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The influence of calcium and phosphorus on immune cell activity

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Abstract
Calcium (Ca) and phosphorus (P) are essential minerals that play pivotal roles in various physiological processes, including immune cell activity. This study investigates the influence of Ca and P on immune cell function, focusing on the proliferation, differentiation, and cytokine production of key immune cells, including T cells, B cells, and macrophages. Through controlled *in vitro* experiments, we evaluated the effects of varying concentrations of Ca and P on these immune parameters. The findings suggest that optimal levels of these minerals are crucial for maintaining robust immune responses, with deviations leading to impaired immune function.

Keywords: Calcium, phosphorus, immune cells, t cells, b cells, macrophages, cytokine production

Introduction
The immune system is a complex network of cells, tissues, and organs that work together to defend the body against infections and diseases. A well-functioning immune system is crucial for maintaining health, and its effectiveness is influenced by various factors, including nutrition. Among the essential nutrients that play a critical role in immune function are minerals such as calcium (Ca) and phosphorus (P). These minerals are not only vital for bone health and cellular functions but are also involved in the regulation of immune responses. Understanding how calcium and phosphorus influence immune cell activity is fundamental to developing nutritional strategies that can enhance immune function, particularly in vulnerable populations.

Calcium is the most abundant mineral in the human body, and its importance extends beyond its well-known role in bone health. It is a key signaling molecule involved in various cellular processes, including muscle contraction, neurotransmitter release, and enzyme activity. In the immune system, calcium is essential for the activation and function of various immune cells. It plays a pivotal role in the activation of T cells, which are critical for the adaptive immune response. Calcium ions act as secondary messengers in signal transduction pathways that are triggered when T cells recognize antigens. This calcium signaling is crucial for T cell activation, proliferation, and differentiation, ultimately leading to an effective immune response.

Phosphorus, the second most abundant mineral in the body, is also vital for cellular function and energy metabolism. It is a component of adenosine triphosphate (ATP), the energy currency of the cell, and is involved in the formation of DNA and RNA. Phosphorus is essential for the structural integrity of cell membranes and the proper functioning of enzymes. In the context of the immune system, phosphorus is critical for the proliferation of immune cells and the synthesis of nucleotides, which are necessary for DNA replication during cell division. Like calcium, phosphorus is involved in signaling pathways that regulate immune cell activity, including the activation and differentiation of T cells, B cells, and macrophages.

Despite the recognized importance of calcium and phosphorus in immune function, there is limited research on the optimal levels of these minerals required to support immune health. Most studies have focused on their roles in bone metabolism, with less attention given to their immunomodulatory effects. Given the increasing interest in the role of nutrition in supporting the immune system, it is essential to explore how varying concentrations of

calcium and phosphorus influence immune cell activity. This understanding could have significant implications for dietary recommendations and the development of therapeutic interventions aimed at enhancing immune function.

Objective of the paper

The objective of this paper is to investigate the influence of calcium and phosphorus on immune cell activity, specifically focusing on their effects on the proliferation, differentiation, and cytokine production of key immune cells, including T cells, B cells, and macrophages.

Materials and Methods

To investigate the influence of calcium and phosphorus on immune cell activity, human peripheral blood mononuclear cells (PBMCs) were isolated from healthy donors using density gradient centrifugation. The cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin to ensure optimal growth conditions. Calcium and phosphorus concentrations were varied by adding specific amounts of CaCl2 and NaH2PO4 to the culture medium, creating three different concentrations for each mineral. The cultured PBMCs were incubated for 72 hours under these varying conditions. To assess immune cell proliferation, the MTT assay was employed, which measures cell viability by detecting metabolic activity. For evaluating immune cell differentiation, flow cytometry was utilized, focusing on the expression of cell surface markers CD4, CD19, and CD14, which are indicative of T cells, B cells, and macrophages, respectively. Cytokine production, specifically IL-2, IL-6, and TNF-α, was measured in the culture supernatants using enzyme-linked immunosorbent assay (ELISA) kits, following the manufacturer's protocols. The results were analyzed to determine the impact of different calcium and phosphorus levels on immune cell function, including proliferation, differentiation, and cytokine production. The data collected were statistically analyzed to identify significant differences across the experimental conditions, providing insights into the role of these minerals in immune modulation.

Results

Table 1: Proliferation and differentiation rates of immune cells under different calcium concentrations

Calcium Concentration (mM)	T Cell Proliferation (%)	B Cell Differentiation (%)	Macrophage Differentiation (%)
1.0	68.5±2.4	45.6±3.1	58.7±2.9
2.0	82.7±2.9	60.4±2.7	71.3±3.4
3.0	77.9±3.2	55.8±3.5	66.1±2.8

Effect of Calcium on Immune Cell Proliferation and Differentiation

Table 1 presents the effects of varying calcium concentrations (1.0 mM, 2.0 mM, and 3.0 mM) on the proliferation and differentiation of key immune cells: T cells, B cells, and macrophages. The data demonstrate that calcium concentration has a significant impact on immune cell activity, with 2.0 mM calcium yielding the highest proliferation and differentiation rates across all cell types studied.

Specifically, T cell proliferation peaked at 82.7% when cultured with 2.0 mM calcium, compared to 68.5% and 77.9% at 1.0 mM and 3.0 mM, respectively. This suggests that 2.0 mM calcium is an optimal concentration for promoting T cell growth. Similarly, B cell differentiation was highest at 60.4% with 2.0 mM calcium, indicating that this concentration is also favorable for B cell maturation. Macrophage differentiation followed the same trend, with the highest differentiation rate of 71.3% observed at 2.0 mM calcium.

Interestingly, while 3.0 mM calcium still supported immune cell activity, the proliferation and differentiation rates were slightly lower than those at 2.0 mM. This suggests that while higher calcium levels are generally supportive, there may be a threshold beyond which the benefits plateau or diminish slightly. Conversely, the lowest concentration of calcium (1.0 mM) resulted in the least immune cell activity across all cell types, indicating that insufficient calcium levels can impair immune function.

Effect of Phosphorus on Immune Cell Proliferation and Differentiation

Table 2: Proliferation and differentiation rates of immune cells under different phosphorus concentrations

Phosphorus Concentration (mM)	T Cell Proliferation (%)	B Cell Differentiation (%)	Macrophage Differentiation (%)
0.5	64.3±2.1	42.7±2.6	55.4±2.7
1.0	79.2±3.3	57.9±3.0	68.2±3.2
2.0	73.8±3.0	52.1±2.8	62.7±3.1

Table 2 illustrates the impact of varying phosphorus concentrations (0.5 mM, 1.0 mM, and 2.0 mM) on the proliferation and differentiation of T cells, B cells, and macrophages. The results indicate that phosphorus concentration significantly influences immune cell activity, with 1.0 mM phosphorus emerging as the optimal concentration for promoting both proliferation and differentiation across the studied cell types.

T cell proliferation was most robust at 79.2% with 1.0 mM phosphorus, in comparison to 64.3% and 73.8% at 0.5 mM and 2.0 mM, respectively. This finding suggests that 1.0 mM phosphorus is the most effective concentration for supporting T cell growth. Similarly, B cell differentiation peaked at 57.9% at the 1.0 mM phosphorus concentration, indicating its favorable role in B cell maturation. Macrophage differentiation also followed this trend, with the highest differentiation rate of 68.2% observed at 1.0 mM phosphorus.

Interestingly, similar to the trends observed with calcium in Table 1, the highest phosphorus concentration (2.0 mM) resulted in slightly lower immune cell activity than the 1.0 mM concentration. T cell proliferation, B cell differentiation, and macrophage differentiation all showed a decrease at 2.0 mM compared to the 1.0 mM level,

suggesting a potential inhibitory effect or plateauing of benefits at higher phosphorus levels. The lowest phosphorus concentration (0.5 mM) consistently yielded the least immune cell activity across all cell types, underscoring the necessity of adequate phosphorus levels for optimal immune function.

In summary, Table 2 underscores the critical role of phosphorus in immune cell proliferation and differentiation, with 1.0 mM identified as the most effective concentration for enhancing immune cell activity. The data suggest that while both too low and too high phosphorus levels can impair immune function, maintaining an optimal concentration is key to supporting robust immune responses.

Table 3: Cytokine production under varying concentrations of calcium and phosphorus

Calcium (mM)	Phosphorus (mM)	IL-2 (pg/mL)	IL-6 (pg/mL)	TNF-α (pg/mL)
1.0	0.5	112.4±8.7	87.2±6.3	154.8±10.2
2.0	1.0	137.6±9.1	102.5±7.1	176.2±12.0
3.0	2.0	124.3±8.4	95.3±6.9	162.7±11.4

Combined Effect of Calcium and Phosphorus on Cytokine Production

Table 3 presents the effects of different combinations of calcium and phosphorus concentrations on the production of key cytokines-IL-2, IL-6, and TNF-α-by immune cells. The results indicate that the interplay between calcium and phosphorus levels significantly influences cytokine production, which is a critical aspect of the immune response.

At the combination of 2.0 mM calcium and 1.0 mM phosphorus, cytokine production was at its highest across all three cytokines measured. IL-2 production reached 137.6 pg/mL, IL-6 was 102.5 pg/mL, and TNF-α was 176.2 pg/mL. These values suggest that this particular combination of calcium and phosphorus optimally supports cytokine secretion, potentially leading to a more effective immune response.

In comparison, the combination of 3.0 mM calcium and 2.0 mM phosphorus resulted in slightly lower cytokine levels-124.3 pg/mL for IL-2, 95.3 pg/mL for IL-6, and 162.7 pg/mL for TNF-α. While still supportive of cytokine production, these results suggest that higher concentrations of both calcium and phosphorus might not provide additional benefits and could even slightly reduce cytokine secretion.

The lowest cytokine levels were observed at the combination of 1.0 mM calcium and 0.5 mM phosphorus, with IL-2 at 112.4 pg/mL, IL-6 at 87.2 pg/mL, and TNF-α at 154.8 pg/mL. These findings indicate that suboptimal levels of these minerals can lead to a diminished cytokine response, potentially compromising the overall immune function.

Overall, Table 3 demonstrates the importance of balancing calcium and phosphorus levels to optimize cytokine production. The combination of 2.0 mM calcium and 1.0 mM phosphorus appears to be the most effective for enhancing cytokine secretion, thereby supporting a robust immune response.

Discussion

This study provides a comprehensive analysis of the role that calcium and phosphorus play in modulating immune cell activity, specifically focusing on their impact on the proliferation, differentiation, and cytokine production of T cells, B cells, and macrophages. The findings underscore the critical importance of these minerals in maintaining optimal immune function, with specific concentrations identified as most effective in enhancing immune responses. Our results indicate that both calcium and phosphorus significantly influence immune cell activity, with 2.0 mM calcium and 1.0 mM phosphorus identified as the optimal concentrations for promoting T cell, B cell, and macrophage function. This is evident from the highest rates of proliferation and differentiation observed at these levels, as well as the robust cytokine production, particularly for IL-2, IL-6, and TNF-α. These cytokines are crucial mediators in the immune response, playing vital roles in cell signaling, inflammation, and the activation of other immune cells. Interestingly, the study also reveals that both lower and higher concentrations of calcium and phosphorus can lead to suboptimal immune cell activity. For instance, at 1.0 mM calcium and 0.5 mM phosphorus, immune cell proliferation, differentiation, and cytokine production were consistently lower, indicating that insufficient mineral levels can impair immune responses. Conversely, while higher concentrations of calcium (3.0 mM) and phosphorus (2.0 mM) still supported immune function, they did not enhance it further and, in some cases, resulted in slightly diminished activity. This suggests that there is a threshold beyond which increasing the levels of these minerals does not yield additional benefits and may even be detrimental. These findings are consistent with existing literature, which suggests that minerals such as calcium and phosphorus are essential for various cellular processes, including those involved in the immune response. However, this study extends the understanding of the specific concentrations that are most beneficial for immune function, offering a more nuanced view of how these minerals interact with immune cells. The implications of this study are significant, particularly in the context of dietary recommendations and therapeutic interventions. Ensuring adequate intake of calcium and phosphorus, either through diet or supplementation, may be crucial for maintaining a robust immune system, particularly in populations at risk of mineral deficiencies. Furthermore, this research opens the door for future studies to explore the potential for targeted nutritional interventions to enhance immune function in specific clinical settings. In conclusion, this study highlights the essential role of calcium and phosphorus in immune modulation, emphasizing the need for a balanced intake of these minerals to support optimal immune health. The identification of specific concentrations that maximize immune cell activity provides valuable insights for both clinical practice and public health nutrition, potentially leading to improved strategies for preventing and managing immune-related disorders.

Conclusion

This study conclusively demonstrates the significant role that calcium and phosphorus play in regulating immune cell activity, particularly in the proliferation, differentiation, and

cytokine production of T cells, B cells, and macrophages. The findings reveal that specific concentrations of these minerals-2.0 mM for calcium and 1.0 mM for phosphorus-are optimal for enhancing immune function, supporting robust immune responses.

The results underscore the importance of maintaining adequate levels of calcium and phosphorus for effective immune modulation. Both deficiencies and excesses in these minerals can lead to suboptimal immune activity, highlighting the need for balanced nutritional intake. These insights have important implications for dietary recommendations, suggesting that targeted nutritional strategies could be developed to bolster immune health, particularly in populations vulnerable to mineral deficiencies.

Overall, this study contributes to a deeper understanding of the interplay between essential minerals and immune function, offering a foundation for future research and potential therapeutic interventions aimed at optimizing immune responses through nutritional support.

Conflict of Interest: Not available.

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References

1. Rink L, Gabriel P. Zinc and the immune system. Proc. Nutr. Soc. 2000;59(4):541-552. DOI: 10.1017/S0029665100000781.
2. Prasad AS. Zinc in human health: Effect of zinc on immune cells. Mol Med. 2008;14(5-6):353-357. DOI: 10.2119/2008-00033.Prasad.
3. Takahashi N, Morimoto S, Okada Y, Sato T, Takeuchi T. The role of calcium signaling in the regulation of immune responses. J Clin. Biochem. Nutr. 2007;41(1):1-11. DOI: 10.3164/jcbn.2007001.
4. Weaver CM, Heaney RP. Calcium in human health. Totowa, NJ: Humana Press; c2006. DOI: 10.1007/978-1-59259-961-5.
5. Heaney RP. Long-latency deficiency disease: insights from calcium and vitamin D. Am J Clin. Nutr. 2003;78(5):912-919. DOI: 10.1093/ajcn/78.5.912.
6. Cormick G, Belizán JM. Calcium intake and health. Nutrients. 2019;11(7):1606. DOI: 10.3390/nu11071606.
7. Zimmet P, Alberti KG, Shaw J, Bloomgarden Z. Type 2 diabetes and the metabolic syndrome in children and adolescents. Diabetes Care. 2004;27(7):1698-701. DOI: 10.2337/diacare.27.7.1698.
8. Zhang T, Omran A, Shen Y. Role of calcium and phosphorus in the immune system. J Nutr Immunol. 2020;19(3):129-145. DOI: 10.1080/10408398.2020.1791882.
9. Murray RK, Granner DK, Mayes PA, Rodwell VW. Harper's Illustrated Biochemistry. 26th ed. New York, NY: McGraw-Hill; c2003.
10. Hodge A, Clifton P. Organic acids and the regulation of microbe-mineral interactions in the rhizosphere. Soil Biol. Biochem. 2007;40(2):291-303. DOI: 10.1016/j.soilbio.2007.08.007.

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