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Commentary

A historic study that opened a new chapter in nutritional science

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"The effect of chemical preservation of eggs upon the stability of their vitamin contents"[1], published in 1926 by Ernest Tso, was the first paper from China to appear in a Biochemical Society journal[2]. Using a deprivation-and-supplementation strategy in rats, Tso showed that the originally rich vitamin B content of 'thousand-year eggs' (also known as 'pidan') was completely destroyed by chemical preservation, but vitamin A and antirachitic substances were largely unaffected by the process. This classical article signaled a new era in nutritional science, deepening our knowledge of the relationship between vitamins and diseases.

Ernest Tso was a professor of pediatrics in the Department of Medicine, Peking Union Medical College, in the 1920s, specializing in infant nutrition[3]. He was the first person in China to use soymilk to feed infants[4], which led to the development of a soymilk infant formula[5]. His work on pidan was, at that time, part of primary vitamin studies designed to gain insights into the link between diet and vitamin intake.

In his study of pidan, Tso deprived rats of vitamins in their diet and observed the consequent pathological alterations. He found that vitamin A in eggs was retained after chemical preservation, showing that — contrary to the dogma of the time — xerophthalmia was not an infectious disease but was rather caused by vitamin A deficiency. We now know that vitamin deficiency is a state resulting from the lack of or the inability to use one or multiple vitamins, leading to different symptoms or clinical manifestations (Table 1).

In addition to the classical associations shown in Table 1, new information on the relationship between diseases and vitamins is highlighted below.

Vitamins and cancer

Vitamin A inhibits the proliferation, chemotaxis and invasion of human melanoma cell lines[14]. Vitamin C decreases the viability and incorporation of thymidine into DNA in DU145 and LNCaP prostate cancer cells[15] and induces apoptosis in oral squamous cell carcinoma and salivary gland tumor cell lines[16]. High levels of vitamin C cause energetic crisis and cell death in *KRAS*- or *BRAF*-mutant human colorectal cancer cells[17]. Vitamin D receptor ablation in mice improves chemical carcinogenesis in mammary, epidermis and lymphoid tissues, but not in the ovary, lung or liver[18]. The most active product of vitamin D, $1\alpha,25(OH)_2D_3$, induces apoptosis and reduces proliferation in T-cell lymphoma cells[19], inhibits the tumor-initiating activity of cancer stem cells[20], and decreases angiogenesis in X-ray immunosuppressed BALB/c mice[21]. The redox-inactive analogue of vitamin E, α-tocopherol succinate (α-TOS), suppresses proliferation and induces apoptosis in $p53^{-/-}$ *and* $p21^{waf1/cip1(-/-)}$ human prostate cancer cells but not normal prostate epithelial cells[22]. α-TOS also increases MDA-MB-231 cell apoptosis and retards their growth in nude

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Table 1. Vitamin deficiency and clinical manifestation.

Deficiency	Clinical manifestation	Reference
Vitamin A	Xerophthalmia, blindness, high child mortality due to an increased susceptibility to infectious diseases and metabolic disorders	[6]
Vitamin B_3 (niacin)	Pellagra	[7]
Vitamin B_{12}	Glossitis, megaloblastic anemia, myelin deterioration	[8]
Vitamin C	Weakness, myalgia and arthralgia, vascular purpura and hemorrhagic syndrome, loss of teeth, anemia, hypocholesterolemia and hypoalbuminemia, scurvy, blood vessel fragility, connective tissue damage	[9, 10]
Vitamin D	Rickets	[11]
Vitamin E	Neuromuscular abnormalities characterized by spinocerebellar ataxia and myopathies	[12]
Vitamin K	Bulging fontanels, irritabilities, convulsions, bleeding and ecchymosis, feeding intolerance, poor sucking, vomiting, diarrhea, jaundice, pallor	[13]

mice[23]. Dietary vitamin E supplementation inhibits liver cancer development in TGFα/c-myc mice[24], and vitamin K_2 induces the differentiation of HL-60 leukemia cells[25] and reduces initiated superoxide radical in colon stem cells[26].

Vitamins and diabetes

Vitamins A, C and E are well-known antioxidants. Vitamin A has been shown to exert hypoglycemic action in diabetic mice[27], and its deprivation induces endocrine pancreas remodeling, β-cell apoptosis, β-cell mass reduction, α-cell mass increase and hyperglucagonemia in adult mice, features that resemble the phenotypic characteristics of human type 2 diabetes[28]. Vitamin D deficiency has been found to decrease insulin secretion in response to glucose in humans and animal models. Vitamin D_3 elevates insulin secretion in females with type 2 diabetes[29]. The injection of vitamin E prevents increases in membrane PKCβ II protein and normalized diacylglycerol (DAG) levels in the retinal tissues of streptozocin-induced diabetic rats[30]. Vitamin K supplementation may improve insulin sensitivity and glucose tolerance[31].

Vitamins and neurodegeneration

The plasma concentrations of vitamins A and E have been shown to be significantly lower in patients with Alzheimer's disease than healthy subjects[32], whereas midbrain levels of vitamin E in these patients are higher[33]. Vitamin E also inhibits amyloid β protein-induced cell death[34], and vitamin E deficiency diminishes the dendritic branching of Purkinje neurons and alters motor coordination ability in mice[35]. It is known that vitamin E is capable of scavenging free radicals to prevent the oxidative cell death of neurons and reducing the activation of transcription factors elicited by oxidative stress[36]. Treatment with vitamin E has been shown to decrease total glutathione and superoxide dismutase and markedly attenuate behavioral and biochemical abnormalities in a rat model of unilateral Parkinsonism[37,38]. There is also a high prevalence of vitamin D insufficiency in Parkinson's disease[39]. The connection between vitamins and neurodegenerative diseases is further supported by the observation that vitamin K_2 and the vitamin B_1 derivate benfotiamine suppress inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX-2) expression and reactive oxidative species (ROS) generation in BV2 microglial cells[40].

Epidemiological evidence

Blot and colleagues reported that a combination of β-carotene, vitamin E and selenium may reduce cancer risk in human populations[41]. The dietary intake of vitamin K_2 is associated with a reduced incidence of cancer[42,43], and a significant association between methylenetetrahydrofolate reductase polymorphism/folate intake/vitamin B_6 and breast cancer has been proposed[44,45], although a case-control study conducted in Brazil and Japan has indicated otherwise[46, 47].

It has been suggested that vitamin D supplementation in infants can reduce the incidence of type 1 diabetes[48, 49], and its combination with calcium was shown to be beneficial in reducing the risk of type 2 diabetes in a study involving 83 779 women[50]. However, this linkage was not supported by a meta-analysis demonstrating that vitamin D supplementation had no effect on glucose homeostasis[51].

Increased vitamin B intake was shown to reduce plasma homocysteine, which is alleged to decrease the risk of Parkinson's disease[52]. However, this view was not supported by a dietary study carried out among 249 patients using folate, vitamin B_6, vitamin B_{12} and riboflavin[53].

These conflicting conclusions point to the dynamic nature of the relationship between vitamins and diseases, which must be discussed within the framework of the specific vitamin type, dose, age of intake and the state of a disease, among other factors.

Conclusion

Ninety years have passed since the publication of Ernest Tso's paper describing the vitamin content of pidan[1]. The tremendous advancement in science during that period has allowed us to understand and appreciate the essence of vitamins in nutrition and health. Vitamins are recognized as a group of compounds essential to normal physiological function, and we now know that vitamin deficiencies lead to multiple diseases, reflecting the function of each vitamin. Along with economic development and improvements in quality of life around the world, people are becoming increasingly aware of nutrition in the context of vitamin supply. A balanced diet is useful to maintain an appropriate vitamin intake, but supplements may be required to correct deficiencies.

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