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Review

Obesity and dysregulated innate immune responses: impact of micronutrient deficiencies

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Obesity is associated with the development of various complications, including diabetes, atherosclerosis, and an increased risk for infections, driven by dysfunctional innate immune responses. Recent insights have revealed that the availability of nutrients is a key determinant of innate immune cell function. Although the presence of obesity is associated with overnutrition of macronutrients, several micronutrient deficiencies, including Vitamin D and zinc, are often present. Micronutrients have been attributed important immunomodulatory roles. In this review, we summarize current knowledge of the immunomodulatory effects of Vitamin D and zinc. We also suggest future lines of research to further improve our understanding of these micronutrients; this may serve as a stepping-stone to explore micronutrient supplementation to improve innate immune cell function during obesity.

Highlights

The presence of obesity is associated with dysregulated innate immune responses, including macrophages.

The research field of immunometabolism dictates that immune cell function is determined by metabolic status.

Obesity is associated with micronutrient deficiencies, including Vitamin D and zinc, which have been attributed widespread immunomodulatory effects.

Limited information is available regarding the immunometabolic effects of micronutrients, including Vitamin D and zinc, on macrophages.

More studies are needed to identify the immunometabolic effects of putative micronutrient interventions that might help improve innate immune cell functions during obesity.

Significance

Metabolism is a crucial determinant of innate immune cell function, including macrophages. The availability of macro- and micronutrients impacts macrophage metabolism and, thus, function. Obesity is often accompanied by innate immune cell dysfunction driving various complications. In contrast to macronutrients, micronutrient deficiencies are frequently observed during obesity. Due to their widespread immunomodulatory effects, targeting micronutrient deficiencies (e.g., vitamin D, zinc, etc.) during obesity may serve to restore innate immune function and prevent or alleviate the development of complications.

Obesity and dysregulated immune responses

It is well established that the presence of obesity impacts the human innate immune system [1,2]. This is illustrated by the development of various obesity-associated complications driven by dysfunctional innate immune responses. For example, multiple studies have provided epidemiological evidence showing a higher incidence and severity of various types of infection in obese individuals compared with lean subjects [3]. This has been shown not only for bacterial infections, including those with *Listeria monocytogenes* or *Klebsiella pneumoniae* [4], but also for viral infections, including Dengue [5], and infections with fungal pathogens, such as *Candida* spp [4]. Recent evidence from the coronavirus disease 2019 (COVID-19) pandemic has clearly demonstrated that the presence of obesity as a comorbidity is associated with higher prevalence and worse outcomes of severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) infection compared with nonobese individuals [6,7]. The increased infection risk during obesity suggests the presence of altered innate immune activation, which may lead to a reduced ability to fight pathogens.

In addition to infections, other obesity-associated complications are also driven by dysfunctional innate immune responses. Obesity is known to lead to a state of chronic low-grade inflammation that partly originates from expanding adipose tissue [8,9]. Moreover, during the development of obesity, adipose tissue is infiltrated by various immune cells, including proinflammatory macrophages [10]. Together with other proinflammatory immune cells, such as effector T cells, which populate obese adipose tissue, macrophages promote the development of a continuous state of inflammation characterized by increased amounts of proinflammatory mediators, such as tumor necrosis factor α (TNFα), interleukin (IL)-6, and IL1β, during obesity [10]. These inflammatory mediators contribute to the development of **insulin resistance** (see Glossary), which may evolve into type 2 diabetes mellitus (T2DM) [11]. Additionally, increased vascular wall inflammation is observed during obesity [12,13], which is largely driven by the presence of proinflammatory macrophages [14]. Following adhesion of monocytes to the vascular wall, these cells subsequently differentiate into macrophages and further develop

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into **foam cells**, which can contribute to driving atherogenesis [15,16], increasing the risk for atherosclerosis development during obesity.

What causes altered innate immune responses during obesity has remained a heavily debated topic. Recently, studies at the interface of immunology and metabolism have emerged as an increasing area of research that may help to better understand dysfunctional innate immunity during obesity. The research field of 'immunometabolism' dictates that the function of immune cells is associated with a specific cellular metabolic state [17,18]. By investigating the importance of key metabolic pathways, including glycolysis and oxidative phosphorylation, ongoing research has revealed that the metabolism of immune cells determines both their functional output and phenotypic fates. In addition to increased uptake and usage of nutrients, intracellular metabolic pathways are altered upon activation. This is exemplified by the treatment of macrophages with lipopolysaccharide (LPS), an agonist of **Toll-like receptor (TLR)-4**, leading to a robust proinflammatory response, including the production of inflammatory cytokines, such as IL6 and TNFα. This functional response is accommodated by an increase in glycolysis and a lowering of oxidative phosphorylation (OXPHOS), also known as the Warburg effect [19,20]. The importance of metabolism in allowing functional changes to immune cells is demonstrated by blocking glycolysis in activated macrophages to subsequently prevent inflammatory cytokine secretion [20]. Follow-up studies showed that the activation of macrophages and other innate immune cells by various stimuli, both endogenous and exogenous, leads to a specific metabolic rewiring of the cells that is required for adequate functional responses [21].

To drive specific metabolic reprogramming, macrophages depend on the availability of nutrients. Indeed, it is well established that the availability of nutrients severely impacts the innate immune response. For example, undernutrition characterized by deficiencies in micro- and macronutrients severely impacts the functionality of the immune response, especially in children, leading to impaired gut-barrier function, reduced plasma concentrations of complement, and cytokine patterns that are skewed toward a T helper (Th)-2 response [22]. Although the presence of obesity is accompanied by overnutrition of macronutrients, the incidence of micronutrient deficiencies is relatively high in individuals with obesity [23–26]. Indeed, deficiencies in several micronutrients, including Vitamin D and zinc, are more frequently observed in individuals with obesity compared with lean subjects [26,27]. These micronutrients also harbor important immunomodulatory properties [28,29], which is supported by recent studies focusing on the supplementation of zinc and Vitamin D to reduce SARS-CoV-2 infection risk and disease severity [30–32].

Several hypotheses exist that aim to explain micronutrient deficiencies observed during obesity, including those of Vitamin D and zinc. For example, micronutrient deficiencies associated with obesity might be partly due to the overconsumption of foods that are high in calories but have low nutrient density [26]. Other potential explanations involve differences in uptake, metabolism, and elimination of micronutrients, resulting in lower nutrient availability [26]. Increased micronutrient needs in relation to body size, and sequestration within adipose tissue, may also explain deficiencies during obesity, yet the specific mechanisms underlying these findings require additional studies.

Here, we summarize current knowledge of these micronutrients and their reported modulatory effects on innate immune function, such as immune cell differentiation, phagocytosis, and cytokine production. We also propose future lines of research that may help improve our understanding of how micronutrients modulate immunometabolic pathways in innate immune cells. A visual representation of this summary is provided in Figure 1 (Key figure). This may be helpful for further exploring the immunomodulatory effects of micronutrient supplementation even beyond Vitamin D

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and zinc to improve innate immune cell metabolism and function during obesity, and prevent or reverse the development of various complications.

Vitamin D

Obesity and Vitamin D deficiency

Vitamin D inadequacy is often observed in individuals with obesity, and prevalence rates as high as 90% in the USA have been reported [33]. Increased adipose tissue mass during obesity in humans can result in lower serum concentrations of Vitamin D [34]. Micronutrients, such as fat-soluble Vitamin D, have been hypothesized to be sequestered in adipose tissue, resulting in decreased availability [35]. Similarly, volumetric dilution of Vitamin D by a larger adipose tissue mass in individuals with obesity has also been suggested to promote lower Vitamin D concentrations in blood [36].

Several obesity-associated complications have been linked to lower Vitamin D status in humans [37]. However, the causative effects of lower Vitamin D status on the development of these complications have not been well established [38]. Additionally, Vitamin D deficiency has been associated with an increased risk of bacterial and viral infections in humans [39].

In addition to the well-established role of Vitamin D as a key regulator of calcium and phosphorus metabolism, which are essential for maintaining bone health, this micronutrient is also reported to harbor robust immunomodulator effects [39]. Vitamin D is available in two different forms, namely Vitamin D2 and Vitamin D3. Here, we mainly focus on the most-studied variant, Vitamin D3, which is formed in the skin, but which may also be obtained via the consumption of fatty fish [40]. To become biologically active, Vitamin D3 requires two sequential hydroxylation steps to form into its active metabolite, $1,25(OH)_2D_3$. The first step primarily occurs in the liver, whereas the second step occurs not only in the kidney, but also in various other cells. Macrophages have been identified as an important producer of $1,25(OH)_2D_3$ since these cells express the enzyme Cyp27B1, facilitating the second hydroxylation step [41]. The widespread effects of Vitamin D on macrophages are further accommodated through expression of the Vitamin D receptor (VDR), which functions as a transcription factor. Upon activation, VDR binds to specific DNA sites, so-called 'Vitamin D response elements' (VDRE), to regulate the expression of its target genes [42]. Various studies have assessed the transcriptional effects of Vitamin D on different immune cells, including macrophages. These transcriptional changes translate into various functional and phenotypic changes induced by Vitamin D. Indeed, the effects of Vitamin D on macrophage differentiation have also been reported (Box 1). Human monocyte-derived macrophages differentiated in the presence of $1,25(OH)_2D_3$, reveal various transcriptional changes in genes involved in inflammatory responses, such as cytokine secretion and cellular stress, compared with macrophages differentiated in the absence of active Vitamin D3 *in vitro* [43]. In addition to regulation of mRNA expression, $1,25(OH)_2D_3$ treatment of macrophages *in vitro* has also led to the differential expression of certain **miRNAs**, adding another layer of regulation via Vitamin D [43,44].

Here, we provide a snapshot of the known modulatory and presumed immunomodulatory effects of active Vitamin D on macrophage function.

Inflammatory signaling

There is significant evidence that macrophage activation and subsequent cytokine production are modulated by Vitamin D. For example, $1,25(OH)_2D_3$ can suppress TLR-mediated inflammation in macrophages. Pretreatment of mouse RAW264.7 macrophages *in vitro* with $1,25(OH)_2D_3$ before LPS exposure led to reduced TNFα and IL6 production compared with cells treated with LPS alone. This inhibition involved activation of suppressor of cytokine signaling 1 (SOCS1), known to block LPS-induced inflammation [45]. Although not in macrophages, $1,25(OH)_2D_3$ can block

Glossary

Acute-phase response (APR): systemic and nonspecific defense response to infection, inflammation, or trauma, characterized by fever, increased numbers of circulating leukocytes, and production of acute phase proteins, such as C-reactive protein.
Autophagy: degradation process used by cells to remove unnecessary or dysfunctional components. The removal involves lysosome-dependent degradation of unwanted material.
Efferocytosis: process of taking up dead endogenous cells by phagocytes, including macrophages.
Foam cells: macrophages that accumulate in the vascular wall during the development of atherosclerosis. By taking up oxidized low-density lipoprotein, they become laden with lipids, leading to a foamy appearance.
Hypozincemia: condition of insufficient zinc in the circulation (plasma concentrations of <74 μg/dl for adult men and <70 μg/dl for adult women).
Insulin resistance: during obesity, long-term exposure of cells to high concentrations of insulin causes resistance toward its effects, ultimately leading to reduced glucose uptake and an increase in plasma glucose.
miRNAs: small noncoding RNA molecules with important roles in the regulation of gene expression.
NLR family pyrin domain-containing 3 (NLRP3) inflammasome: cytosolic multiprotein complex involved in innate inflammatory responses, comprising a pattern recognition receptor (NLRP3), an adaptor protein (ASC), and caspase 1, which mediates cleavage of pro-interleukin (pro-IL)-1β) and pro-IL18.
Pathogen-associated molecular patterns (PAMPs): small molecular motifs specific for a class of microbes; recognized by various pattern recognitions receptors, including TLRs, and inducing a proinflammatory response.
Pathogen recognition receptor (PRR): class of proteins that recognize molecular patterns, such as PAMPs, to initiate innate immune responses.
Single cell energetic metabolism by profiling translation inhibition (SCENITH): flow cytometry-based technique allowing the study of metabolic responses of multiple cells in parallel.
Toll-like receptor 4 (TLR4): transmembrane protein expressed on innate immune cells and a member of

NF-κB translocation to the nucleus in murine endothelial cells *in vitro*, thereby preventing transcription of proinflammatory cytokine genes [46]. Other modes of action involve downregulated expression of TLR2 and TLR4 by Vitamin D3 in human primary monocytes, leading to reduced responsiveness to **pathogen-associated molecular patterns** (PAMPs) [47]. These anti-inflammatory effects appear to be relevant in preventing obesity-associated complications, including insulin resistance [48]. VDR activation using the Vitamin D analog calcipotriol reduced inflammatory cytokine production by hepatic macrophages isolated from obese mice. This was accompanied by their improved hepatic insulin sensitivity relative to controls [48]. Hence, active Vitamin D has been attributed anti-inflammatory properties in macrophages via various molecular pathways; indeed, it has been speculated that its concentrations observed during obesity lead to increased inflammatory responses.

the Toll-like receptor family. TLR4 can recognize LPS, activating a proinflammatory response.

Phagocytosis

Various lines of evidence have revealed that the phagocytic capacity of macrophages is enhanced by Vitamin D, leading to increased pathogen uptake. For instance, human primary monocyte-derived macrophages differentiated *in vitro* in the presence of $1,25(OH)_2D_3$ displayed increased expression of the complement receptor immunoglobulin (CRIg); moreover, higher amounts of CRIg were associated with increased phagocytosis of complement-opsonized *Staphylococcus aureus* and *Candida albicans* relative to untreated cells [49]. Similar results were obtained using human THP1-derived macrophages infected with *K. pneumoniae*. Accordingly, the treatment of macrophages with $1,25(OH)_2D_3$ enhanced their bactericidal activity and reduced the expression of TNFα and IL6 [50].

In addition to pathogens, the uptake of endogenous unwanted material known, as **efferocytosis**, can be enhanced with Vitamin D. Specifically, Vitamin D treatment reduced the number of apoptotic cells in the experimental autoimmune encephalomyelitis (EAE) mouse model of multiple sclerosis. This effect was mediated via enhanced macrophage efferocytotic activity with Vitamin D treatment [51]. Together, these results suggest an important role for Vitamin D in the uptake and killing of pathogens, as well as in the restoration and maintenance of tissue homeostasis, by supporting the clearance of apoptotic endogenous cells.

Antimicrobial activity

Several lines of evidence have demonstrated that Vitamin D impacts the antimicrobial activity of macrophages. This effect involves enhanced regulation of the antimicrobial peptide cathelicidin in primary human-derived macrophages *in vitro* [52]. In addition, the antimicrobial peptide defensin β2 (DEFB4) has been identified as a direct target of $1,25(OH)_2D_3$ in human primary monocytes *in vitro* [53]. Bacterial killing in primary human monocytes and macrophages was also promoted with $1,25(OH)_2D_3$ treatment of cells [54]. The underlying mechanism involved enhanced **autophagy**, leading to enhanced intracellular isolation of the pathogen and subsequent killing by antibacterial proteins. Moreover, $1,25(OH)_2D_3$-induced antimicrobial activity might also involve PI3K- and NADPH oxidase-mediated regulation leading to enhanced generation of reactive oxygen species (ROS) in human THP1-derived macrophages [55]. Hence, multiple lines of evidence support an important role for Vitamin D in killing pathogens with the induced production of specific antimicrobial peptides. Thus, lower Vitamin D amounts during obesity might impact the efficiency of host defense against various pathogens.

Immunometabolism

Whereas $1,25(OH)_2D_3$ may harbor widespread immunomodulatory roles in macrophages, its immunometabolic effects have remained understudied. However, several recent reports have shed some light on the potential metabolic effects of $1,25(OH)_2D_3$ in innate immune cells, including dendritic cells (DCs).

Key Figure

Visual summary of the effects of Vitamin D and zinc on myeloid effector functions

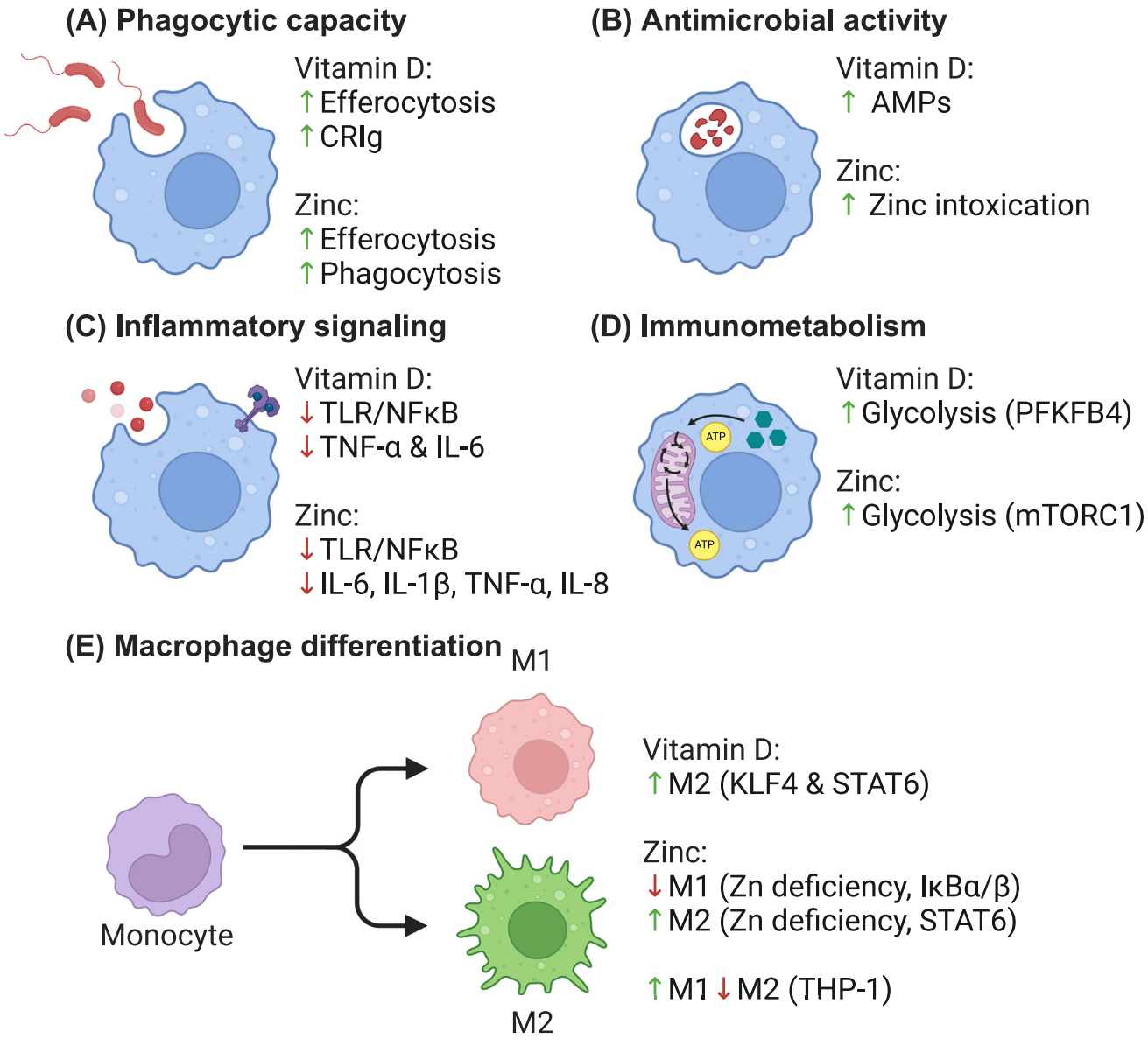

Figure 1. (A) Phagocytosis: Vitamin D [1,25(OH)$_2$D$_3$] increased expression of the complement receptor immunoglobulin (CRIg) in primary monocyte-derived macrophages, leading to increased phagocytosis of opsonized *Staphylococcus aureus* and *Candida albicans* [49]. Furthermore, Vitamin D treatment augmented efferocytosis in an experimental autoimmune encephalomyelitis (EAE) model [51]. Zinc concentrations in bronchoalveolar lavage (BAL) fluid positively correlated with macrophage efferocytosis in patients with chronic obstructive pulmonary disease (COPD) [92]. Multiple studies suggest that zinc deficiency decreases macrophage bacterial phagocytosis, which could be improved by zinc supplementation [90,94,95,134]. (B) Antimicrobial activity: Vitamin D can support direct antimicrobial responses in monocytes and macrophages through production of antimicrobial peptides (AMPs), such as cathelicidin [52] and defensin β2 [53]. Macrophages can mobilize high concentrations of zinc intracellularly to eradicate pathogens through zinc intoxication [71]. (C) Inflammatory signaling: 1,25(OH)$_2$D$_3$ treatment reduces Toll-

(Figure legend continued at the bottom of the next page.)

Box 1. Vitamin D and macrophage differentiation/phenotypes

Depending on the tissue of residency or signals that they have encountered, macrophages display great plasticity, allowing for the execution of a variety of different functions. A comparison of various human macrophage phenotypes revealed differences in the amount of $1,25(OH)_2D_3$ they produce. All macrophage subtypes express Cyp24a1, allowing for degradation of Vitamin D through the 24-hydroxylase pathway. However, reparative human monocyte-derived macrophages (MDMs) display higher concentrations of $1,25(OH)_2D_3$ compared with other MDM subtypes, including naïve and activated macrophages [117]. Hence, various macrophage phenotypes display different Vitamin D metabolic rates. Thus, it will be interesting to compare macrophages that reside in different tissues to evaluate the importance of Vitamin D metabolism on their functional output. In addition, direct effects on polarization are regulated by Vitamin D-dependent activation of KLF4 and STAT6, which is also accompanied by improved macrophage autophagy in human THP1 cells, mediated by Vitamin D-induced KLF4 activation [118].

Of note, Vitamin D appears to promote a specific macrophage phenotype that differs from the conventionally defined M1-like (proinflammatory) or M2-like macrophages, yet with superior functional properties related to inhibition of *Mycobacterium tuberculosis* growth. This effect was illustrated by the observation that human monocyte-derived cells that were differentiated into macrophages using the active form of Vitamin D, displayed an induction of the antimicrobial peptide LL37, as well as various inflammatory cytokines, such as TNFα and IL1β, in this infection model [119].

A recent study examined the effects of $1,25(OH)_2D_3$ treatment on the Akt/mTOR and glycolytic pathways in mouse bone marrow-derived DCs (BMDCs). $1,25(OH)_2D_3$ treatment led to suppressed Akt/mTOR signaling accompanied by increased expression of genes related to glycolysis, including *Glut1*, *Pfkfb4*, and *Hif1A* [56]; the latter is a key transcriptional regulator of glycolytic rate in innate immune cells, including macrophages [20]. Upregulated glycolysis after $1,25(OH)_2D_3$ treatment was associated with more tolerogenic BMDCs compared with untreated cells [56]. Similar metabolic effects of active Vitamin D3 have been reported in primary human DCs, which led to the identification of PFKFB4 as a transcriptional target of $1\alpha,25(OH)_2D_3$, promoting glucose oxidation [57]. On a functional level, this was also associated with a tolerogenic DC profile, as illustrated by the expression of inhibitory surface markers and secretion of regulatory cytokines [57].

In addition to molecular regulators of metabolism, treatment with active Vitamin D can also influence the abundance of several intracellular metabolites, supporting its role as an immunometabolic modulator. Using metabolomics, differences in the metabolic profiles of cells exposed to the TLR2/1 ligand Pam3CSK4, $1,25(OH)_2D_3$, or a combination of Pam3CSK4/$1,25(OH)_2D_3$, were observed compared with untreated cells. Indeed, 32 differentially regulated metabolites, such as isoleucine and glutathione, were identified and linked to bioenergy production, redox regulation, inflammation, and protein synthesis in U937 cells [58], a human promonocytic cell line. These results suggest a modulatory role for active Vitamin D in the metabolism of innate immune cells, which might ultimately lead to functional cellular changes.

Moreover, various studies have linked active Vitamin D treatment with its effects on lipid metabolism in macrophages. For example, Vitamin D3 can reduce fatty acid accumulation in human THP1-derived macrophages exposed to fatty acids *in vitro*; these effects appear to involve the

like receptor (TLR) and NF-κB responses and subsequent production of the proinflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-6 [45,46]. Similarly, zinc uptake through ZIP8 and ZIP14 can inhibit Toll-like receptor (TLR)-4 and NF-κB signaling and reduce the production of proinflammatory cytokines (IL6, IL1β, TNF-α, and IL-8) in macrophages [84,85]. However, complete depletion of intracellular zinc disrupted TLR4-mediated LPS signaling in human monocytes [76], demonstrating that intracellular zinc is also essential for maintaining this inflammatory response. (D) Immunometabolism: $1,25(OH)_2D_3$ treatment increased the expression of glycolysis-related genes, including a key transcriptional regulator of glycolytic rate (*Pfkfb4*) in dendritic cells [56,57]. Intracellular zinc inhibited the activity of PP2A, leading to increased mTORC1-induced glycolysis and IL1β secretion in activated human monocytes and macrophages [87]. (E) Vitamin D was reported to promote alveolar macrophage M2-like activation through induction of KLF4 and STAT6 signaling [118]. Zinc deficiency promoted M1-like differentiation both in THP1 cells [131] and a murine colitis model [133], while zinc uptake through ZIP9 supported M2-like activation via enhanced STAT6 signaling and inhibited M1-like differentiation by suppressing IκBα/β phosphorylation. By contrast, studies of ZIP7 knockdown and zinc supplementation showed opposite effects of zinc on M1-like/M2-like polarization in THP1 cells [95,131]. Created using BioRender (BioRender.com). Abbreviation: Zn, zinc.

regulation of proteins involved in lipid transport and clearance [59]. Similarly, $1,25(OH)_2D_3$ can inhibit the formation of macrophage foam cells that can contribute to atherogenesis. Mechanistically, $1,25(OH)_2D_3$ treatment suppressed foam cell formation by T2DM [60]. In line with these findings in mouse and human macrophages, Vitamin D3 can attenuate the accumulation of lipids through increased autophagy-mediated lipid breakdown [61].

Although more work is needed to further identify potential immunometabolic properties of active Vitamin D in macrophages, current available studies have clearly demonstrated its effects on multiple metabolic pathways, including glucose and lipid metabolism.

Zinc

Obesity and zinc deficiency

Another relevant immunomodulator is zinc (Box 2), the second most abundant trace metal in humans, and an essential micronutrient for immune function. Zinc deficiency is most often caused by malnutrition, with an estimated 17.3% of the global population being at risk of insufficient zinc intake [62]. A meta-analysis reported lower serum zinc concentrations in obese individuals compared with lean subjects [27]. Serum zinc concentrations have also showed an inverse association with waist circumference [63]. In addition, low zinc concentrations are also associated with increased prevalence of obesity in humans [64–67], which might partly be explained by the increased activity of zinc transporters during obesity, which results in reduced plasma zinc [68]. Furthermore, research has reported decreasing circulating zinc concentrations with aging [69].

The detrimental effects of zinc deficiency were first described in severe cases of hepatosplenomegaly, dwarfism, and hypogonadism during the 1960s [70]. A major consequence of human zinc deficiency is increased susceptibility to infections and sepsis [71]. Moreover, recent studies have drawn attention to a potential association between zinc deprivation and worse outcomes in patients with COVID-19 [72–74]. Additionally, excessive adiposity, inflammation, insulin resistance, and pro-atherogenic changes are associated with lower adipose tissue zinc status in humans [75]. Zinc not only has effects on monocyte/macrophage differentiation (Box 3), but also harbors important roles for various aspects of macrophage function.

Inflammatory signaling

Zinc is a modulator of many inflammatory pathways in myeloid cells, including the TLR4 signaling pathway; activation of TLR4 by LPS triggers an immediate increase in free Zn^{2+} [76]. Moreover, depletion of intracellular zinc using the membrane-permeable zinc-specific chelator TPEN completely disrupts TLR4-mediated LPS response in human monocytes and subsequent cytokine release [76]. Mechanistically, immunoblot analysis demonstrated that zinc can prevent the dephosphorylation of p38 MAPKs, MEK1/2, and ERK1/2, which are phosphorylated downstream of TLR4 binding to the adaptor protein MyD88 [76]. Through this pathway, zinc supports activation of NF-κB and production of the proinflammatory cytokines TNFα, IL1β, and IL6 [76]. Of note, chelation of zinc augments Toll/

Box 2. Zinc

The average healthy human body contains ~2–4 g of zinc, most of which is localized to muscle and bone [120]. Zinc import in cells is regulated by zinc transporters, of which 24 are identified in humans [121]. Together, they constitute two solute carrier (SLC) gene families encoding SLC39 (ZIP proteins) and SLC30 (ZnT proteins). ZIP transporters are responsible for cytosolic zinc uptake from the microenvironment and intracellular compartment, while ZnT proteins regulate zinc efflux from the cytosol. However, many of these proteins do not exclusively transport zinc, but also facilitate transfer of other trace metals, such as iron and manganese [121]. Given that free labile zinc ions (Zn^{2+}) are inherently toxic, most circulating and intracellular zinc is bound to proteins, such as albumin and metallothioneins, from which it can be subsequently transferred to other zinc-binding proteins [122]. Zinc is mostly known for its role during gene transcription, because it is an important cofactor of ~750 transcription factors known as zinc finger proteins [123]. Additionally, zinc is a catalytic component in more than 300 enzymatic reactions [124].

Box 3. Zinc and macrophage differentiation/phenotypes

While lymphopoiesis is decreased by the effects of zinc deficiency, myelopoiesis has been reportedly increased in zinc-deficiency animal models, resulting in higher numbers of polymorphonuclear neutrophils and monocytes compared with controls [125]. Given that infection and inflammation are associated with a period of **hypozincemia** [126], these results align with increased myelopoiesis during the **acute-phase response** (APR) [127]. Accordingly, chemical chelation of zinc with TPEN increases the expression of monocyte surface markers CD11b and CD14 during differentiation of human myeloid leukemia (HL60) and monocytic THP1 cells *in vitro* [128]. By contrast, one study reported inhibition of phorbol 12-myristate 13-acetate-(PMA)-induced macrophage differentiation in HL60 cells after zinc chelation with TPEN [129]. This discrepancy should be resolved but might be the result of a difference in chelation severity between both studies, namely moderate versus extreme zinc chelation, respectively [128].

Literature on the direct effects of zinc on primary macrophage differentiation is limited. Recent work demonstrated differential expression of *Zip9* in M1-like and M2-like polarized murine peritoneal macrophages, with higher expression found in M2-like cells [130]. The authors additionally found that *ZIP9* expression was reduced in tumor-associated macrophages (TAMs) from human liver cancer tissues [130]. Mechanistically, *Zip9* promoted M2-like polarization through enhanced STAT6 signaling, while simultaneously inhibiting M1-like differentiation by suppressing IκBα/β phosphorylation [130]. Hence, small interfering (si)RNA knockdown of *Zip9* increased the expression of M1-like associated genes (*iNos*, *Il1b*, and *Tnfa*) while that of M2-like markers (*Arg1*, *Ym1*, and *Fizz1*) was reduced [130]. These results suggested that increased intracellular zinc concentrations through upregulation of *Zip9* support anti-inflammatory macrophage differentiation. By contrast, knockdown of *ZIP7* (SLC39A7) increased gene expression of the M2-like marker *CD206* in THP1 cells, while decreasing the M1-like marker *NOS2* and proinflammatory cytokine production [95]. Conversely, physiological zinc supplementation strongly inhibited THP1 cell M2-like polarization, as evidenced by reduced STAT6 phosphorylation and cell surface expression of Dectin 1 [131]. However, complete zinc deficiency decreased splenic M2-like $CD163^+$ macrophage numbers in rats [132] and promoted M1-like differentiation in both THP1 cells [131] and a murine model of 2,4,6-trinitrobenzene sulfonic acid-induced colitis [133]. Together, these results demonstrate that zinc can act as a modulator of macrophage differentiation, although many questions remain.

IL1R domain-containing adapter-inducing IFN-β (TRIF) signaling, the second major signaling arm downstream of TLR4, leading to increased IFNβ (*IFNB*) transcription [77]. Zinc was shown to prevent phosphorylation of IFN regulatory factor 3 (IRF3), thus acting as a negative regulator of TRIF signaling [77]. Similar results were obtained for TLR3, which also triggers TRIF-dependent signaling [77], indicating that zinc is broadly involved in PAMP responses.

Zinc deficiency has differential effects on inflammation and cytokine production. In promyeloid cells, zinc deficiency through chelation with the resin Chelex increased production of IL1β and TNFα in a ROS-dependent manner [78]. Accordingly, zinc depletion by Chelex enhanced IL1β and IL6 production following LPS stimulation in THP1 cells, which was paralleled by the increased expression of activation markers ICAM1, MHC class II, and CD86, relative to non-zinc-depleted controls [79]. However, these results have to be interpreted with caution because another study reported that Chelex could augment cytokine production independently from its effect on zinc [80]. These authors found decreased production of TNFα and IL6 in zinc-depleted human monocytes, while bacterial killing through phagocytosis and oxidative burst was elevated [80].

Another inflammatory pathway impacted by zinc deficiency is the **NLR family pyrin domain-containing 3 (NLRP3) inflammasome** pathway, which is required for the maturation and secretion of IL1β [81]. Analysis of lysosomal integrity using Lysotracker showed that zinc depletion resulted in lysosomal damage in murine peritoneal macrophages, which in turn activated the NLRP3 inflammasome and IL1β production *in vivo* [82]. By contrast, short-term (15 min) zinc depletion with TPEN inhibited caspase 1 activation and IL1β release, suggesting that acute and sustained zinc depletion have opposing effects on macrophage inflammatory responses [83].

From another perspective, inflammatory signaling in myeloid cells is also tightly linked to the activity of several zinc transporters. ZIP8 (SLC39A8) and ZIP14 (SLC39A14) are highly induced upon LPS activation or infection of monocytes and macrophages in humans and animal models [84–87] (Box 2). This constitutes a highly conserved immunological response, because both

transporters are also upregulated upon LPS stimulation in a macrophage-like cell line derived from Atlantic salmon (SHK-1) [88]. Seminal work demonstrated that ZIP8 acts as a regulator of NF-κB activity; indeed, *ZIP8* expression is strongly induced by *in vitro* LPS stimulation in human monocytes and macrophages, leading to increased intracellular zinc concentrations, subsequently downregulating NF-κB activity through direct suppression of IκB kinase (IKK) [84]. This results in the decreased production of proinflammatory cytokines (IL6, IL1β, TNFα, and IL8) relative to controls. Accordingly, small interfering (si)RNA knockdown of *ZIP14* decreased LPS-induced production of IL6 and TNFα in primary human macrophages [85]. In an *in vitro* model of zinc supplementation, LPS-driven zinc uptake decreased the release of the anti-inflammatory cytokine IL10 through inhibition of C/EBPβ activity, which was abrogated by *ZIP8* knockdown [89]. Importantly, a myeloid-specific *Zip8*-knockout mouse model (*Zip8*$^{flox/flox}$;LysM^{Cre}) was associated with increased inflammation and mortality following infection with *Streptococcus pneumoniae* as a result of overactive NF-κB signaling [90]. ZIP8 was also implicated in the maintenance of the gut microbiome, with transplantation of microbiota (fecal matter transplant; FMT) from myeloid-specific *Zip8*-knockout mice to wild-type mice resulting in intestinal dysbiosis and impaired host defense against *S. pneumoniae* in the lung, compared with wild-type mice receiving FMT with the microbiota from wild-type controls [91]. This combined work demonstrates the importance of zinc transporters in macrophages during inflammation.

Phagocytosis

Several lines of research suggest that zinc affects macrophage phagocytic capacity. In patients with chronic obstructive pulmonary disease (COPD), zinc concentrations in bronchoalveolar lavage (BAL) fluid were positively correlated with efferocytosis [92]. Similarly, alveolar macrophages from patients with alcoholism displayed reduced concentrations of intracellular zinc and bacterial phagocytosis compared with macrophages from healthy controls [93]. In a murine model of juvenile polymicrobial sepsis, peritoneal macrophages from zinc-supplemented mice showed improved phagocytosis of *Escherichia coli* and *S. aureus* following infection [94]. Knockdown of the zinc transporter ZIP7 reduced phagocytosis in THP1 cells, which could be rescued by additional zinc supplementation [95]. Additionally, bone marrow-derived macrophages (BMDMs) isolated from myeloid specific *Zip8*-knockout mice showed decreased phagocytosis and killing of *S. pneumoniae* relative to wild-type controls [90]. By contrast, reduced intracellular zinc concentrations did not change the phagocytic capacity of BMDMs after knockdown of the ZIP10 transporter (SLC39A10) [96], and zinc supplementation did not affect RAW 264.7 cell uptake of phagocytosis beads, suggesting that zinc concentrations alone are not enough to achieve a phagocytic response [97]. However, zinc supplementation in children with diarrhea resulted in a slight increase in phagocytosis of *E. coli* by monocytes and polymorphonuclear neutrophils relative to healthy controls [134], suggesting that zinc supplementation provides benefits to humans. In addition, increased zinc concentrations through **pattern recognition receptor** (PRR)-induced metallothionein gene expression was shown to support bacterial killing of *S. aureus* and *Salmonella enterica* serovar Typhimurium through autophagy in macrophages [98]. Thus, zinc deficiency is often negatively correlated with phagocytosis, and the available literature suggests that zinc supplementation could improve phagocytic capacity in certain cases.

Antimicrobial function

In addition to its modulatory effect on macrophage effector functions and inflammatory signaling, zinc is important during infections because of its antimicrobial properties. Innate immune cells, such as macrophages, can mobilize high concentrations of zinc to phagosomes in response to certain pathogens, leading to clearance through zinc intoxication [71]. The antimicrobial capacity of zinc is mediated through mismetallation of essential proteins and deregulation of trace metal homeostasis, directly impacting key survival processes, such as metabolic pathways. Dietary

zinc restriction interfered with the bacterial-killing capacity of phagocytic cells (alveolar macrophages, monocytes, and neutrophils) in a mouse model of *S. pneumoniae* infection due to their inability to use zinc as a direct antimicrobial agent, which was further corroborated in THP1-derived macrophages [99]. Other pathogens targeted for zinc intoxication include *Mycobacterium tuberculosis*, *S. enterica* serovar Typhimurium, and *E. coli*, all of which have developed evasion strategies to subvert zinc-mediated killing, such as the expression of zinc-detoxification systems [100–102]. Zinc transporters are implicated in this antimicrobial zinc intoxication pathway. In human primary macrophages, LPS can induce ZnT1 (*SLC30A1*) mRNA and protein expression, which has demarcated zinc-containing intracellular compartments in THP1 cells infected with *E. coli* [103]. Inducible overexpression of ZnT1 in THP1 cells promoted antimicrobial zinc stress and bacterial clearance following *E. coli* infection. ZIP8 is also expected to have a role in *M. tuberculosis* infection, because it is highly expressed on the bacterium-containing phagosome [104]. However, similar to many other pathogens, *M. tuberculosis* can actively scavenge zinc from the microenvironment to persist in host macrophages [105] and it is currently unclear whether this expression benefits the host or the microbe. As a result, macrophages have also evolved sequestration strategies to resolve infections through zinc starvation [106].

Immunometabolism

Given that the potential immunometabolic effects of zinc have only recently come under increased attention, current literature on this subject is relatively limited. Recent work showed that intracellular zinc promoted IL1β secretion by augmenting mTORC1-induced glycolysis in activated human monocytes and macrophages [87]. Zinc inhibited the activity of PP2A, a phosphatase that regulates phosphorylation of S6 kinase (S6K) via mTORC1 [87]. Furthermore, the authors detected increased gene expression of zinc transporter *ZIP8* as well as increased amounts of phosphorylated S6K in monocytes from patients with rheumatoid arthritis (RA) [107]. *ZIP8* expression correlated with RA severity, suggesting that ZIP8-mediated zinc transport has a role in RA-associated inflammation. Treatment of murine BMDMs with a well-known inhibitor of glucose metabolism, the glucose analog 2-deoxy-D-glucose (2-DG), increased clearance of the fungal pathogen *Histoplasma capsulatum* by decreasing cellular zinc import, leading to zinc starvation [107]. Moreover, preculturing *H. capsulatum* with zinc rescued the pathogen from the effects of 2-DG in this model.

Of note, T cells are also sensitive to zinc status [108]. T cell activation through the T cell receptor (TCR) is accompanied by extensive metabolic rewiring, hallmarked by the Warburg effect [109]. Both zinc supplementation and depletion supported glucose uptake and insulin receptor signaling in mixed lymphocyte cultures following anti-CD3/CD28 antibody stimulation, indicating that intracellular zinc concentrations can modulate a T cell metabolic shift [110]. Finally, zinc homeostasis is essential for mitochondrial integrity and function in both *Caenorhabditis elegans* and human cells [111], with free intracellular zinc overload being implicated in neurodegenerative processes through inhibited energy production [112]. These pioneering studies suggest that zinc has a role in regulating immune cell metabolism; however, more in-depth studies are needed to fully elucidate its importance in macrophage immunometabolism.

Concluding remarks

While counterintuitive at first glance, micronutrient deficiency is increasingly being recognized as a serious complication of malnutrition during obesity [24]. The high prevalence of obese patients in intensive care units during the COVID-19 pandemic has further underscored the impact of nutritional status on the ability of the human immune system to combat infections [113]. Given that micronutrients are inherently connected to metabolic processes in tissues and cells, deficiencies could disrupt immune cell function through alterations in immunometabolism. Here, we have

Outstanding questions

What metabolic pathways are affected by Vitamin D and zinc in macrophages and how do these metabolic effects underly functional changes?

What is the impact of micronutrient deficiencies, including Vitamin D and zinc, during obesity on macrophage metabolism and function and most likely also on other immune cells?

Can complications during obesity be directly attributed to micronutrient deficiencies leading to dysfunctional innate immune cells, including macrophages?

How do we target innate immune cells *in vivo* using micronutrient supplementation and how do we personalize these interventions? Can we identify those individuals with dysfunctional macrophages and micronutrient deficiencies to optimize efficiency of supplementation?

provided a snapshot of the immunomodulating effects of two micronutrients that are habitually reduced in obese individuals, Vitamin D and zinc, on macrophages. However, in many instances, direct connections between obesity, zinc/Vitamin-D deficiencies, and altered innate immune functions remain elusive (see Outstanding questions). Moreover, the cumulative effect of obesity on micronutrient status is far broader and more variable, encompassing numerous additional vitamins, minerals, and trace elements. This may also help explain the lack of metabolic improvements after supplementation of single micronutrients. For instance, combinations of multiple deficiencies might underlie specific obesity subpopulations, which would require a more targeted supplementation approach. Additionally, the available literature on the immunometabolic effects of individual micronutrient deficiencies is relatively limited, and often not based on state-of-the-art techniques. To elucidate the impact of micronutrients on immunometabolic parameters, future studies using innovative tools that are available in the immunologist's toolbox [114] are needed, including single-cell metabolic profiling using, for example, **Single cell energetic metabolism by profiling translation inhibition (SCENITH)** [115]. Single-cell techniques allow more precise identification of metabolic adaptations associated with micronutrient restriction in either circulating or tissue-resident immune cell populations, such as macrophage subpopulations associated with obesity or respiratory infections. This knowledge is also crucial for determining the optimal route of supplementation, given that oral supplements do not always reach the required concentrations in target cells *in vivo*. For example, CD163-binding lipid nanoparticles might be envisaged to specifically deliver Vitamin D to macrophages in certain tissues [116].

Overall, to develop effective micronutrient-based interventions to counteract innate immune dysfunction during obesity, we need to overcome several hurdles. First, we must robustly characterize the immunometabolic effects of micronutrients on macrophage functions. Second, we need to determine the impact of micronutrient deficiencies, including Vitamin D and zinc, during obesity on macrophage metabolism and function and, most likely, on other immune cells. Third, we need to identify the specific micronutrient deficiencies that exist in individuals with obesity to intervene with the appropriate (combination of) micronutrient supplementation. Finally, targeted approaches are needed to deliver specific micronutrients to immune cells of interest.

Together, these approaches might lead to personalized nutrition approaches for the management of micronutrient deficiencies, aiming to improve dysfunctional innate immune responses, and, ideally, prevent obesity-associated complications.

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Declaration of interests

None declared by authors.

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