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Vitamin and co-factor biosynthesis pathways in *Plasmodium* and other apicomplexan parasites

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Abstract

Vitamins are essential components of the human diet. By contrast, the malaria parasite *Plasmodium falciparum* and related apicomplexan parasites synthesise certain vitamins, *de novo*, either completely or in parts. The occurrence of the various biosynthesis pathways is specific to different apicomplexan parasites, emphasising their distinct requirements for nutrients and growth factors. The absence of vitamin biosynthesis from the human host implies that inhibition of the parasite pathways may be a way to interfere specifically with parasite development. However, the precise role of biosynthesis and potential uptake of vitamins for the overall regulation of vitamin homeostasis in the parasites needs to be established first. In this review Sylke Müller and Barbara Kappes focus mainly on the procurement of vitamin B₁, B₅ and B₆ by *Plasmodium* and other apicomplexan parasites.

Keywords

Malaria; drug target; pantothenate; thiamine; pyridoxal phosphate; vitamins

Exploring the metabolism of protozoan parasites

The intracellular lifestyle of various protozoan parasites has advantages, but also can cause problems, for instance in terms of nutrient acquisition - particularly when considering the high growth rates of some parasitic protozoa. *Plasmodium* intraerythrocytic stages have solved some of these problems by inducing the new permeation pathway (NPP). The NPP allows transport of a variety of low molecular mass molecules and ions across the host cell membrane, with subsequent transit of these nutrients across the parasitophorous vacuole membrane into the parasite through transporters in their plasma membrane¹⁻³. *P. falciparum* growth *in vitro* absolutely depends on an external supply of pantothenate (vitamin B₅), calcium and isoleucine, but the addition of other amino acids significantly improves parasite growth rates⁴⁻⁶. Thus, minimal growth *in vitro* is achieved with relatively few external additions of nutrients to the growth medium, implying that some of the essential growth factors are either not important to sustain parasite growth, or that the parasites might be able to generate them in a sufficient amount to allow functional metabolism. Indeed, it was shown that *Plasmodium* synthesises vitamin B₁ and B₆ and it is well established that folate biosynthesis occurs in the parasites

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7-10. The fact that the parasites can synthesise some of the metabolites known to be vitamins in humans (see Box 1) potentially makes them excellent targets for the development of new antimalarials. This has already been proven for folate metabolism which will not be discussed here because recent excellent reviews cover the role of this pathway for *Plasmodium* survival and its potential as drug target^{8, 9}. Here we will report and discuss the potential of vitamin B₁, B₅ and B₆ biosynthesis/acquisition as new drug targets and current knowledge about these metabolic pathways in the related apicomplexans *Toxoplasma gondii* and *Cryptosporidium* will also be addressed.

Vitamin B₁

Vitamin B₁ is an essential nutrient for mammals (see Box 1), but plants, bacteria and fungi can synthesise it *de novo*¹¹. Mammals salvage thiamine (THI) from their diet and convert it into its active form, thiamine diphosphate (THI-PP), using thiamine diphosphokinase (TPK). The biosynthesis of thiamine in bacteria is well characterised and differs from the reactions in eukaryotes^{12, 13}. Thiamine phosphate (THI-P) is synthesised from 4-methyl-5-(2-phosphoethyl)-thiazole (THZ-P) and 4-amino-2-methyl-5-hydroxymethyl pyrimidine diphosphate (HMP-PP) (see Figure 1).

Analyses of the *P. falciparum* genome revealed the presence of genes encoding proteins with similarities to bacterial and yeast thiamine biosynthesis enzymes (Table 1), such as 4-amino-2-methyl-5-hydroxymethyl pyrimidine phosphate (HMP)/HMP-P kinase (ThiD)¹⁴. ThiD catalyses the phosphorylation of HMP and HMP-P, however, the specific activity for HMP-P is extremely low. The first phosphorylation step is also supported by the parasite's pyridoxal kinase¹⁰. So far the precursors for HMP-biosynthesis were not identified in the parasites and it was suggested that the intraerythrocytic stages rely primarily on HMP uptake¹⁰. In agreement with this, no genes encoding either 'no message in thiamine' (NMT1) or *thiC* gene product, the enzymes responsible for the formation of HMP in eukaryotes or prokaryotes, respectively, were identified in the parasite genome (<http://sites.huji.ac.il/malaria/>; Figure 1; Table 1)^{11, 15}. The importance of the ThiD reactions for parasite survival was investigated using the naturally occurring HMP analogue bacimethrin. In bacteria, bacimethrin is converted into 2-methoxythiamine which is subsequently phosphorylated to 2-methoxythiamine pyrophosphate and replaces thiamine diphosphate from its target proteins¹⁶. These reactions appear to also occur in *P. falciparum*, however, bacimethrin had no adverse effect on parasite survival *in vitro*¹⁰. These findings do not exclude that the HMP biosynthesis is of significance for *Plasmodium* survival because the lack of an antiparasmodial effect of bacimethrin could for instance be explained by poor uptake of the compound.

Despite the fact that most of the genes encoding proteins that are involved in THZ-P biosynthesis are not conserved or could not be identified in the *Plasmodium* (see Table 1), it was suggested that the parasites are able to generate the metabolite using yet unknown enzymatic reactions¹⁰. In addition to a potential biosynthesis of THZ-P, the presence of the gene encoding 4-methyl-5-β-hydroxyethylthiazole kinase (ThiM) suggests that the metabolite is salvaged by the parasites^{10, 14}. HMP-PP and THZ-P are merged into thiamine phosphate (THI-P) by thiamine phosphate synthase (ThiE). A gene encoding this protein was also identified in the parasite genome (Table 1)¹⁴. Its deduced amino acid sequence revealed similarity to ThiE from other organisms only in the N-terminal 235 of the 538 amino acids, whereas the C-terminus does not show any similarity to other proteins of known function¹⁰. To become the active co-factor, thiamine monophosphate needs to be dephosphorylated and subsequently activated by thiamine diphosphokinase (TPK) to form thiamine diphosphate. A specific phosphatase acting on thiamine monophosphate was not identified but a gene potentially encoding an ortholog of TPK appears to be present (Table 1).

In addition to the reactions described above, it is likely that thiamine is taken up by the parasite¹⁰. So far, the precise contributions of biosynthesis and uptake to the overall vitamin B₁ homeostasis of the parasite are not clear. Yet, the lack of these pathways in the mammalian host and the fact that bacimethrin acts in a similar way on parasite ThiD as it does on the bacterial counterpart suggests that thiamine biosynthesis in *Plasmodium* might be exploitable for the design of new antimalarials¹⁷.

Genome analyses suggest that the related apicomplexan parasites *T. gondii* and *Cryptosporidium parvum/hominis* cannot synthesise vitamin B₁ – genes encoding proteins of the pathways described above seem to be absent from these parasite genomes (Table 1). A gene potentially encoding TPK was identified in the genome of *T. gondii*, supporting the hypothesis that, like their human host, they probably fully rely on thiamine uptake. No clear orthologs could be found in the *Cryptosporidium* (unpubl. observations), leaving the question how *Cryptosporidium* acquires this essential co-factor (Table 1). However, the lack of obvious orthologs in both parasites does not necessarily mean the lack of this pathway because it might be very distinct from those characterised in other organisms so far and therefore was not recognised to date.

Pantothenate (vitamin B₅) and CoA-biosynthesis

Pantothenate and pantotheine are precursors of the essential co-factor coenzyme A (CoA) (see Box 1). CoA is synthesised from aspartate and ketovalerate in bacteria, fungi and plants via the formation of pantothenic acid. Animals and some pathogenic bacteria cannot generate pantothenate, thus lacking the first part of the CoA-biosynthesis pathway. They therefore rely on pantothenate uptake from their diet. *T. gondii* possesses genes encoding proteins which potentially synthesise pantothenate which are lacking in *Plasmodium* and *Cryptosporidium* (Figure 2; Table 1). This is in agreement with the finding that pantothenate supplementation is beneficial for growth of malaria parasites^{4, 18}. Mammals take up pantothenate via a family of Na⁺:pantothenate symporters¹⁹, whereas, in *P. falciparum*, it is taken up by a H⁺:pantothenate symporter²⁰. The *Plasmodium*-specific vitamin B₅ uptake system differs from that of the host not only with respect to its cation dependence, but also by its affinity for pantothenate. This transporter represents a low affinity uptake system as opposed to the high affinity transport system characterised in other organisms^{21, 22}. By contrast, *T. gondii* contains a gene (locus 541.m01156) with a high degree of similarity to the mammalian Na⁺-dependent multivitamin symporter (MVT). These symporters are often found to be responsible for the transport of biotin and potentially lipoic acid as well as pantothenate²³. From the gene sequence it is impossible to predict its primary function and thus, it is possible that this predicted *T. gondii* MVT not only confers the ability to take up pantothenate but also is important for biotin uptake especially as the parasites are able to generate pantothenate *de novo*.

Once inside the parasite, pantothenate is transformed into CoA via 5 enzymatic steps (see Figure 2; Table 1). The first is catalysed by pantothenate kinase (PanK). Humans contain 4 distinct PanKs whereas in *P. falciparum* only one activity was identified. The kinetic parameters of the *P. falciparum* PanK are quite distinct from the mammalian counterpart with a K_m for pantothenate in the nM range showing its high specificity for the substrate^{20, 24}. In bacteria and mammals, PanK regulates CoA-biosynthesis²⁴⁻²⁶, and mutations in PanK2, one of the four human PanKs, results in the development of pantothenate kinase-associated neurodegeneration^{27, 28}. In *Drosophila melanogaster*, a PanK termed ‘fumble’ is not only important for CoA-biosynthesis, but also for progression through the cell cycle by actively associating with the metaphase and telophase spindle during cell division²⁹. These data suggest an important role for PanK also in malaria and other apicomplexan parasites which makes it an interesting target for future drug design. Indeed, recent work has identified a number of pantothenate analogues that show antimalarial activity and act through inhibition of the

parasite PanK^{6, 30}. In the second step, 4'-phosphopantothenate is conjugated to cysteine via phosphopantothenoylecysteine synthetase (PPCS) (Figure 2). *P. falciparum* appears to possess two genes encoding potential homologues of eukaryotic ATP-dependent PPCS (Table 1). They are annotated as hypothetical proteins and their authenticity and functionality is yet to be determined. This is in contrast to *T. gondii* and *C. parvum/hominis*, where only 1 gene potentially encoding PPCS was identified (Table 1, Figure 2). The following step of the CoA-biosynthesis requires the decarboxylation of phosphopantothenoylecysteine, by phosphopantothenoylecysteine decarboxylase (PPCDC). In *Plasmodium*, *T. gondii* and *Cryptosporidium*, genes encoding orthologs of eukaryotic PPCDC have been identified (Table 1)^{31, 32}. Apicomplexan CoA-biosynthesis, therefore, is more similar to that found in mammals, because bacteria contain a bifunctional PPCS/PPCDC (Table 1)^{31, 32}. The following two steps of the CoA-biosynthesis are catalysed by a bifunctional protein in mammals – pantetheine phosphate adenylyltransferase/de-phospho-CoA kinase (PPAT/DPCK)³¹. In contrast, these steps are catalysed by separate enzymes in the malaria parasite. It was suggested that the lack of sequence similarity between the *Plasmodium* and host PPAT has some potential for drug development^{31, 32} (Figure 2, Table 1). Similarly *Cryptosporidium* possesses two genes potentially catalysing these last two reactions of CoA biosynthesis whereas in *T. gondii* a single gene encoding a polypeptide similar to the mammalian protein was identified (Table 1, Figure 2).

Vitamin B₆

The term 'vitamin B₆' collectively refers to the vitamers pyridoxal, pyridoxine, pyridoxamine, and their respective phosphate esters (Figure 3). The metabolically active form is pyridoxal-5'-phosphate (PLP) (see Box 1). Proteobacteria synthesise vitamin B₆ via the 1-desoxyxylulose 5-phosphosphate (DOXP)-dependent pathway³³, whereas other bacteria and eukaryotes generate PLP using Pdx1 and Pdx2, which form a typical class I glutamine amidotransferase (GATase). In addition PLP can be acquired by salvage which requires the activity of a pyridoxine/pyridoxamine oxidase (PdxH) and a pyridoxal kinase (PdxK)^{33, 34}. The GATase formed by Pdx1 and Pdx2 acts as a PLP synthase using glutamine, ribose-5-phosphate (or ribulose-5-phosphate) and glyceraldehyde-3-phosphate (or dihydroxyacetone phosphate) to synthesise PLP^{35, 36}. Crystal structures of two bacterial PLP synthase complexes were recently solved leading to a better understanding of the reaction mechanism of this enzyme complex and offering great opportunities for future drug development not only for antibacterials but also antimalarials^{37, 38}.

Single genes encoding Pdx1 and Pdx2 have been identified in *P. falciparum* and are also present in *T. gondii* [39, 40] (see Table 1). The *Plasmodium* proteins form a functional PLP synthase complex with parasite-specific properties and substrate specificities⁷. Both proteins are expressed throughout the intraerythrocytic and gametocyte development of *P. falciparum*, suggesting that these parasitic stages possess a functional vitamin B₆ biosynthesis of the DOXP-independent type⁷. In addition, PdxK has been described in *Plasmodium* and a gene encoding a potential ortholog is also present in *T. gondii* [40] (see Table 1). The *Plasmodium* metabolic map of vitamin B₆ (<http://sites.huji.ac.il/malaria>) predicts the presence of a potential pyridoxine/pyridoxamine oxidase (PF14_0570). However, the predicted gene product has the highest similarity to apolipoprotein-A1 binding protein albeit it also has some similarity with a predicted pyridoxine/pyridoxamine oxidase from rice. These ambiguous results emphasise that often it is impossible to reliably predict gene product functions without experimental evidence. The potential lack of pyridoxine/pyridoxamine oxidase in the malaria parasite needs therefore to be experimentally confirmed before making final assumptions about how the parasites maintain their crucial PLP homeostasis although the potential lack of the oxidase could make this parasite pathway particularly vulnerable to inhibition.

Biotin

Generally, bacteria, plants and some fungi are able to synthesise biotin, but animals have to obtain it through their diet (see Box 1). Biotin is covalently attached to biotin-dependent proteins such as acetyl-CoA carboxylase by a biotin-protein ligase (BPL). In a first step, these ligases activate biotin to form a biotinyl-AMP intermediate before biotin is ligated to a specific lysine residue of the acceptor protein⁴¹. *Plasmodium* parasites lack the ability to synthesise biotin, but they possess at least one biotin-dependent protein, acetyl-CoA carboxylase, representing the first step of fatty acid biosynthesis in the apicoplast. Inhibition of this protein in *T. gondii* by aryloxyphenoxypropionate herbicides has an adverse effect on parasite growth⁴². Searching the *Plasmodium* genome database revealed two orthologs of genes potentially encoding BPLs (Table 1). In contrast, *T. gondii* and *Cryptosporidium* only possess one gene encoding BPL (Table 1). The apicomplexan gene products have similarity to the bacterial biotin operon repressor BirA that was also shown to act as transcriptional regulator in bacteria, yeast and mammals by sensing of intracellular biotin levels via the biotinyl-AMP bound to BPL^{43, 44}. It would be highly interesting to investigate the hypothesis that biotin concentrations within the parasite are sensed by a similar mechanism.

Vitamin status of the host and its effect on *Plasmodium* development

An infection with *Plasmodium* causes an immune response resulting in the activation of macrophages leading to the production and release of reactive oxygen species (ROS). The vitamins C (L-ascorbic acid) and E (α -tocopherol) (see Box 1) play pivotal roles in protection against oxidative stress. It was postulated that the increased concentrations of both vitamins in *Plasmodium*-infected cells in a mouse model is the consequence of the increased oxidative stress that the animal encounters when its immune system is activated by the malaria infection⁴⁵⁻⁴⁸. This simultaneous increase in concentration of both vitamins in erythrocytes apparently offered an enhanced protection against lipid peroxidation where lipid peroxides are detoxified by vitamin E which itself is then reduced by the water-soluble ascorbate. The resulting dehydroascorbate is recycled enzymatically or non-enzymatically in a glutathione-dependent way⁴⁸. The increase of vitamin E and C concentrations in *Plasmodium*-infected erythrocytes of rodents led to the suggestion that the parasites are able to synthesise the two vitamins. However, scrutiny of the genome did not support this suggestion – we could not find genes encoding proteins involved in the biosynthesis of ascorbate or tocopherol. Interestingly, vitamin E or vitamin C deficiencies have a protective effect in malaria patients apparently because the lack of this antioxidant renders the parasites more vulnerable to the damage by ROS⁴⁵. Indeed, it was postulated that ascorbic acid not only acts as an antioxidant but also has strong pro-oxidant features resulting in the generation of free radicals in the presence of oxygen and free transition metals according to the Fenton reaction⁴⁹.

There is evidence that β -carotene and vitamin A have protective effects against malaria^{50, 51}. Malaria infection seems to be accompanied by decreases of vitamin A concentrations in the serum from ≥ 120 mmol/L to ≤ 70 μ mol/L^{52, 53}, and, in rodent models, there appears to be an inverse correlation between parasitemia and vitamin A concentrations in the host. Providing vitamin A supplements partially protected against malaria infection, particularly in immunologically naïve patients⁵⁰. This was suggested to be mediated by an increased clearance of parasitized erythrocytes and a reduced pro-inflammatory cytokine response, which is generally high in malaria patients. Vitamin A supplementation in a randomised and controlled trial showed a marked benefit with ~ 70 % lower parasite densities in young children⁵¹. One of the underlying mechanisms was found to be an upregulation of the phagocytotic receptor CD36, and the downregulation of cytokines such as TNF α by binding of 9-cis-retinoic acid to the peroxisome proliferator-activated receptor γ (PPAR γ) or retinoid-x-receptor^{50, 54}. How the vitamin deficiency is caused in the first place is not entirely clear, and the

suggestion that the parasites take up the vitamin and therefore remove it from its host could not be substantiated⁵⁵.

Vitamin D₃ is pivotal for calcium homeostasis, but also exerts a number of other functions, which are mediated through its receptor – vitamin D₃ receptor (VDR) belonging to the family of nuclear hormone receptors^{56, 57}. Genes involved in the generation of vitamin D₃ have been characterised in mammals, but none of those appears to be present in *Plasmodium* (www.PlasmDB.org; unpubl. observations); neither is a gene encoding VDR. In the context of infectious diseases, vitamin D₃ immunomodulatory functions are of particular interest⁵⁸. It has been shown that VDR is expressed in antigen-presenting cells (e.g. macrophages and dendritic cells) and expression is inducible in lymphocytes. However, there is no clear evidence that vitamin D₃ contributes in a beneficial or adverse way to a malaria infection⁵⁹.

One way in which vitamin D₃ might affect *Plasmodium* is through its involvement in phospholipid metabolism and signalling pathways⁶⁰. Vitamin D₃ and analogues have pronounced inhibitory effects on *P. falciparum* erythrocytic late stage development possibly because the phospholipid biosynthesis pathways of the parasite is affected by these compounds⁶¹. Inhibition of phospholipid biosynthesis by other classes of inhibitors (for instance choline analogues) has been followed up extensively^{62, 63} and it is likely that these inhibitors will be developed as new drugs against malaria in the near future⁶⁴. Thus the activity of vitamin D₃ analogues merits further attention.

Conclusions

This review has concentrated primarily on those growth factors that are likely to be required for the development of *Plasmodium* and other apicomplexan parasites such as vitamin B₁, B₅ and B₆, and which are absolutely necessary as dietary additions in humans. The finding that some vitamins are generated fully or partially by apicomplexans is of great interest with respect to the potential of these parasite-specific pathways as drug targets. Indeed the suitability of CoA biosynthesis as target for antimalarials as a concept has been shown as pantothenate analogues act as inhibitors of *P. falciparum* viability probably through PanK inhibition^{6, 30, 65}. ThiD, one of the enzymes involved in thiamine biosynthesis of bacteria, is the target of the naturally occurring HMP analogue bacimethrin and Wrenger and colleagues showed that bacimethrin is metabolised by the parasite ThiD protein in a similar way suggesting that it acts similarly in the parasites¹⁰. Unfortunately this could not be shown unambiguously because bacimethrin had no effect on parasite viability *in vitro*. This is disappointing but might be due to poor uptake of the inhibitor into the parasite, which certainly could be improved by medicinal chemistry¹⁰. The occurrence of vitamin B₆ biosynthesis in apicomplexa and the recently available structural information of two bacterial PLP synthase complexes will aid to identify strategies for the design of inhibitors against the parasite PLP synthase given their similarity and the presumed similar mode of action between bacterial and parasite proteins^{37, 38}. Inhibitors could either be substrate analogues, reaction intermediates or peptidomimetics interfering with protein-protein interaction that is absolutely required for PLP synthase activity⁷.

The data available about some of the vitamin and co-factor biosynthesis pathways to date are extremely promising and support the idea that these pathways have indeed potential for disease control against apicomplexa.

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Box 1: Role of vitamins described in this review

<p>Vitamins are micronutrients that are essential in small quantities for the normal functioning of the human body. All natural vitamins are organic food substances found solely in plants, fungi, bacteria and animals. Generally, humans cannot synthesize vitamins, so they must be supplied in the diet. There are two types of vitamins: fat-soluble and water-soluble. Fat soluble vitamins are stored in the body, primarily in the liver, and include vitamins A, D, E and K. Water-soluble vitamins, which include the B vitamins and vitamin C, are, with the exception of vitamin B₁₂, not stored by the body and need constant replacement⁶⁶⁻⁶⁸.</p>	
<p>Vitamin B₁ (Thiamine)</p> <p>Thiamine diphosphate, the metabolically important form, is a co-factor of α-keto acid dehydrogenase complexes and the glycine cleavage system as well as enzymes like transketolase and pyruvate decarboxylase.</p>	
essential for	<ul style="list-style-type: none"> Carbohydrate and amino acid metabolism. Normal functioning of the nervous system, muscles and heart.
clinical deficiency	<ul style="list-style-type: none"> Systemic thiamine deficiency can lead to myriad problems including neurodegeneration, wasting, and death. Well-known syndromes include Wernicke-Korsakoff syndrome and beriberi. Beriberi affects the cardiovascular and nervous system.
<p>Vitamin B₅ (Pantothenic acid)</p> <p>Pantothenic acid is the pre-cursor of coenzyme-A (CoA). CoA acts as acyl-chain carrier in numerous important cellular functions.</p>	
essential for	<ul style="list-style-type: none"> Fatty acid metabolism and tricarboxylic acid cycle and thus is critical for in the metabolism and synthesis of carbohydrates, proteins, and fats. The synthesis of the neurotransmitter acetylcholine.
clinical deficiency	<ul style="list-style-type: none"> Symptoms include fatigue, increased incidence of allergies, nausea and abdominal pain. In a few rare circumstances adrenal insufficiency and hepatic encephalopathy.
<p>Vitamin B₆ complex</p> <p>The vitamin B₆ complex consists of pyridoxamine, pyridoxine, pyridoxal and the related phosphateesters. The active form is pyridoxal phosphate (PLP).</p>	
essential for	<ul style="list-style-type: none"> Amino acid metabolism. In bacteria it is also important for the biosynthesis of antibiotics.
clinical deficiency	<ul style="list-style-type: none"> Is unusual in humans manifesting itself as peripheral neuropathy, glossitis and anaemia. Children fed with pyridoxine deficient milk display symptoms such as weakness, irritability, nervousness, susceptibility to noise, weight loss and insomnia.
<p>Vitamin H or B₇ (biotin)</p> <p>Biotin functions as the carboxyl carrier for biotin-dependent carboxylases. In humans there are only five biotin-dependent carboxylases.</p>	
essential for	<ul style="list-style-type: none"> Key reactions in gluconeogenesis (maintaining a steady blood sugar level), fatty acid metabolism and amino acid catabolism. Maintaining metabolic homeostasis.
clinical deficiency	<ul style="list-style-type: none"> Is extremely rare mainly because intestinal bacteria produce an excess of the daily requirement for the body.
<p>Vitamin C</p>

Almost all animals and plants synthesize ascorbate, but primates (including humans) need an external source of vitamin C.

essential for • Antioxidative defence, the production and maintenance of collagen, the metabolism of the amino acids tyrosine and tryptophane and cholesterol biosynthesis.

clinical deficiency • Vitamin C deficiency is known as scurvy

Vitamin A (Retinol)

The term 'vitamin A' refers to a group of fat-soluble compounds known as the 'retinoids'.

essential for • The maintenance of epithelial surfaces, immune competence, the normal functioning of the retina, as well as for growth, embryonic development and reproduction.

• Antioxidative defence.

clinical deficiency • Vitamin A and its metabolites retinal and retinoic acid appear to serve a number of critical roles in physiology, as evidenced by the large number of disorders that accompany deficiency or excess states.

• Night blindness as one of the first signs is a problem largely in developing countries where it is the major cause of morbidity and mortality in childhood.

• Growth retardation and increased susceptibility to infections.

• In almost every infectious disease studied (including malaria), vitamin A deficiency increases the frequency and severity of disease. This "anti-infective" effect is partly due to the key role of vitamin A for normal immune responses. Additionally, many infections are associated with inflammatory reactions that reduce synthesis of retinol-binding protein and thus, reduced circulating levels of retinol.

Vitamin D (calciferol)

The term vitamin D refers to a group of five chemically related compounds (D₁-D₅). Vitamin D₃, also known as cholecalciferol, is a form of vitamin D that is made by in the skin the human body, when 7-dehydrocholesterol reacts with ultraviolet light (UVB; 290 to 315 nm). Vitamin D is not a true vitamin since human skin can create vitamin D in some circumstance. It may be better described as a conditional vitamin.

essential for • The maintenance of an intact and strong skeleton

• Regulation of the calcium and phosphorus concentrations in the blood by ensuring correct intake from intestines and secretion.

• Regulation of the growth of skin cells.

clinical deficiency • Vitamin D deficiency causes rickets, osteoporosis and osteomalacia.

Vitamin E (d-α-tocopherol)

Vitamin E is a generic term for a group of eight chemically-similar compounds sharing the tocopherol and tocotrienol structures, which are lipid-soluble.

essential for • Protection of cell membranes against lipid peroxidation.

clinical deficiency • Vitamin E deficiency is rare in humans.

• If it occurs it is usually characterized by neurological problems associated with nerve degeneration in hands and feet.

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Thiamine biosynthesis

Figure 1. Vitamin B1 biosynthesis

The left panel shows the biosynthesis of 4-methyl-5-(2-phosphoethyl)-thiazole (THZ-P). Plants and *E. coli* use 1-deoxyxylulose-5-phosphate (DOXP), tyrosine (Tyr) and cysteine (Cys) as precursors whereas *Bacillus subtilis* and yeast use a pentulose, glycine (Gly) and cysteine (Cys). In order to generate THZ-P, a number of enzymatic reactions are required and the best investigated systems are the bacterial pathways whereas the eukaryotic pathways are still poorly understood. In bacteria it is established that the ubiquitin-like protein ThiS-CoSH acts as sulphur carrier; ThiF catalyses the adenylation of ThiS; NifS or IscS transfer sulphur from cysteine to ThiS; in *B. subtilis* ThiO oxidises glycine and ThiG catalyses the formation of the thiazole phosphate ring^{13, 69}. The only enzyme shown to be involved in THZ-P biosynthesis in eukaryotes is Thi4^{70, 71}. On the right, the biosynthesis of 4-amino-2-methyl-5-hydroxymethyl pyrimidine diphosphate (HMP-PP) is shown. Again different substrates are used in prokaryotes and eukaryotes. Bacteria generate HMP-PP from an intermediate of purine biosynthesis (AIR; 5-amino-imidazole ribonucleotide) whereas yeast uses pyridoxine and histidine (His) as precursors^{72, 73}. The enzymes that catalyse the reactions that lead to the formation of HMP-PP are ThiD (4-amino-2-methyl-5-hydroxymethyl pyrimidine kinase) and possibly pyridoxal kinase (PdxK). Red letters indicate that a gene has been identified in *P. falciparum* whereas black letters mean that orthologous genes have not been found in the *Plasmodium* genome databases. Abbreviations used: AIR, 5-amino-imidazole ribonucleotide; Cys, cysteine; Gly, glycine; DOXP, 1-deoxy-D-xylulose-5-phosphate; His, histidine; HMP, 4-amino-2-methyl-5-hydroxymethyl pyrimidine; HMP-P, 4-amino-2-methyl-5-hydroxymethyl

pyrimidine phosphate; HMP-PP, 4-amino-2-methyl-5-hydroxymethyl pyrimidine diphosphate; NMT1, no message in thiamine; THZ, thiazole; THZ-P, 4-methyl-5-(2-phosphoethyl)-thiazole; THI, thiamine; THI-P, thiamine phosphate; THI-PP, thiamine pyrophosphate; ThiC, *thiC* gene product; ThiD, 4-amino-2-methyl-5-hydroxymethyl pyrimidine kinase; ThiE, thiamine phosphate synthase; ThiM, 4-methyl-5- β -hydroxyethylthiazole kinase TPK, thiamine diphosphokinase. This figure was generated following Bodzech and Ginsburg¹⁴ and <http://sites.huji.ac.il/malaria/>.

Pantothenate/CoA metabolism

Figure 2. Vitamin B5/CoA biosynthesis

Genes encoding proteins potentially synthesising pantothenate from valine and aspartate were identified in *T. gondii* when searching the ApiDB database (www.ApiDB.org). *P.*

falciparum and *C. parvum/hominis* do not possess the ability to generate vitamin B₅. All three apicomplexans contain genes encoding for the enzymes necessary for CoA biosynthesis. In *T. gondii* PPAT and DPCK form a bifunctional protein whereas in *Plasmodium* and *Cryptosporidium* these reactions are catalysed by distinct enzymes. Abbreviations used: BCAT, branched-chain amino acid transaminase; PanB, 3-methyl-2-oxobutanoate hydroxymethyltransferase; PanE, 2-dehydropantoate 2-reductase; PanC, pantothenate synthase; PanK, pantothenate kinase; PPCS, phosphopantothenoyl synthase; PPCDC, phosphopantothenoyl cysteine decarboxylase; PPAT, pantetheine-phosphate adenylyltransferase; DPCK, de-phospho-CoA kinase.

Vitamin B6 biosynthesis/metabolism

Figure 3. Vitamin B₆ metabolism

The expression vitamin B₆ comprises pyridoxal, pyridoxine and pyridoxamine and their phosphate esters. In eukaryotes such as fungi and plants and most bacteria (except the γ -subdivision of proteobacteria including *E. coli*) pyridoxal-phosphate (PLP) is synthesised by the enzyme complex Pdx1/Pdx2 from ribose or ribulose-5-phosphate, glyceraldehyde-3-phosphate and glutamine. Crystal structures of both proteins have been solved and they reveal that Pdx1 forms a doughnut-like hexameric structure, which binds the glutamine amidotransferase Pdx2 to form the PLP-synthase complex^{7, 37, 71, 74, 75}. Regulation of PLP levels is crucial and can be achieved in several ways. PLP is supplied by biosynthesis, oxidation from pyridoxamine phosphate or pyridoxine phosphate as well as from pyridoxal by phosphorylation. Whether apicomplexan parasites can convert pyridoxamine phosphate and pyridoxine phosphate into PLP is not clear, because a gene encoding an ortholog of pyridoxamine/pyridoxine phosphate oxidase (PdxH) was not unambiguously identified in the *Plasmodium* genome (question mark in Figure). Therefore this figure deviates from that published on <http://sites.huji.ac.il/malaria/>. Thus it is possible that PLP levels in apicomplexans may solely depend on PLP biosynthesis, pyridoxal uptake and phosphorylation as well as on the degree of PLP catabolism. Abbreviations used: PdxH, pyridoxamine/pyridoxine phosphate oxidase; PdxK, pyridoxal kinase.

Table 1

Genes potentially encoding proteins involved in vitamin biosynthesis and co-factor binding in apicomplexan parasites^a

	<i>P. falciparum</i>	<i>T. gondii</i>	<i>C. parvum/hominis</i>	Reference
Vitamin B1 biosynthesis				
<i>(A) HMP-biosynthesis</i>				
4-Amino-2-methyl-5-hydroxymethyl pyrimidine kinase (ThiD)	yes (PFE1030c)	no ^b	no	10, 14
4-Amino-2-methyl-5-hydroxymethyl pyrimidine kinase (ThiD)	yes (PFE1030c)	no	no	10, 14
<i>(B) Thiazole biosynthesis</i>				
sulfur carrier protein ThiS	potential genes belonging to the ThiS/Urm/ubiquitine-like family were identified			^c
ThiF	yes (PF13_0344)	yes (28.m00291)	yes (cgd2_1120)	^c
ThiI	no	no	no	^c
Cysteine desulfurase (IscS)	yes (MAL7P1.150)	yes (27.m00002)	yes (cgd4_3040/Chro.40346)	76, ^c
ThiO/ThiH	no	no	no	^c
ThiG	no	no	no	^c
Thi4	no	no	no	^c
<i>(C) Thiazole salvage</i>				
ThiM	yes (PFL1920c)	no	no	10, 14
<i>(D) Thiamine-diphosphate biosynthesis</i>				
Thiamine phosphate synthase (ThiE)	yes (PFF0680c)	no	no	10, 14
Thiamine pyrophosphate kinase (TPK)	yes (PFI1195c)	yes (33.m02662)	no	http://sites.huji.ac.il/malaria ; ^c
Pantothenate and CoA biosynthesis				
Branched-chain amino acid transaminase	maybe (PF14_0557)	yes (113.m01283)	? ^d	^c
3-Methyl-2-oxobutanoate hydroxymethyltransferase	no	yes (55.m04671)	no	^c
2-Dehydropantoate 2-reductase	no	yes (55.m04671)	no	^c
Pantothenate synthetase	no	yes (57.m01763)	no	^c
Pantothenate kinase (PanK)	yes (PF14_0200)	yes (551.m00221)	yes (cgd7_4950)	31;
Phosphopantothenoylcysteine synthetase (PPCS)	yes (PF11_0036/PFD0610)	yes (641.m01494)	yes (cgd4_2250/Chro.40256)	http://sites.huji.ac.il/malaria ; ^c
Phosphopantothenoylcysteine decarboxylase (PPCDC)	yes (MAL8P1.81)	yes (49.m00067)	yes (cgd4_2250)	31;
Adenylyltransferase (PPAT)	yes (PF13_0159)/(PF07_0018)	yes (44.m02629)	yes (cgd7_3480/Chro.70388)	http://sites.huji.ac.il/malaria ; ^c
De-phospho-CoA kinase (DPCK)	yes (PF14_0415)	yes (44.m02629)	yes (cgd2_380/Chro.20045)	31;
Vitamin B6 biosynthesis/metabolism				
Pdx1	yes (PFF1025c)	yes (46.m00006)	no	7, 39, 40, ^c
Pdx2	yes (PF11_0169)	yes (74.m00760)	no	7, 39, 40, ^c
Pyridoxal kinase (PdxK)	yes (PFF0775w)	yes (113.m00756)	no	40, ^c
Pyridoxal phosphate phosphatase	yes (PF07_0059)	yes (80.m02257)	no	^c
Pyridoxamine/pyridoxine-phosphate oxidase (PdxH)	?? (PF14_0570) ^f	no	no	http://sites.huji.ac.il/malaria ; ^c
Pyridoxal dehydrogenase /aldehyde oxidase	yes (PF14_0088)	yes (25.m01796)	no	^c
Biotin				
Biotin protein ligase	yes (PF14_0573/PF10_0409)	yes (80.m02241)	yes (cgd2_2890/Chro.20303)	^c

^aBlast searches (TBLASTN and BLASTN) were performed in ApiDB.org (www.ApiDB.org) using bacterial or plant homologous of the respective genes; numbers in brackets give gene identifiers of gene loci in ApiDB.org. Potential genes were used to search the Swissprot database to corroborate the results.

^bNo – No gene/protein with convincing similarity was identified

^cunpublished observations from ^a by the authors

$d_{??}$ – Gene possibly present but similarity does not allow clear annotation or their predicted amino acid sequences have been annotated differently

$f_{??}$ – Gene present on <http://sites.huji.ac.il/malaria>, but questioned by the authors of this review