Fat-Soluble Vitamin Deficiencies and Disruption of the Immune System in Pancreatic Cancer

*A Vicious Cycle*

*Mohammad Hosein Aziz, MD,\* Jan van der Meulen, MD, PhD,*† *Dana A.M. Mustafa, PhD,*†‡ *and Casper H. J. van Eijck, MD, PhD*†‡

Abstract: Pancreatic ductal adenocarcinoma (PDAC) is currently an in- creasing contributor to cancer-related mortality. Despite advances in cancer treatment, PDAC survival rates have remained roughly unchanged over the years. Specifically, late diagnosis and insensitivity to currently available therapeutic regimens have been identified as the main causes for its poor survival. Pancreatic exocrine insufficiency (PEI) is a typical complication associated with PDAC diagnosis and pancreatic surgery. Pancreatic exo- crine insufficiency, a major contributor to maldigestion in PDAC, is often not treated because it remains undetected because of lack of overt signs and symptoms. In this review, we will focus on the major consequences of PEI, including the inadequacy of lipase excretion, which results in defi- ciency of fat-soluble vitamins. Because PDAC is known for its immune- high jacking mechanisms, we describe key features in which deficiencies of fat-soluble vitamins may contribute to the aggressive biological behavior and immune evasion in PDAC. Because PEI has been shown to worsen sur- vival rates in patients with PDAC, detecting PEI and the related fat-soluble vitamin deficits at the time of PDAC diagnosis is critical. Moreover, timely supplementation of pancreatic enzymes and fat-soluble vitamins may im- prove outcomes for PDAC patients.

Key Words: pancreatic cancer, pancreatic exocrine insufficiency, fat-soluble vitamin deficiency

Abbreviations: PDAC – pancreatic ductal adenocarcinoma, PEI –

pancreatic exocrine insufficiency

n 1969, 4.4% of all cancer-related deaths in the Netherlands was due to pancreatic ductal adenocarcinoma (PDAC).1 Fifty years later, the contribution of PDAC to cancer-related mortality had in- creased to 6.6%.2 This increase is largely explained by the late di- agnosis and poor response to conventional therapy in comparison to other cancer types.3,4 Five-year survival rate is roughly 6% and pancreatic cancer is still one of the cancers with the worst progno- sis, with little increase in survivability in recent decades.5 Hence, a better understanding of the biological derangements induced by this cancer might unveil novel therapeutic possibilities to improve

I

patient outcomes.6

A typical sign of PDAC is pancreatic exocrine insufficiency (PEI), which is strongly correlated with reduced survival, whereas

enzyme replacement therapy has shown survival benefits.7,8 Clini- cal manifestations of PEI include abdominal discomfort, bloating, malnutrition, and steatorrhea. The latter manifestation, steatorrhea, is however very uncommon. It occurs in less than 20% of patients with advanced PDAC and severe PEI.9 Pancreatic exocrine insuffi- ciency occurs because of damage of the secreting components of the pancreas by the carcinoma itself, because of bile duct obstruc- tion, and after pancreatic surgery. Importantly, PEI is characterized by a diminished excretion of several enzymes, such as amylase, pro- tease, and lipase. Insufficient lipase excretion, in combination with reduced bile salts, results in improper digestion of fat with as conse- quence malabsorption of the fat-soluble vitamins A, D, E, and K.

Among their integral functions in a multitude of physiolog- ical processes, these fat-soluble vitamins are also known to assist the immune system to attack tumor cells by stimulating cyto- toxic immune cells and blocking the synthesis of tolerogenic cy- tokines.10 Thus, deficiencies of these vitamins may play a role in the intrusive pathobiology of PDAC.11 Unfortunately, the preva- lence of deficiencies of the fat-soluble vitamins at the time of PDAC diagnosis is not well known. In addition, the current Dutch PDAC guidelines restrict the measurement of fat-soluble vitamins only to patients with symptoms of malabsorption.12 However, there are several studies describing patients without symptoms of malabsorption or nutritional deficiencies, whereas labora- tory tests showed PEI with these vitamin deficiencies.9,13 Thus, PEI and vitamin deficiencies may be present before clinically relevant malabsorption is noticed, especially in the case of ob- structive jaundice.14 Hence, the focus of this review will be on the current difficulties in identifying fat-soluble vitamin defi- ciencies, as well as how these deficiencies contribute to pancre- atic tumor immune evasion and aggressiveness.

# DIAGNOSIS AND MANAGEMENT OF PEI

The pancreas consists of 2 compartments, the endocrine and the exocrine compartment. The exocrine compartment, where PDAC arises, produces bicarbonate and enzymes for the digestion of carbohydrates, proteins, and fat. More than 90% of the pan- creas is composed of the exocrine compartment.15 Tumor growth within this compartment will replace these bicarbonate and enzyme-producing cells, which will result in PEI. Progressive PEI will lead to malnutrition, specifically inadequacy of fat- soluble vitamins, and micronutrients.16

The prevalence of PEI after diagnosis of PDAC is 70%. Moreover, 70% of those affected do not exhibit clinical signs of malabsorption.17 Clinical manifestations of PEI are usually non- specific and can lead to a lack of timely diagnosis. However, there are several diagnostic tests for PEI. Previously, exocrine pancreas insufficiency was diagnosed by a stool collection for 72 hours while patients were given a daily fat intake of 100 g. When there was more than 7 g of fat per 100 g of stool each day, fat malabsorp- tion was suspected. When there was more than 15 g of fat per

100 g of stool per day, severe PEI was diagnosed.18 However, this test is not very patient-friendly due to many side effects, such as bloating, worsening steatorrhea, and flatulence.19 Nowadays, the fecal elastase test is commonly used because of its simplicity.19,20 Pancreatic elastase 1 is produced by the pancreas and is known to be a stable enzyme during the intestinal passage.21 An enzyme- linked immunosorbent assay is used to measure elastase in fecal samples, and there is a good relationship between pancreatic exo- crine secretion and elastase levels.22 When formed stools are stud- ied, fecal elastase testing has a high negative predictive value and a high sensitivity in individuals with mild to severe PEI.23–25 There- fore, the test should not be conducted in watery stools, because fe- cal elastase is measured as a concentration.26

In general, for the diagnosis of PEI, a fecal elastase of 100 to 200 μg/g feces reflects moderate to mild PEI and a fecal elastase of

<100 μg/g feces reflects severe PEI.20,21,24,25 The advantages of fecal elastase testing make it an appropriate test for screening PDAC patients for PEI.27,28 Furthermore, because porcine en- zymes do not cross-react with the human antibodies, administra- tion of pancreatic enzymes does not have to be discontinued for testing. In case of PEI, enteric-coated capsules with pancreatic en- zymes are administered to aid in digestion right before food con- sumption. Usually, the lipase units for adults vary between 40,000 and 50,000 lipase units per main meal, and smaller units are pre- scribed for snack consumption. Starting supplementation of fat- soluble vitamins and pancreatic enzymes together should be con- sidered because most patients suffering from PEI after pancreatic surgery do not ingest enough pancreatic enzymes and eventually will develop vitamin deficiencies.29

# FAT-SOLUBLE VITAMINS

## Vitamin A

Vitamin A, also called retinol, is required for the growth of epithelial and red blood cells and aids in building adequate im- mune activity.30 It comes in the form of retinol, b-carotene, or retinyl ester in the diet. Fish oils, dairy products, egg yolk, and the liver are the main sources of retinol. B-carotene is present in vegetables and the liver converts it into retinol. Retinol, a form of vitamin A, is oxidized to retinaldehyde by aldehyde dehydroge- nase protein, which is then converted to retinoic acid (RA).31 Se- rum retinol concentrations lower than 0.35 mM reflect deficiency, and adequate concentrations are between 1.0 and 2.2 mM. AWest- ern diet is believed to contain more than enough vitamin A.32

## Vitamin A in Pancreatic Cancer

Retinoic acid plays a central role in the immune system, and lack of RA can cause poor immune regulation and decreased cel- lular and humoral responses, but also a weak response to vaccines and poor lymphoid organ development.33 Sixteen percent of pa- tients with PEI due to chronic alcohol-induced pancreatitis, but without steatorrhea, had vitamin A deficiency.34 Another cause of low serum retinol concentration is inflammation. In this situa- tion, the liver starts to produce C-reactive protein with a concom- itantly decreased production of retinol-binding protein. Reduced retinol-binding protein levels have also been linked to an elevated neutrophil-to-lymphocyte ratio,35 a potent inflammatory biomarker used in pancreatic as well as other cancer prognostic studies.36,37 Decrease in retinol-binding protein results in an increase of un- bound retinol, which is excreted in the urine.34 However, there are ways of correcting the interference from inflammation.38 Retinoic acid also regulates cellular development, differentiation, proliferation, and apoptosis. Its pleiotropic impact is mediated by nuclear receptors known as retinoic acid receptors and retinoic X

receptors. In cancer-associated fibroblast (CAF) cells, RA has been demonstrated to inhibit the production of interleukin-6 (IL-6).39 In several forms of cancers, including pancreatic cancer, IL-6 is linked to tumorigenesis, as well as cancer progression, and treatment resistance.40–42 It was demonstrated that the suppres- sion of tumor cell migration and epithelial mesenchymal transi- tion (EMT) was linked to reduced IL-6 release from CAFs.43 Cancer-associated fibroblasts, which are abundant in the stroma of pancreatic tumors, help tumor cells proliferate, migrate, metas- tasize, and resist chemotherapy. Subsequently, CAFs treated with RA became immobile because of the low expression of *ɑ*-smooth muscle actin, fibroblast activation protein, as well as decreased ex- tracellular matrix formation. Cancer-associated fibroblasts are one of the main components of desmoplastic tissue, which surrounds the tumor cells and prevents immune cell infiltration, as well as preventing vascularization, and thus limiting exposure to conven- tional systemic therapy in pancreatic cancer.44–46 Although until now no clinical trials have published an objective response with RA in PDAC, CAFs response to RA seems promising.46 Further- more, pancreatic stellate cells (PSC), which are precursor cells of CAFs, store RA. When triggered by malignancy or inflammation, PSC lose their RA reserves and take on the characteristics of an ac- tivated myofibroblast. In mice, restoring RA depots inside PSC re- duced desmoplasia and slowed cancer progression.45 As a result, RA seems to be a suitable drug for reducing the activation of sev- eral pathways in PDAC.44,47 Noteworthy, it seems that RA levels do not always correspond with retinol levels. In patients with lung cancer, RA levels, and not retinol levels, were lower than normal.48 Thus, in patients with PDAC, not only retinol but also RA levels should be measured for the diagnosis of vitamin A deficiency.

## Vitamin D

Ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3) are the most significant vitamin D molecules (vitamin D3). The ending “ol” of both compounds indicates that the compounds con- tain a hydroxyl group with the chemical formula –OH. The major natural source is cholecalciferol, which is synthesized in the skin by sun exposure. Vitamin D is biologically inactive and must un- dergo 2 stages of hydroxylation involving protein enzymes. The first step is in the liver where D3 becomes calcidiol (25(OH)D). In the second step, which occurs predominantly in the kidneys, 25(OH)D becomes calcitriol (1,25(OH)D). Whereas sun exposure in the population is variable and a safe amount of sun exposure in view of skin cancer is uncertain, vitamin D supplementation is ad- vised for a large part of the population.49 The European Calcified Tissue Society defines vitamin D-deficiency as a serum concen- tration of 25(OH)D <50 nmol/L independent of the time of the year the measurement is performed. However, the Australian guidelines state “The target level should be >50 nmol/L at the end of winter (10–20 nmol/L higher at the end of summer, to al- low for seasonal decrease) for optimal musculoskeletal health.”50 The spread of 10 to 20 nmol/L reflects the different latitudes, Perth in Australia is 32°S and Melbourne 38°S. The closer to the equa- tor, the smaller the difference between summer and winter. In the United Kingdom, which has the same latitude as the Netherlands, the difference between summer and winter is 25 nmol/L.51

## Vitamin D in Pancreatic Cancer

Besides being the principal factor *for optimal musculoskeletal health, vitamin D has* many other biological effects.52 There are suggestions that higher serum concentrations, that is, >75 nM of vitamin D are needed for proper innate and adaptive immune re- sponses.53 Interestingly, inflammation lowers serum 25(OH)D levels. As the liver starts to produce C-reactive protein during

inflammation, the production of vitamin D binding protein decreases. This decreased production may increase unbound 25(OH)D, which is redistributed in the tissues.54

The following evidence supports vitamin D's significance in pancreatic cancer biology. The enzyme involved in the conversion of calcifediol to calcitriol has also been observed in adenocarcino- matous ductal cells and their proliferation is inhibited by the pres- ence of calcitriol.55 In vitro, vitamin D analogs reduce the prolifer- ation of pancreatic cancer cells, induce differentiation, and enhance apoptosis, and in vivo, they are known to inhibit pancreatic tumor growth.56,57 Calcitriol also improves the antitumor efficacy of gem- citabine in vitro and in vivo.58 Cholecalciferol and calcitriol radio- sensitize cancer cells.59 Thus, early suppletion of activated vitamin D, that is, calcitriol is a promising adjuvant in pancreatic cancer pa- tients treated with chemotherapy and radiotherapy. Moreover, the receptor for vitamin D is present on almost all cells of the immune system, and therefore it has a wide and complex effect on the im- mune cells.60 In pancreatic tumor stroma, a topical vitamin D ana- log (calcipotriol) showed a decrease in CAF proliferation and re- duction of the pro-tumorigenic factors, such as IL-6.61 Increased levels of inflammatory biomarkers have recently been related to vi- tamin D deficiency,62 and as a result, shorter overall survival in PDAC patients at all stages.63 Vitamin D deficiency could therefore have important clinical implications in pancreatic cancer patients' survival. However, a proper definition of vitamin D deficiency is needed, especially in the complex pathobiology of cancer patients.

## Vitamin E

Vitamin E is not 1 vitamin, it consists of 4 tocopherols and 4 tocotrienols (a-, b-, d-, and g-). Plants make these 8 different forms of vitamin E, and they are all known to be potent antioxidants. A-tocopherol serum concentrations in persons older than 18 years are typically 15 to 35 mol/L. The prevalence of a-tocopherol defi- ciency is low because the Western diet contains sufficient a- tocopherol.64 Because tocotrienols are far less frequent in plants, deficiencies appear to be much more common in the general pop- ulation.65,66 However, measuring of tocotrienols is not commonly performed because the high-performance liquid chromatography method is recommended.67 Thus, tocotrienols are only mentioned in about 3% of all vitamin E publications.68

## Vitamin E in Pancreatic Cancer

Although vitamin a-tocopherol deficiency is uncommon, animal and human studies suggest that intake above currently suggested levels can help the immune system age more slowly and improve the body's immunological response to cancer.69 Supplementing with vitamin E on a daily basis can improve the ac- tivity of neutrophils, lymphocytes, and natural killer cells.70 Al- though there are no data on tocotrienol serum levels in PDAC, vita- min d-tocotrienol has been demonstrated to enhance gemcitabine's anti-tumor effects and decrease nuclear factor kappa B (NF-κB) ac- tivation in pancreatic cancer.69 Because it is also recognized to be a critical transcription factor for inflammatory pathways, NF-κB has recently been explored as a possible conduit between cancer and inflammation. In 67% of human pancreatic tumors, NF-κB is activated, but not in normal pancreatic tissues.71 Vitamin d-tocotrienol reduces NF-κB activity, cell proliferation, cell sur- vival, and tumor formation in nude mice. By Western blot, NF-κB inhibition causes a reduction in phosphorylated IkBa ex- pression in pancreatic cancer cells (AsPc-1 and MIA PaCa-2 cells) and tumor tissues.69 In the cytosol, vitamin b-, d-, and g-tocotrienol considerably reduced ReIA (p65) IkBa subunit binding to DNA, whereas g- and d-tocotrienol dramatically reduced p65 binding to DNA in the nucleus.72 Inhibition of NF-κB has also been shown

to improve gemcitabine activity in pancreatic cancer cells.69,71,73–76 These findings show that tocotrienols' anti-pancreatic cancer ef- fect stems in part from their suppression of the NF-κB transcrip- tion factor. Furthermore, before evaluating the clinical importance of vitamin E administration, more studies assessing tocotrienol levels in PDAC are warranted.77

## Vitamin K

Vitamin K (VK), like vitamin E, is not 1 vitamin. It consists of phylloquinone (VK-1), menaquinone (VK-2), and menadione (VK-3). Plants produce VK-1, which is used to treat bleeding dis- orders because of certain anticoagulants. Leafy green vegetables are a good source of VK-1, because VK-1 is directly involved in photosynthesis. VK-2 is synthesized by certain intestinal bacteria and food is a minor source.78,79 Menaquinone consists of several re- lated chemical subtypes and the 2 most studied are menaquinone-4 (MK-4) and menaquinone-7 (MK-7). Menaquinone-3, MK-5, and MK-6 make up the category of VK-3, these are synthetic vita- mins.11 An indirect test for VK-1 is the prothrombin time and sev- eral methods are available for direct quantification of serum VK-1, but not for serum VK-2.79,80 Reference values for serum concentra- tions of VK-1 are 0.8 to 5.3 nmol/L. However, VK-1 is usually not measured because its levels respond to dietary changes within 24 hours, whereas effects on the vitamin K–dependent proteins appear much later.81

## Vitamin K in Pancreatic Cancer

Phylloquinone and dihydrophylloquinone in the diet, but not menaquinone, have been linked to a lower risk of pancreatic can- cer.82 However, in a large prospective study, the opposite was found. Dietary intake of menaquinone and not phylloquinone was inversely related to all-cause mortality.83 The explanation for the protective effect of VK-1 and VK-2 in PDAC could be the role they play in apoptosis. The lack of sensitivity to apoptotic stimuli is the primary cause of PDAC formation and progression, as well as its subsequent resistance to conventional therapies.84 The intrinsic, or mitochondrial, process of apoptosis is separated from the extrinsic, or death receptor pathway. Phylloquinone and VK-2 both triggered apoptosis in pancreatic cell lines, but VK-2 seemed more effective. Phylloquinone and VK-2 could thus assist in patients with pancreatic cancer as a single medication or in con- junction with chemotherapy for treatment or to prevent recurrence of pancreatic cancer after resection.85

Ras is the most frequently mutated oncogene. The exchange of guanosine diphosphate for guanosine triphosphate activates the enzyme and Raf kinase is then activated. Subsequently, Raf kinase activates Raf/mitogen-activated protien kinase, which in turn acti- vates extracellular signal-regulated kinase (ERK).86 Activation of ERK promotes tumor growth through an important autocrine growth loop.87 Phylloquinone is directly involved with the MEK- ERK pathway in pancreatic cancer, where it can induce apoptosis through inhibition of phosphor-MEK and phosphor-ERK.88 ERK phosphorylation and growth suppression were also observed after injections of menadione (VK-3) into pancreatic tumor tissues.89 Menaquinone has also been investigated for the treatment of liver cancer.90,91 In a prospective randomized controlled trial, a signif- icant risk reduction regarding the development of hepatocellular carcinoma occurred in Japanese women with viral cirrhosis of the liver who received 45 mg/d of vitamin K2.92 However, to our knowledge no clinical trials on VK-2 and pancreatic cancer treatment have been conducted. Furthermore, the typical range for VK-2 should be determined before such studies can be con- ducted, as it is unknown in the European population.93

# DISCUSSION

The late disease presentation and the aggressive nature of PDAC made this cancer the 5th leading cause of cancer-related deaths in 2008.94 Typical for this aggressiveness is the tendency of PDAC to cause the immune system to become tolerable to can- cerous cells instead of attacking them. Pancreatic ductal adenocar- cinoma is, therefore, characterized as a poorly immunogenic tu- mor. Myeloid derived suppressive cells (MDSCs), T regulatory cells, and M2 macrophages make up the majority of its microen- vironment.10 The tolerance to cancer in PDAC is also due to the upregulation of negative T-cell costimulatory molecules, such as PDL1, PDL2, and CTLA4. To date, immunotherapeutic regimes have failed in clinical trials mainly due to this complex landscape within tumor and stroma, and tangled combination of immune cells, chemokines, and receptor-ligands. By accepting this expla- nation, the question arises: “Why does this immune evasion hap- pen in PDAC?” We hypothesize that one of the reasons could be the fact that PDAC causes PEI, which starts a vicious cycle of im- mune modulation due to fat-soluble vitamin deficiencies. In this review, we describe these potent immune-modulating effects of fat-soluble vitamins in PDAC.

The prevalence of vitamin A deficiency at the time of diag-

nosis of PDAC is not well known, most likely it is around 20% be- cause after pancreatoduodenectomy, it is 21%.95 However, it may be higher because 50% of patients with PDAC have signs of a systemic inflammatory response,36 and during an inflammatory response a temporary vitamin A deficiency could exist.32 The im- portance of this temporary phenomenon needs to be investigated because an increased inflammatory response in PDAC is also as- sociated with poor prognosis.36 During this response low serum retinol concentrations are caused by increased urinary losses and decreased mobilization from the liver. These low serum reti- nol concentrations may have adverse effects on retinol availabil- ity to target malignant tissues, such as PDAC.96 Multiple, en- hanced, embryonic, context-specific signaling cascades triggered in PDAC appear to be dampened by RA. Retinoic acid also in- hibits cardiotoxicity of doxorubicin, a chemotherapeutic that shows promising results in PDAC, and RA combats the adverse effects of radiotherapy.97,98 Exogenous RA also abrogates the generation of MDSCs with negligible impact on myeloid differ- entiation.99 Finally, the all-trans retinoic acid, the vitamin A me- tabolite, seems to inhibit the proliferation of M2 macrophages.100 Therefore, retinol and RA supplementation, as soon as the diag- nosis of PDAC is made, could contribute to better survival. How- ever, the effectiveness of RA supplementation can only be effec- tively assessed once RA and retinol levels are determined.

It seems logical to exclude vitamin D deficiency in the case of PEI because the major natural source of vitamin D is the skin.101 This statement is correct if one considers a full year. In the Netherlands the diet supplies about 4 μg vitamin D per day and sun exposure 11 μg vitamin D per day in the months April un- til September. Thereafter, in the months October until March, the sun contributes only 2 μg vitamin D per day.102 For maintaining healthy bones, which have an average turnover of 10% per year, a yearly average intake of 10 μg vitamin D per day seems suffi- cient.103 In contrast, the turnover of the adult pancreas has been estimated to take approximately 4 months.104 When this period falls within the winter months, the major source of vitamin D is the diet and in the case of PEI, a vitamin D deficiency is likely to be present.105 It is unknown how common vitamin D deficiency is at the time of PDAC diagnosis, however, after pancreatoduode- nectomy 40% of the patients had a serum concentration of 25(OH)D

<50 nmol/L.95 One may assume that the same percentages may ap- ply to PDAC patients at the time of diagnosis. Whereas proliferation

of PDAC ductal cells is inhibited by the presence of calcitriol, and intraperitoneal injections of calcitriol abrogated the recruitment of MDSCs induced by IL-6 stimulation,106 vitamin D plus calcitriol supplementation as soon as the diagnosis PDAC is made, could contribute to improvement in PDAC survival.107 Yet, the results of suppletion can only be evaluated properly when 25(OH)D levels are corrected for the time of the year they are measured, as well as the presence or absence of inflammation.

Vitamin E deficiency has the following practical problem; there are 2 groups of vitamin E, the tocopherol and the tocotrienol group. The prevalence of a-tocopherol deficiency at the time of di- agnosis of PDAC is not known, after pancreatoduodenectomy, it is 3%.95 Serum tocotrienol levels are mostly not measured and no data are available regarding tocotrienol deficiency in PDAC. Tocotrienols have been shown to harbor anti-tumor activity, en- hance gemcitabine activity, and d-tocotrienols have been shown to suppress NF-κB activation in pancreatic cancer. Experimental studies have also shown a reduction of the T regulatory cells infil- tration in tumors by feeding mice with gamma-tocotrienol,108 and intraperitoneal injections of alpha-tocopherol seem to abrogate the recruitment of MDSCs.109 Thus, supplementation of, a-tocopherol and d-tocotrienol as soon as the diagnosis PDAC is made, should be considered, also because low side effects are expected.68,110 In this situation, too, the effects of d-tocotrienol supplementation can only be effectively assessed once d-tocotrienol levels are determined. Vitamin K deficiency has the same practical problems as vitamin E. There are 2 groups of vitamin K, the phylloquinone and the menaquinone group. Data on the prevalence of vitamin K deficiency at the time of diagnosis of PDAC are rare and when present it regards VK-1. One study reported that VK-1 defi- ciency was common in patients with hepatobiliary and pancre- atic disorders. A deficiency was found in 50.6% of 90 patients, of whom 8 with PDAC.111 Other studies reported that a decrease in pancreatic elastase is associated with serious deterioration of the microbiome, which is the major source of VK-2.112,113 Al- though menaquinone induced apoptosis in pancreatic cancer cell lines, menaquinone suppletion, as soon as the diagnosis PDAC is made, should be considered after evaluation of the

menaquinone levels.88

# FUTURE PERSPECTIVES

Fat-soluble vitamins are usually described with the 4 capital letters A, D, E, and K, suggesting that there would be only 4 fat-soluble vitamins. In reality, there are at least 6 fat-soluble vita- mins, retinol (A), cholecalciferol (D), tocopherol (E), tocotrienol (E), phylloquinone (K-1), and menaquinone (K-2). The next problem is the definition of a deficiency. Minimum serum values of the vitamins, although not always a good representation of a deficiency, are usually used as such. However, here too, only ref- erence values are known for the vitamins A, D, tocopherol, and phylloquinone, but not for tocotrienols and menaquinones, which are showing promising results in treating PDAC. Therefore, se- rum levels of tocotrienol and menaquinone, both of which have promising immunologically mediated anti-tumor activity, should be measured in future studies. Also, randomized trials are needed to compare standard supplementation of fat-soluble vitamins in PDAC patients, especially the active form of retinol (retinoic acid also called tretinoin), the active form of ergocalciferol (calcitriol), tocopherol plus tocotrienol, and menaquinone. Furthermore, there is evidence to suggest that there is an association between PEI and body composition indices in patients with pancreatic dis- ease.114 Therefore, future studies should also focus on the effect of treating PEI and changes in body composition.

# CONCLUSION

To conclude, PEI is a major contributor to malnutrition in pancreatic cancer. In this review, we provide an overview of the role of fat-soluble vitamin deficiencies in PDAC and highlight critical gaps of knowledge that should be addressed in future stud- ies. Indeed, because deficiencies of fat-soluble vitamins appear to play a crucial role in the immune disturbance and pathobiological behavior of PDAC, suppletion of these vitamins may represent an additional simple but effective strategy for the treatment of patients with PDAC.

REFERENCES

1. Hoogendoorn D. Trends in kankersterfte [Trends in cancer mortality]. [Article in Dutch]. *Ned Tijdschr Geneeskd*. 1983;127:1661–1668.
2. Integraal Kankercentrum Nederland. Kankersoorten, percentage van de totale kankersterfte in 2019. Available at: <https://iknl.nl/>. Accessed February 2, 2021.
3. Boz G, De Paoli A, Innocente R, et al. Radiotherapy and chemotherapy in pancreatic cancer. Topical issues and future perspectives. *JOP*. 2006;7: 122–130.
4. Neoptolemos JP, Kleeff J, Michl P, et al. Therapeutic developments in pancreatic cancer: current and future perspectives. *Nat Rev Gastroenterol Hepatol*. 2018;15:333–348.
5. Bengtsson A, Andersson R, Ansari D. The actual 5-year survivors of pancreatic ductal adenocarcinoma based on real-world data. *Sci Rep*. 2020;10:16425.
6. Wang S, Zheng Y, Yang F, et al. The molecular biology of pancreatic adenocarcinoma: translational challenges and clinical perspectives. *Signal Transduct Target Ther*. 2021;6:249.
7. Roberts KJ, Bannister CA, Schrem H. Enzyme replacement improves survival among patients with pancreatic cancer: results of a population based study. *Pancreatology*. 2019;19:114–121.
8. Partelli S, Frulloni L, Minniti C, et al. Faecal elastase-1 is an independent predictor of survival in advanced pancreatic cancer. *Dig Liver Dis*. 2012; 44:945–951.
9. Lindkvist B. Diagnosis and treatment of pancreatic exocrine insufficiency.

*World J Gastroenterol*. 2013;19:7258–7266.

1. Sideras K, Braat H, Kwekkeboom J, et al. Role of the immune system in pancreatic cancer progression and immune modulating treatment strategies. *Cancer Treat Rev*. 2014;40:513–522.
2. Davis-Yadley AH, Malafa MP. Vitamins in pancreatic cancer: a review of underlying mechanisms and future applications. *Adv Nutr*. 2015;6:774–802.
3. Integraal Kankercentrum Nederland. Pancreaskanker. 2017. Available at: [https://www.oncoline.nl/uploaded/docs/voeding/Pancreaskanker.pdf?u=](https://www.oncoline.nl/uploaded/docs/voeding/Pancreaskanker.pdf?u=1WFlTS) [1WFlTS](https://www.oncoline.nl/uploaded/docs/voeding/Pancreaskanker.pdf?u=1WFlTS). Acessed March 5, 2017.
4. Dominguez-Muñoz JE. Management of pancreatic exocrine insufficiency.

*Curr Opin Gastroenterol*. 2019;35:455–459.

1. Rigby SH, Schwarz KB. Nutrition and Liver Disease. In: Coulston AM, Rock CL, Monsen ER, eds. *Nutrition in the Prevention and Treatment of Disease*. 1st ed. London, UK: Academic Press; 2001.
2. Rhim AD, Stanger BZ. Molecular biology of pancreatic ductal adenocarcinoma progression: aberrant activation of developmental pathways. *Prog Mol Biol Transl Sci*. 2010;97:41–78.
3. Vujasinovic M, Valente R, Del Chiaro M, et al. Pancreatic exocrine insufficiency in pancreatic cancer. *Nutrients*. 2017;9:183.
4. Gong J, Guan M, Forsmark CE, et al. Fecal elastase, an assay for exocrine pancreatic insufficiency, has clinical utility in patients with pancreatic ductal adenocarcinoma. *Ther Adv Gastroenterol*. 2020; 13:1756284820964319.
5. Struyvenberg MR, Martin CR, Freedman SD. Practical guide to exocrine pancreatic insufficiency—breaking the myths. *BMC Med*. 2017;15:29.
6. Beharry S, Ellis L, Corey M, et al. How useful is fecal pancreatic elastase 1 as a marker of exocrine pancreatic disease? *J Pediatr*. 2002;141:84–90.
7. Sziegoleit A, Krause E, Klör HU, et al. Elastase 1 and chymotrypsin B in pancreatic juice and feces. *Clin Biochem*. 1989;22:85–89.
8. Löser C, Möllgaard A, Fölsch UR. Faecal elastase 1: a novel, highly sensitive, and specific tubeless pancreatic function test. *Gut*. 1996;39: 580–586.
9. Witt H, Apte MV, Keim V, et al. Chronic pancreatitis: challenges and advances in pathogenesis, genetics, diagnosis, and therapy. *Gastroenterology*. 2007;132:1557–1573.
10. Gullo L, Ventrucci M, Tomassetti P, et al. Fecal elastase 1 determination in chronic pancreatitis. *Dig Dis Sci*. 1999;44:210–213.
11. Nissler K, Von Katte I, Huebner A, et al. Pancreatic elastase 1 in feces of preterm and term infants. *J Pediatr Gastroenterol Nutr*. 2001;33:28–31.
12. Lieb JG 2nd, Draganov PV. Pancreatic function testing: here to stay for the 21st century. *World J Gastroenterol*. 2008;14:3149–3158.
13. Fischer B, Hoh S, Wehler M, et al. Faecal elastase-1: lyophilization of stool samples prevents false low results in diarrhoea. *Scand J Gastroenterol*. 2001;36:771–774.
14. Domínguez-Muñoz JE, D Hardt P, Lerch MM, et al. Potential for screening for pancreatic exocrine insufficiency using the fecal elastase-1 test. *Dig Dis Sci*. 2017;62:1119–1130.
15. Takeda M, Shiratori K, Hayashi N, et al. [Fecal elastase-1 test: clinical evaluation of a new noninvasive pancreatic function test]. [Article in Japanese]. *Rinsho Byori*. 2002;50:893–898.
16. Sikkens EC, Cahen DL, van Eijck C, et al. The daily practice of pancreatic enzyme replacement therapy after pancreatic surgery: a northern European survey: enzyme replacement after surgery. *J Gastrointest Surg*. 2012;16:1487–1492.
17. Bono MR, Tejon G, Flores-Santibañez F, et al. Retinoic acid as a modulator of T cell immunity. *Nutrients*. 2016;8:349.
18. Belyaeva OV, Adams MK, Popov KM, et al. Generation of retinaldehyde for retinoic acid biosynthesis. *Biomolecules*. 2019;10:5.
19. Allen LH, Haskell M. Estimating the potential for vitamin A toxicity in women and young children. *J Nutr*. 2002;132(9 Suppl):2907S–2919S.
20. Marotta F, Labadarios D, Frazer L, et al. Fat-soluble vitamin concentration in chronic alcohol-induced pancreatitis. Relationship with steatorrhea. *Dig Dis Sci*. 1994;39:993–998.
21. Stephensen CB, Alvarez JO, Kohatsu J, et al. Vitamin A is excreted in the urine during acute infection. *Am J Clin Nutr*. 1994;60:388–392.
22. Gonda K, Shibata M, Sato Y, et al. Elevated neutrophil-to-lymphocyte ratio is associated with nutritional impairment, immune suppression, resistance to S-1 plus cisplatin, and poor prognosis in patients with stage IV gastric cancer. *Mol Clin Oncol*. 2017;7:1073–1078.
23. Aziz MH, Sideras K, Aziz NA, et al. The systemic-immune-inflammation index independently predicts survival and recurrence in resectable pancreatic cancer and its prognostic value depends on bilirubin levels: a retrospective multicenter cohort study. *Ann Surg*. 2019;270:139–146.
24. Cupp MA, Cariolou M, Tzoulaki I, et al. Neutrophil to lymphocyte ratio and cancer prognosis: an umbrella review of systematic reviews and meta-analyses of observational studies. *BMC Med*. 2020;18:360.
25. Thurnham DI, Mburu AS, Mwaniki DL, et al. Micronutrients in childhood and the influence of subclinical inflammation. *Proc Nutr Soc*. 2005;64:502–509.
26. Guan J, Zhang H, Wen Z, et al. Retinoic acid inhibits pancreatic cancer cell migration and EMT through the downregulation of IL-6 in cancer associated fibroblast cells. *Cancer Lett*. 2014;345:132–139.
27. Chang Q, Daly L, Bromberg J. The IL-6 feed-forward loop: a driver of tumorigenesis. *Semin Immunol*. 2014;26:48–53.
28. Lesina M, Kurkowski MU, Ludes K, et al. Stat3/Socs3 activation by IL-6 transsignaling promotes progression of pancreatic intraepithelial

neoplasia and development of pancreatic cancer. *Cancer Cell*. 2011;19: 456–469.

1. Zhang H, Wu H, Guan J, et al. Paracrine SDF-1α signaling mediates the effects of PSCs on GEM chemoresistance through an IL-6 autocrine loop in pancreatic cancer cells. *Oncotarget*. 2015;6:3085–3097.
2. Erdogan B, Webb DJ. Cancer-associated fibroblasts modulate growth factor signaling and extracellular matrix remodeling to regulate tumor metastasis. *Biochem Soc Trans*. 2017;45:229–236.
3. Kocher HM, Basu B, Froeling FEM, et al. Phase I clinical trial repurposing all-trans retinoic acid as a stromal targeting agent for pancreatic cancer. *Nat Commun*. 2020;11:4841.
4. Froeling FE, Feig C, Chelala C, et al. Retinoic acid-induced pancreatic stellate cell quiescence reduces paracrine Wnt-β-catenin signaling to slow tumor progression. *Gastroenterology*. 2011;141:1486–1497, 1497. e1-e14.
5. Recchia F, Sica G, Candeloro G, et al. Chemoradioimmunotherapy in locally advanced pancreatic and biliary tree adenocarcinoma: a multicenter phase II study. *Pancreas*. 2009;38:e163–e168.
6. Gupta S, Pramanik D, Mukherjee R, et al. Molecular determinants of retinoic acid sensitivity in pancreatic cancer. *Clin Cancer Res*. 2012;18:

280–289.

1. Moulas AN, Gerogianni IC, Papadopoulos D, et al. Serum retinoic acid, retinol and retinyl palmitate levels in patients with lung cancer. *Respirology*. 2006;11:169–174.
2. Bischoff-Ferrari HA, Giovannucci E, Willett WC, et al. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr*. 2006;84:18–28.
3. Nowson CA, McGrath JJ, Ebeling PR, et al. Vitamin D and health in adults in Australia and New Zealand: a position statement. *Med J Aust*. 2012;196:686–687.
4. Webb AR, Kift R, Durkin MT, et al. The role of sunlight exposure in determining the vitamin D status of the U.K. White adult population. *Br J Dermatol*. 2010;163:1050–1055.
5. Nair R, Maseeh A. Vitamin D: The “sunshine” vitamin. *J Pharmacol Pharmacother*. 2012;3:118–126.
6. Aranow C. Vitamin D and the immune system. *J Investig Med*. 2011;59: 881–886.
7. Waldron JL, Ashby HL, Cornes MP, et al. Vitamin D: a negative acute phase reactant. *J Clin Pathol*. 2013;66:620–622.
8. Schwartz GG, Eads D, Rao A, et al. Pancreatic cancer cells express 25-hydroxyvitamin D-1 alpha-hydroxylase and their proliferation is inhibited by the prohormone 25-hydroxyvitamin D3. *Carcinogenesis*. 2004;25:1015–1026.
9. Schwartz GG, Eads D, Naczki C, et al. 19-nor-1 alpha,25- dihydroxyvitamin D2 (paricalcitol) inhibits the proliferation of human pancreatic cancer cells in vitro and in vivo. *Cancer Biol Ther*. 2008;7: 430–436.
10. Persons KS, Eddy VJ, Chadid S, et al. Anti-growth effect of 1,25- dihydroxyvitamin D3-3-bromoacetate alone or in combination with

5-amino-imidazole-4-carboxamide-1-beta-4-ribofuranoside in pancreatic cancer cells. *Anticancer Res*. 2010;30:1875–1880.

1. Yu WD, Ma Y, Flynn G, et al. Calcitriol enhances gemcitabine anti-tumor activity in vitro and in vivo by promoting apoptosis in a human pancreatic carcinoma model system. *Cell Cycle*. 2010;9:3022–3029.
2. Nuszkiewicz J, Woźniak A, Szewczyk-Golec K. Ionizing radiation as a source of oxidative stress-the protective role of melatonin and vitamin D. *Int J Mol Sci*. 2020;21:5804.
3. Martens PJ, Gysemans C, Verstuyf A, et al. Vitamin D's effect on immune function. *Nutrients*. 2020;12:1248.
4. Gorchs L, Ahmed S, Mayer C, et al. The vitamin D analogue calcipotriol promotes an anti-tumorigenic phenotype of human pancreatic CAFs but reduces T cell mediated immunity. *Sci Rep*. 2020;10:17444.
5. Wang SY, Shen TT, Xi BL, et al. Vitamin D affects the neutrophil-to- lymphocyte ratio in patients with type 2 diabetes mellitus. *J Diabetes Investig*. 2021;12:254–265.
6. Rasmussen LS, Yilmaz MK, Falkmer UG, et al. Pre-treatment serum vitamin D deficiency is associated with increased inflammatory biomarkers and short overall survival in patients with pancreatic cancer. *Eur J Cancer*. 2021;144:72–80.
7. Kemnic TR, Coleman M. Vitamin E deficiency. In: *StatPearls [Internet]*. Treasure Island, FL: StatPearls Publishing; 2021.
8. Rasool AH, Yuen KH, Yusoff K, et al. Dose dependent elevation of plasma tocotrienol levels and its effect on arterial compliance, plasma total antioxidant status, and lipid profile in healthy humans supplemented with tocotrienol rich vitamin E. *J Nutr Sci Vitaminol (Tokyo)*. 2006;52:

473–478.

1. Horvath G, Wessjohann L, Bigirimana J, et al. Differential distribution of tocopherols and tocotrienols in photosynthetic and non-photosynthetic tissues. *Phytochemistry*. 2006;67:1185–1195.
2. Desai ID. Vitamin E analysis methods for animal tissues. *Methods Enzymol*. 1984;105:138–147.
3. Peh HY, Tan WS, Liao W, et al. Vitamin E therapy beyond cancer: tocopherol versus tocotrienol. *Pharmacol Ther*. 2016;162:152–169.
4. Wu D, Meydani SN. Age-associated changes in immune function: impact of vitamin E intervention and the underlying mechanisms. *Endocr Metab Immune Disord Drug Targets*. 2014;14:283–289.
5. De la Fuente M, Hernanz A, Guayerbas N, et al. Vitamin E ingestion improves several immune functions in elderly men and women. *Free Radic Res*. 2008;42:272–280.
6. Husain K, Francois RA, Yamauchi T, et al. Vitamin E δ-tocotrienol augments the antitumor activity of gemcitabine and suppresses constitutive NF-κB activation in pancreatic cancer. *Mol Cancer Ther*. 2011;10:2363–2372.
7. Wang W, Abbruzzese JL, Evans DB, et al. The nuclear factor-kappa B RelA transcription factor is constitutively activated in human pancreatic adenocarcinoma cells. *Clin Cancer Res*. 1999;5:119–127.
8. Arlt A, Gehrz A, Müerköster S, et al. Role of NF-kappaB and Akt/PI3K in the resistance of pancreatic carcinoma cell lines against

gemcitabine-induced cell death. *Oncogene*. 2003;22:3243–3251.

1. Kong R, Sun B, Jiang H, et al. Downregulation of nuclear factor-kappaB p65 subunit by small interfering RNA synergizes with gemcitabine to inhibit the growth of pancreatic cancer. *Cancer Lett*. 2010;291:90–98.
2. Kong R, Sun B, Wang SJ, et al. [An experimental study of gemcitabine inducing pancreatic cancer cell apoptosis potentiated by nuclear factor- kappa B P65 siRNA]. [Article in Chinese]. *Zhonghua Wai Ke Za Zhi*. 2010;48:128–133.
3. Pan X, Arumugam T, Yamamoto T, et al. Nuclear factor-kappaB p65/relA silencing induces apoptosis and increases gemcitabine effectiveness in a subset of pancreatic cancer cells. *Clin Cancer Res*. 2008;14:8143–8151.
4. Sen CK, Khanna S, Roy S. Tocotrienols in health and disease: the other half of the natural vitamin E family. *Mol Asp Med*. 2007;28:692–728.
5. Conly JM, Stein K. The production of menaquinones (vitamin K2) by intestinal bacteria and their role in maintaining coagulation homeostasis. *Prog Food Nutr Sci*. 1992;16:307–343.
6. Hill MJ. Intestinal flora and endogenous vitamin synthesis. *Eur J Cancer Prev*. 1997;6(suppl 1):S43–S45.
7. Bruno EJ. The prevalence of vitamin k deficiency/insufficiency, and recommendations for increased intake. *J Hum Nutr Food Sci*. 2016; 4:1077.
8. Fusaro M, Gallieni M, Rizzo MA, et al. Vitamin K plasma levels determination in human health. *Clin Chem Lab Med*. 2017;55:789–799.
9. Yu DW, Li QJ, Cheng L, et al. Dietary vitamin K intake and the risk of pancreatic cancer: a prospective study of 101,695 American adults. *Am J Epidemiol*. 2021;190:2029–2041.
10. Geleijnse JM, Vermeer C, Grobbee DE, et al. Dietary intake of menaquinone is associated with a reduced risk of coronary heart disease: the Rotterdam study. *J Nutr*. 2004;134:3100–3105.
11. Westphal S, Kalthoff H. Apoptosis: targets in pancreatic cancer.

*Mol Cancer*. 2003;2:6.

1. Showalter SL, Wang Z, Costantino CL, et al. Naturally occurring K vitamins inhibit pancreatic cancer cell survival through a

caspase-dependent pathway. *J Gastroenterol Hepatol*. 2010;25:738–744.

1. Hennig A, Markwart R, Esparza-Franco MA, et al. Ras activation revisited: role of GEF and GAP systems. *Biol Chem*. 2015;396:831–848.
2. Roberts PJ, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. *Oncogene*. 2007;26: 3291–3310.
3. Wei G, Wang M, Carr BI. Sorafenib combined vitamin K induces apoptosis in human pancreatic cancer cell lines through RAF/MEK/ERK and c-Jun NH2-terminal kinase pathways. *J Cell Physiol*. 2010;224: 112–119.
4. Osada S, Tomita H, Tanaka Y, et al. The utility of vitamin K3 (menadione) against pancreatic cancer. *Anticancer Res*. 2008;28:45–50.
5. Ha TY, Hwang S, Hong HN, et al. Synergistic effect of sorafenib and vitamin K on suppression of hepatocellular carcinoma cell migration and metastasis. *Anticancer Res*. 2015;35:1985–1995.
6. Yamamoto T, Nakamura H, Liu W, et al. Involvement of

hepatoma-derived growth factor in the growth inhibition of hepatocellular carcinoma cells by vitamin K(2). *J Gastroenterol*. 2009;44:228–235.

1. Habu D, Shiomi S, Tamori A, et al. Role of vitamin K2 in the development of hepatocellular carcinoma in women with viral cirrhosis of the liver. *JAMA*. 2004;292:358–361.
2. Klapkova E, Cepova J, Dunovska K, et al. Determination of vitamins K1, MK-4, and MK-7 in human serum of postmenopausal women by HPLC with fluorescence detection. *J Clin Lab Anal*. 2018;32:e22381.
3. Jemal A, Bray F, Center MM, et al. Global cancer statistics. *CA Cancer J Clin*. 2011;61:69–90.
4. Latenstein AEJ, van Gerven R, Grevers F, et al. Micronutrient deficiencies and anaemia in patients after pancreatoduodenectomy. *Br J Surg*. 2021; 108:e74–e75.
5. Rubin LP, Ross AC, Stephensen CB, et al. Metabolic effects of inflammation on vitamin a and carotenoids in humans and animal models. *Adv Nutr*. 2017;8:197–212.
6. Yang L, Luo C, Chen C, et al. All-trans retinoic acid protects against doxorubicin-induced cardiotoxicity by activating the ERK2 signalling pathway. *Br J Pharmacol*. 2016;173:357–371.
7. Matos A, Nogueira C, Franca C, et al. The relationship between serum vitamin a and breast cancer staging before and after radiotherapy. *Nutr Hosp*. 2014;29:136–139.
8. Sun HW, Chen J, Wu WC, et al. Retinoic acid synthesis deficiency fosters the generation of polymorphonuclear myeloid-derived suppressor cells in colorectal cancer. *Cancer Immunol Res*. 2021;9:20–33.
9. Liu Z, Ren G, Shangguan C, et al. ATRA inhibits the proliferation of DU145 prostate cancer cells through reducing the methylation level of HOXB13 gene. *PLoS One*. 2012;7:e40943.
10. Saraff V, Shaw N. Sunshine and vitamin D. *Arch Dis Child*. 2016;101: 190–192.
11. Health Council of the Netherlands. *Evaluation of dietary reference values for vitamin D*. The Hague, The Netherlands: Health Council of the Netherlands; 2012.
12. Manolagas SC, Parfitt AM. What old means to bone. *Trends Endocrinol Metab*. 2010;21:369–374.
13. Kong B, Michalski CW, Erkan M, et al. From tissue turnover to the cell of origin for pancreatic cancer. *Nat Rev Gastroenterol Hepatol*. 2011;8:467–472.
14. Boonman-de Winter LJ, Albersen A, Mohrmann K, et al. Hoge prevalentie van vitamine D-deficiëntie in Zuidwest-Nederland [High prevalence of vitamin D deficiency in the south-west Netherlands]. [Article in Dutch]. *Ned Tijdschr Geneeskd*. 2015;159:A8167.
15. Chen PT, Hsieh CC, Wu CT, et al. 1α,25-Dihydroxyvitamin D3 inhibits esophageal squamous cell carcinoma progression by reducing IL6 signaling. *Mol Cancer Ther*. 2015;14:1365–1375.
16. Moz S, Contran N, Facco M, et al. Vitamin D prevents pancreatic cancer-induced apoptosis signaling of inflammatory cells. *Biomolecules*. 2020;10:1055.
17. Subramaniam S, Anandha Rao JS, Ramdas P, et al. Reduced infiltration of regulatory T cells in tumours from mice fed daily with gamma-tocotrienol supplementation. *Clin Exp Immunol*. 2021;206:161–172.
18. Kang TH, Knoff J, Yeh WH, et al. Treatment of tumors with vitamin E suppresses myeloid derived suppressor cells and enhances CD8+ T cell-mediated antitumor effects. *PLoS One*. 2014;9:e103562.
19. Springett GM, Husain K, Neuger A, et al. A phase I safety, pharmacokinetic, and pharmacodynamic presurgical trial of vitamin E

δ-tocotrienol in patients with pancreatic ductal neoplasia. *EBioMedicine*. 2015;2:1987–1995.

1. Fisher L, Byrnes E, Fisher AA. Prevalence of vitamin K and vitamin D deficiency in patients with hepatobiliary and pancreatic disorders. *Nutr Res*. 2009;29:676–683.
2. Frost F, Kacprowski T, Rühlemann M, et al. Impaired exocrine pancreatic function associates with changes in intestinal microbiota composition and diversity. *Gastroenterology*. 2019;156:1010–1015.
3. Palermo A, Tuccinardi D, D'Onofrio L, et al. Vitamin K and osteoporosis: myth or reality? *Metabolism*. 2017;70:57–71.
4. Shintakuya R, Uemura K, Murakami Y, et al. Sarcopenia is closely associated with pancreatic exocrine insufficiency in patients with pancreatic disease. *Pancreatology*. 2017;17:70–75.