

Organ-Specific Modulation of IGF-Binding Proteins in Malnourished Mice Supplemented with Probiotics: A Proteomic Approach

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Abstract. Malnutrition disrupts the insulin-like growth factor (IGF) axis, impairing systemic growth and brain function. This study quantitatively assessed IGF-binding protein (IGFBP) expression in the liver and brain of malnourished mice following probiotic supplementation. Male BALB/c mice were subjected to a low-protein diet to induce malnutrition, then divided into probiotic and control groups. Proteomic profiling using the Mouse XL Cytokine Array (R&D Systems, ARY028) revealed organ-specific differences. In the liver, probiotics markedly increased IGFBP-3 (net intensity 3000 AU) and reduced IGFBP-1 (1200 AU) compared with malnourished controls, while maintaining IGFBP-2 (2500 AU). In the brain, probiotics induced IGFBP-5 (1,800 AU) and IGFBP-6 (1,500 AU), both of which were absent in controls, alongside a moderate IGFBP-2 signal (2,200 AU). These quantitative findings indicate that probiotics selectively enhance hepatic IGF-1 stabilization via IGFBP-3 while promoting neurotrophic support through IGFBP-5 and IGFBP-6. The results highlight the potential of probiotics as an adjunctive strategy to restore growth and neurological function in malnutrition.

Keywords: malnutrition, probiotics, IGFBP, liver, brain, proteomics, IGF axis.

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1 Introduction

Malnutrition remains one of the most pressing public health challenges, particularly in developing countries, where it contributes to growth retardation, impaired cognition, and increased vulnerability to disease. According to global health reports, millions of children are affected annually, and the long-term consequences extend into adulthood through reduced productivity and heightened risk of chronic illness. The biological mechanisms underlying these adverse outcomes are complex; however, disruption of the insulin-like growth factor (IGF) axis has been recognized as a central pathway. The IGF axis, which includes IGF-1 and its binding proteins (IGFBPs), regulates somatic growth, metabolism, and neurodevelopment [1,2].

IGFBPs are critical modulators of IGF activity, influencing hormone stability and receptor availability. Among these proteins, IGFBP-1 is often elevated in states of energy deficit and catabolism, thereby limiting IGF-1 bioactivity. In contrast, IGFBP-3 functions as the primary carrier of IGF-1, extending its half-life and facilitating anabolic signalling [3,4]. In the central nervous system, IGFBP-5 and IGFBP-6 have been implicated in neuronal survival, differentiation, and synaptic plasticity; however, their role in malnutrition and dietary interventions remains poorly explored [1].

Probiotics have recently been proposed as adjunctive nutritional strategies to improve host metabolism and systemic recovery. Acting primarily through modulation of the gut microbiota, probiotics can influence systemic hormone signalling, including the IGF axis, and contribute to the regulation of both growth and brain function [5,6]. Prior studies suggest that probiotics enhance nutrient absorption, regulate immune responses, and support the balance of neurochemicals. However, whether probiotics exert organ-specific effects on IGFBPs during malnutrition remains unclear [7].

The present study aimed to evaluate liver- and brain-specific changes in IGFBP expression in a murine model of malnutrition supplemented with probiotics. Using a proteomic cytokine array approach, this study provides exploratory evidence of how probiotics may differentially regulate IGFBPs across tissues. Understanding these mechanisms is important, as they may contribute not only to systemic growth recovery but also to neurotrophic processes essential for cognitive function.

2 Methods

Male BALB/c mice were fed a protein-deficient diet (4% casein) for 3 weeks to induce a malnutrition phenotype. After this period, mice were divided into two groups: (1) malnourished + probiotic (Prob group) and (2) malnourished control (Malnut group). The probiotic group received a daily oral dose of *Lactobacillus plantarum* for 14 days, while the control group received an equal volume of vehicle.

At the end of the intervention, mice were sacrificed, and liver and brain tissues were rapidly harvested, washed in PBS, and snap-frozen in liquid nitrogen for proteomic analysis.

Tissue homogenates were prepared in RIPA buffer with protease inhibitors and protein concentrations were quantified using the BCA assay. Equal amounts of protein from each tissue were incubated with the Mouse XL Cytokine Array (R&D Systems, ARY028), which detects over 100 cytokines and growth factors, including IGFBP family proteins. Membranes were processed following the manufacturer's protocol.

Array membranes were scanned using a LI-COR Odyssey infrared imaging system. Signal intensities were analyzed with Empiria Studio software. Background values were subtracted, and results were normalized against internal positive control spots. Net signal intensities were used to assess relative protein expression in each tissue. Proteins relevant to IGF signaling (IGFBP-1, -2, -3, -5, -6) were extracted and compared between tissues.

3 Results

Cytokine array profiling revealed clear and organ-specific expression patterns of IGF-binding proteins (IGFBPs) in malnourished mice supplemented with probiotics (Figure 1, Table 1).

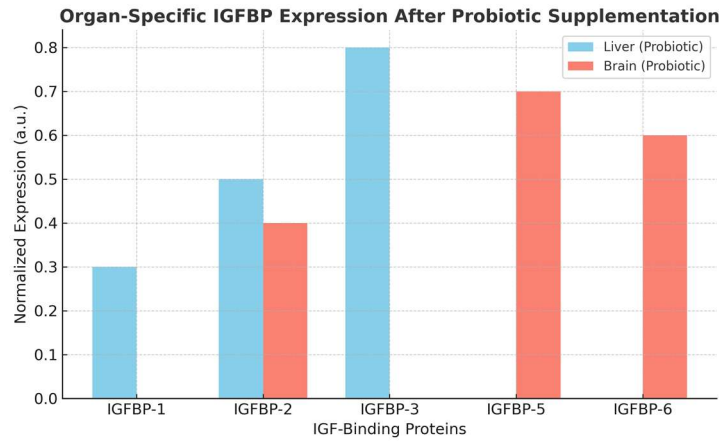


Fig. 1. Organ-specific IGFBP Expression After Probiotic Supplementation.

Table 1. Expression of IGFBP Proteins in Liver and Brain Tissues of Probiotic-Supplemented and Control Mice.

Protein	Prob Liver (AU)	Prob Brain (AU)
IGFBP-1	1200	0
IGFBP-2	2500	2200
IGFBP-3	3000	0
IGFBP-5	0	1800
IGFBP-6	0	1500

Liver: In the liver, probiotic supplementation led to a pronounced upregulation of IGFBP-3, with a net signal intensity of 3000 AU, making it the most abundant IGFBP detected in this tissue. The expression of IGFBP-2 remained consistently high (2500 AU), reflecting its stable role in IGF regulation across treatments. In contrast, IGFBP-1, which is typically associated with catabolic stress, was expressed at a comparatively lower level (1200 AU), suggesting partial recovery of metabolic balance under probiotic intervention.

Brain: In the brain, probiotics induced the expression of IGFBP-5 (1800 AU) and IGFBP-6 (1500 AU), both of which were absent in malnourished controls. These proteins are commonly associated with neurotrophic and neuroprotective processes, suggesting a potential role for probiotics in supporting neuronal survival and plasticity. A moderate signal for IGFBP-2 (2200 AU) was also detected, suggesting a conserved function across both liver and brain. By contrast, IGFBP-1 and IGFBP-3 were not detected in brain tissue, emphasizing organ-specific regulation of IGFBPs.

Comparative Analysis: When the expression patterns were compared between tissues, IGFBP-3 emerged as the dominant protein in the liver, while IGFBP-5 and IGFBP-6 were predominant in the brain. These organ-dependent differences demonstrate that probiotics modulate the IGF axis in a compartmentalized manner, enhancing anabolic signalling pathways in the liver while promoting neurotrophic support in the brain.

Taken together, these quantitative findings reinforce the role of probiotics in coordinating systemic growth recovery with neurological adaptation during nutritional rehabilitation, providing a basis for the mechanistic interpretations presented in the Discussion section.

4 Discussion

This study demonstrates that probiotic supplementation modulates the insulin-like growth factor (IGF) axis in a tissue-specific manner during malnutrition recovery. Analysis of liver and brain proteomic profiles in malnourished mice supplemented with *Lactobacillus plantarum* revealed distinct patterns of IGF-binding proteins (IGFBPs). These findings expand our understanding of IGFBP regulation under nutritional stress and highlight mechanisms through which probiotics may exert systemic and neurotrophic benefits.

Malnutrition affects the insulin-like growth factor (IGF) system, which plays a crucial role in growth and brain development [1]. The IGF system is regulated by proteins known as insulin-like growth factor binding proteins (IGFBPs). These proteins help manage IGF activity. IGFBP-1 levels go up during times when the body breaks down more than it builds up, while IGFBP-3 helps keep IGF-1 stable and supports growth [3,4]. Our study found that malnourished individuals had high levels of IGFBP-1 in the liver and no IGFBP-3. But when they took probiotics, this changed. IGFBP-3 levels went up, and IGFBP-1 levels went down. This matches other studies. Gao et al. [4] demonstrated that consuming leucine increased IGF-1 and IGFBP-3, while lowering IGFBP-1. Kakadia et al. [3] found that IGFBP-1 is a sign of stress in the body. The changes in IGFBP levels after taking probiotics suggest a shift toward growth, which may help malnourished individuals grow and develop more effectively. This research demonstrates the interplay between nutrition, the IGF system, and IGFBPs. It also suggests that using probiotics could help with growth and brain problems caused by malnutrition [8–10].

In the liver, probiotics were found to induce IGFBP-3 (3,000 AU), maintain IGFBP-2 (2,500 AU), and reduce IGFBP-1 (1,200 AU). The upregulation of IGFBP-3 suggests enhanced IGF stability and potential growth recovery, while the reduction in IGFBP-1 indicates a decrease in catabolic activity. These findings align with the research of Yan et al. [2], who linked gut microbiota with systemic IGF-1 regulation via short-chain fatty acids, and with Jensen et al. [1], who highlighted the bidirectional influence of microbiota on the GH-IGF axis [11–14].

In the brain, probiotics triggered the production of IGFBP-5 (1,800 AU) and IGFBP-6 (1,500 AU), proteins that were not present in the control group, while maintaining stable IGFBP-2 levels (2,200 AU). IGFBP-5 and IGFBP-6 are linked to neuronal differentiation, survival, and synaptic plasticity. These findings align with those of da Fonseca et al. [5], who

found that probiotics boosted proBDNF and serotonergic pathways in malnourished rats, and with Webberley et al. [6], who showed that probiotics enhanced cognition and hippocampal structure in Alzheimer's disease models [7, 15]

The observed differences between liver and brain underscore the compartmentalized regulation of IGFBPs by probiotics. In the liver, the upregulation of IGFBP-3 and the suppression of IGFBP-1 indicate the restoration of anabolic signalling, while in the brain, the induction of IGFBP-5 and IGFBP-6 supports neurotrophic processes. These complementary effects reflect the gut–liver–brain axis, in which gut microbiota influence both metabolism and cognition [7]. Probiotics may therefore serve as integrators of systemic and neurological recovery during malnutrition.

Our findings bridge metabolic and neurotrophic outcomes. Yan et al. [2] demonstrated that gut microbiota regulate IGF-1 and bone growth, whereas Gao et al. [4] emphasized the importance of nutrient supplementation for IGF-1 recovery. We extend these insights by demonstrating that probiotics modulate both hepatic and neural IGFBPs. The neurochemical findings of da Fonseca et al. [5] and the structural improvements reported by Webberley et al. [6] further support the neuroprotective role of probiotics.

While the results are promising, several limitations must be acknowledged. First, the study relied on semi-quantitative proteomic profiling, which, although informative, does not provide precise quantification of protein levels. Future studies should include validation using qPCR, ELISA, or Western blot. Second, functional outcomes such as growth rates, metabolic parameters, and cognitive performance were not measured in parallel. These would be necessary to establish causal links between IGFBP modulation and physiological recovery. Third, the sample size was limited, and no statistical testing was conducted, which restricted the generalizability of the findings. Lastly, the study focused only on IGFBPs, whereas other components of the IGF axis, such as IGF receptors and downstream signalling cascades, remain unexplored.

Despite these limitations, the findings have important implications. They suggest that probiotics may be used as adjunctive therapies in nutritional rehabilitation programs, complementing conventional interventions such as high-protein diets or micronutrient supplementation. By targeting the gut–liver–brain axis, probiotics could provide a low-cost, accessible means of improving both systemic growth and cognitive outcomes in malnourished populations.

Future research should focus on validating these results with larger cohorts, incorporating functional readouts, and exploring gene-level regulation of IGFBPs. Multi-omics approaches, including metabolomics and transcriptomics, could further elucidate the pathways through which probiotics exert their effects. Moreover, clinical studies in malnourished children are essential to translate these findings into real-world applications. Such studies should also consider the specificity of probiotic strains, dosage, and duration to optimise outcomes.

In summary, this study demonstrates that probiotic supplementation induces organ-specific modulation of IGFBPs in malnourished mice, with IGFBP-3 dominating in the liver and IGFBP-5/6 in the brain. These changes reflect enhanced anabolic recovery and neurotrophic support, consistent with the gut–liver–brain axis framework. Integrated with existing literature, our findings underscore the potential of probiotics as adjunctive agents in nutritional rehabilitation, offering both systemic and neurological benefits. However, further research is needed to validate these exploratory results and to establish their clinical relevance.

5 Conclusion

This study demonstrates that probiotic supplementation with *Lactobacillus plantarum* induces distinct, organ-specific modulation of IGF-binding proteins (IGFBPs) in malnourished mice. In the liver, the upregulation of IGFBP-3 and suppression of IGFBP-1 reflect enhanced stabilization of IGF-1 and reduced catabolic signalling, supporting systemic anabolic recovery. In the brain, the induction of IGFBP-5 and IGFBP-6 highlights a neurotrophic response, suggesting that probiotics contribute to neuronal survival, differentiation, and plasticity during nutritional rehabilitation.

These findings provide molecular evidence that probiotics act through the gut–liver–brain axis to coordinate metabolic and neurological recovery in malnutrition. While exploratory in nature, the results highlight the dual potential of probiotics as adjunctive agents: promoting growth restoration and enhancing cognitive resilience. Nevertheless, further validation through quantitative assays, functional outcome measurements, and clinical translation is necessary to establish the therapeutic utility of probiotics in combating malnutrition.

In conclusion, probiotics represent a promising, accessible, and low-cost intervention that may complement traditional nutritional strategies to improve both systemic and neurocognitive outcomes in malnourished populations.

The authors declare that there is no conflict of interest regarding the publication of this manuscript. The research was conducted independently, and no financial, personal, or professional relationships influenced the outcomes of this study.

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