researchers to experiment with WSVs in the treatment of metabolic diseases. It is disappointing to find that clinical trials in this regard are still few and have been conducted considering WSVs as active pharmaceutical ingredients, testing them individually. This is an important limitation of current evidence: often clinical studies investigate one or a few WSVs. This is likely a consequence of an attempt to oversimplify and exposes to some risk. An example can clarify the situation: in the past, a relationship was highlighted between a high level of homocysteine in the blood and an increased cardiovascular risk. Insisting on simplification, it was realized that the level of homocysteine could be reduced by administering only folic acid. After numerous clinical studies, analyzing the collected data, it was found that folic acid administration, despite being effective in reducing homocysteine levels, was not effective in

reducing cardiovascular risk. The temptation was to abandon homocysteine and focus on other factors, but some researchers formulated new questions to revive research: does the elevation of homocysteine levels perhaps hide a deficiency of other WSVs, in addition to folate, involved not only in homocysteine metabolism but in the functioning of the entire organism? Is the insufficient intake of these vitamins conditioning health and increasing cardiovascular risk? To answer these questions, it is useful to point out that a high level of homocysteine has been linked to multiple diseases and that various WSVs are involved in the metabolism of homocysteine: folate, vitamin B2, vitamin B3, vitamin B6, and vitamin B12 which, coincidentally, are frequently lacking in the population. It has therefore been proposed to carry out studies in which the serum levels of all WSVs are investigated and in which all WSVs, or at least those involved in homocysteine metabolism, are administered for therapeutic purposes. Starting from the complex biochemistry of cellular metabolism and reflecting on the experience with homocysteine, it can be suspected that the therapeutic use of a single WSV, neglecting all the others, is probably destined to fail or at least be unsatisfactory in the prevention and treatment of metabolic diseases such as adiposity, frank obesity, IR, T2DM, hepatic steatosis, dyslipidemia, and MetS.

In-depth investigation of the WSVs status of each patient with carbohydrate and lipid metabolism disorders appears to be an impractical and perhaps pointless approach, given the ample existing evidence. In any case, it does not seem rational to focus solely on working hypotheses involving one, two, or at most three WSVs, to the detriment of working hypotheses involving a broader group of closely related vitamins, which is the entire complex of WSVs. Furthermore, the metabolic disorders addressed in this article are related to a restriction of the availability of the entire complex of WSVs, and metformin, H2RAs, PPIs, and statins, which are used to treat these disorders, are proven or suspected to induce a restriction of the availability of various WSVs.

In conclusion, the preservation of the correct cellular biochemical balance is a prerequisite for the preservation or restoration of health, to allow the therapeutic strategies undertaken to succeed and to avoid that the therapeutic strategies undertaken can become a source of additional pathological states.

Based on the available data, the relevance of administering the inseparable prophylactic and therapeutic unit consisting of all WSVs cannot be overlooked to interrupt what appears, in all respects, a vicious cycle. The administration of WSVs is safe, very economical, can benefit patients with metabolic diseases and related disorders, can improve the therapeutic performance of prescribed drugs, and allow control of their adverse effects.

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