Gut microbiota modulation via probiotics and its effects on nocturnal hypertension: a randomized controlled trial

Modulación del microbiota intestinal mediante probióticos y sus efectos sobre la hipertensión nocturna: un ensayo controlado aleatorizado

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Abstract

Nocturnal hypertension as an independent predictor of cardiovascular complications most often fails to respond well to conventional treatments. This randomized, double-blind, placebo-controlled clinical trial investigated the effect of multi-drug probiotic supplementing (Lactobacillus plantarum, Bifidobacterium lactis, and Lactobacillus acidophilus, combined 10^10 CFU) on gut microbiota modulation and nocturnal hypertension control in 116 non-dipping/rising pattern patients. At 12 weeks of therapy, the probiotic group had a considerable and clinically relevant reduction in nocturnal systolic blood pressure (-9.3 mmHg vs. -1.2 mmHg in placebo group; p<0.001) and prevalence of non-dipping pattern (25.9% vs. 79.3%). These alterations were linked with increased microbial richness, increased growth of butyrate-producing bacteria (+1.9%), increased Lactobacillus counts (+2.7%), and decreased inflammatory biomarkers (hs-CRP: -1.8 mg/L, IL-6: -2.1 pg/mL, TNF-α: -1.5 pg/mL; p<0.001). Multivariate regression modeling corroborated a significant negative association between increased beneficial bacteria and decreased nocturnal blood pressure. The findings show that probiotics are a safe and effective option for the targeted management of resistant nocturnal hypertension via modulation of the gut-vascular axis and systemic inflammation suppression.

Keywords: Probiotics, Nocturnal Hypertension, Gut Microbiota, Systemic Inflammation.

Resumen

La hipertensión nocturna, como predictor independiente de complicaciones cardiovasculares, suele no responder adecuadamente a los tratamientos convencionales. Este ensayo clínico aleatorizado, doble ciego y controlado con placebo investigó el efecto de la suplementación con probióticos multifármacos (Lactobacillus plantarum, Bifidobacterium lactis y Lactobacillus acidophilus, combinados 10^10 UFC) sobre la modulación del microbiota intestinal y el control de la hipertensión nocturna en 116 pacientes con patrón de no descenso/ascenso. A las 12 semanas de tratamiento, el grupo probiótico presentó una reducción considerable y clínicamente relevante de la presión arterial sistólica nocturna (-9,3 mmHg frente a -1,2 mmHg en el grupo placebo; p < 0,001) y la prevalencia del patrón de no descenso (25,9 % frente a 79,3 %). Estas alteraciones se relacionaron con una mayor riqueza microbiana, un mayor crecimiento de bacterias productoras de butirato (+1,9%), un mayor recuento de Lactobacillus (+2,7%) y una disminución de los biomarcadores inflamatorios (PCR-as: -1,8 mg/L, IL-6: -2,1 pg/mL, TNF-α: -1,5 pg/mL; p<0,001). El modelo de regresión multivariante corroboró una asociación negativa significativa entre el aumento de bacterias beneficiosas y la disminución de la presión arterial nocturna. Los hallazgos muestran que los probióticos son una opción segura y eficaz para el tratamiento específico de la hipertensión nocturna resistente mediante la modulación del eje intestino-vascular y la supresión de la inflamación sistémica.

Palabras clave: Probióticos, Hipertensión Nocturna, Microbiota Intestinal, Inflamación Sistémica.

Introduction

Hypertension is recognized as a main modifiable risk factor for cardiovascular disease and death globally. In the meantime, abnormal blood pressure patterns, especially nocturnal hypertension, marked by target organ damage and more adverse cardiovascular complications, have become a focus of growing interest among practitioners[1,2]. The condition not only acts as an independent predictor of cardiovascular events, but its control by employing traditional therapeutic approaches is generally challenging[3,4]. Gut-brain axis and the role of the gut microbiota in influencing body physiological systems, including the cardiovascular system, has been of interest to research workers in recent times. Cumulating evidence suggests that deterioration in the structure and function of the gut microbiota (dysbiosis) can contribute to hypertensive pathophysiology[5] and non-dipping nocturnal blood pressure profiles through various different mechanisms, such as the stimulation of inflammatory pathways, altered bile acid metabolism, and the production of vascular-acting microbial metabolites[6].

Probiotics, as live microbial preparations that provide health benefits when administered in sufficient numbers, have shown considerable potential in altering the balance and function of the gut microbiota[7]. This treatment can lead to restoration of balance to the microbiota, reduction in intestinal permeability, maintenance of systemic immune and inflammatory responses, and ultimately an improvement in vascular function[8]. However, the specific effect of probiotic treatments on blood pressure control during sleep has not been thoroughly examined, especially in the form of vigorous controlled trials[9]. Therefore, scientific and systematic studies on the potentiality of probiotics in manipulating gut microbiota and evaluating clinical effects of such manipulation on nocturnal blood pressure profile are of significant clinical and scientific importance. Such studies could be a first step towards the development of new and complementary strategies for targeted treatment of one of the resistant hypertension subtypes[10,11].

Nocturnal hypertension refers to an autonomous pathologic process of a disturbance of the normal dip in nocturnal blood pressure (non-dipping) or paradoxical increase of nocturnal blood pressure. It is associated with target organ injury, including left ventricular hypertrophy, kidney impairment, and increased arterial stiffness, and enhances stroke, heart failure, and cardiovascular mortality risks independently[12]. Its pathophysiologic processes are multifactorial and include autonomic nervous system dysregulation, chronic activation of the renin-angiotensin-aldosterone system, endothelial dysfunction, and systemic inflammation. The gut-heart-vascular axis is a

newly identified area of research that emphasizes the gut microbiota as a controller of blood pressure[13]. Proof suggests that the composition of gut microbiota in patients with primary and resistant hypertension is varied in changes, including reduced microbial diversity, a reduction in the Firmicutes/Bacteroidetes ratio, and reduced abundance of short-chain fatty acid-producing bacteria such as butyrate. These fatty acids work through their action on specific receptors (GPR41/GPR43) on endothelial and renal cells to cause vasodilation, renin-angiotensin system suppression, and sodium excretion[14,15]. Aside from that, gut dysbiosis is also implicated in the development of hypertension by increasing intestinal permeability, transporting bacterial endotoxins such as LPS into systemic circulation, and causing subclinical inflammation via the NF-κB and NLRP3 inflammasome pathways[16].

Probiotics have been shown to modulate blood pressure through several mechanisms including competition with pathobionts, enhancement of the barrier function of the intestinal epithelium, synthesis of protective metabolites (e.g., SCFA), inactivation of pressor peptides from food, modulation of the host immune system, and inhibition of oxidative stress[17,18]. Significant reductions in systolic and diastolic blood pressure after oral administration of some Lactobacillus and Bifidobacterium strains have been reported in animal studies. In humans, meta-analyses have also demonstrated modest but certain blood pressure reductions with the administration of several probiotics, though these effects have mainly referred to diurnal blood pressure[19]. Despite this data, the causal connection between probiotic-induced microbiota modulation and nocturnal blood pressure rhythms has not been well investigated. Less research has really measured the effect of probiotics on 24-hour ambulatory blood pressure (ABPM) parameters aimed at the nocturnal period[20], and the lack of consistency in selecting strains, dose, intervention duration, and approaches to microbiota assessment has limited the ability to draw an overall conclusion. This is a sign of the need for properly designed controlled trials to assess concomitantly changes in microbiota, markers of inflammation, and 24-h blood pressure profiles[21].

Materials and methods

Study Design

The trial was designed as a randomized, double-blind, placebo-controlled clinical trial. Follow-up of the participants was for a duration of 12 weeks. The study protocol was approved by the medical ethics committee of the university, and all the participants provided written informed consent.

Participant Selection and Allocation Criteria

The study population was adults aged 45 to 70 years with primary hypertension with a nocturnal non-dipping (nocturnal systolic blood pressure dip of less than 10%) or rising (nocturnal increase in blood pressure) pattern established by 24-hour ambulatory blood pressure monitoring twice. Reasons for exclusion included advanced cardiovascular disease, renal failure, uncontrolled diabetes, history of having taken antibiotics within the last three months, prior intestinal surgery, and daily probiotic or prebiotic supplement intake. After initial screening, participants to be enrolled were randomly assigned to two groups of control and intervention by a block randomization method.

Intervention and Placebo

The intervention group received one capsule daily of a combination multiple probiotic preparation of Lactobacillus plantarum, Bifidobacterium lactis, and Lactobacillus acidophilus strains (total 10^10 colony-forming units). The control group received an equivalent placebo capsule of microcrystalline cellulose. All capsules were strictly identical in color, taste, and texture. Treatment adherence was assessed by capsule counts and participants' daily entry.

Clinical and anthropometric measurements were documented at baseline and at study completion. Ambulatory blood pressure monitoring over 24 hours was completed with standardized, calibrated equipment at three points in time (pre-intervention, week 6, and termination of week 12). Nocturnal blood pressure data were examined specifically. Stool samples for gut microbiota were drawn at baseline and study completion and stored at -80°C. Microbiota structure was compared by next-generation sequencing of the 16S rRNA gene. ELISA was applied to determine serum levels of inflammatory markers including TNF-α, IL-6, and hs-CRP.

Statistical analysis

Data analysis was performed using the SPSS statistical software version 26. Independent t-test and repeated-measures ANOVA for intra- and intergroup comparisons were applied. Nonparametric data were compared with Mann-Whitney and Friedman tests. Multivariate linear regression was used to establish a stage where microbiota changes, inflammatory markers, and blood pressure parameters were related to each other. A stage of significance of 0.05 was set.

Results

The trial successfully randomized 120 eligible participants with confirmed nocturnal hypertension. Four subjects discontinued due to personal reasons unrelated to the intervention, resulting in 116 completers (58 per group) included in the final analysis. Both groups demonstrated comparable demographic and clinical characteristics at baseline, confirming effective randomization.

Table 1: Participant Flow

Phase	Probiotic	Placebo
Assessed	185	185
Randomized	60	60
Completed	58 (96.7%)	58 (96.7%)
Discontinued	2 (3.3%)	2 (3.3%)

A balanced distribution of baseline variables was observed between groups. Participants averaged 58 years of age with mean 24-hour systolic blood pressure of 141 mmHg and nocturnal systolic pressure of 134 mmHg, confirming the presence of the target pathology.

Table 2: Baseline Characteristics

Characteristic	Probiotic	Placebo	p-value
Age (years)	58.3±6.2	57.8±5.9	0.64
Male (%)	62.1	58.6	0.69
24-h SBP (mmHg)	142.3±8.7	140.9±9.1	0.38
Nocturnal SBP (mmHg)	134.6±7.9	133.2±8.3	0.34

Following the 12-week intervention, the probiotic group exhibited significant reductions in nocturnal blood pressure parameters compared to placebo. The mean reduction in nocturnal systolic pressure reached 9.3 mmHg, nearly eight times greater than observed in controls.

Table 3: Primary Outcome (Nocturnal BP Changes)

Parameter	Probiotic Δ	Placebo Δ	p-value
Nocturnal SBP (mmHg)	-9.3±3.1*	-1.2±2.4	<0.001
Nocturnal DBP (mmHg)	-5.1±2.2*	-0.8±1.9	<0.001
Non-dipping pattern (%)	25.9%*	79.3%	<0.001

Microbiological analyses revealed substantial shifts in gut microbiota composition exclusively in the probiotic group. We observed increased microbial diversity and enrichment of beneficial taxa, particularly butyrate-producing bacteria, which increased by nearly 2% relative abundance.

Table 4: Gut Microbiota Shifts

Microbiota Metric	Probiotic Δ	Placebo Δ	p-value
Shannon diversity	+0.38±0.12*	-0.05±0.08	<0.001
Lactobacillus (%)	+2.7±0.6*	+0.1±0.3	<0.001
Bacteroidetes/Firmicutes	+0.31±0.09*	-0.04±0.05	<0.001
Butyrate producers	+1.9±0.4*	+0.2±0.2	<0.001

Concomitant with these changes, significant attenuation of systemic inflammation markers occurred in the probiotic group. High-sensitivity CRP decreased by 1.8 mg/L, while pro-inflammatory cytokines IL-6 and TNF-α showed reductions exceeding 2 pg/mL and 1.5 pg/mL respectively.

Table 5: Inflammatory Markers

Biomarker	Probiotic Δ	Placebo Δ	p-value
hs-CRP (mg/L)	-1.8±0.5*	+0.2±0.3	<0.001
IL-6 (pg/mL)	-2.1±0.7*	+0.3±0.4	<0.001
TNF-α (pg/mL)	-1.5±0.4*	+0.1±0.2	<0.001

Multivariate regression modeling indicated that microbial and inflammatory changes significantly predicted nocturnal blood pressure improvements. Increases in *Lactobacillus* abundance and butyrate producers demonstrated the strongest inverse associations with systolic reductions, while baseline non-dipping status positively correlated with treatment responsiveness.

Table 6: Multivariate Regression for Nocturnal SBP Reduction

Predictor	β-coefficient	95% CI	p-value
Δ *Lactobacillus*	-0.41	-0.57 to -0.25	<0.001
Δ Butyrate producers	-0.33	-0.49 to -0.17	0.003
Δ hs-CRP	+0.28	+0.13 to +0.43	0.008
Baseline non-dipper	+0.19	+0.05 to +0.33	0.04

The intervention demonstrated excellent safety with no serious adverse events. Mild transient bloating was infrequently reported and did not differ significantly between groups (6.9% probiotic vs 3.4% placebo, p=0.47).

Discussion

The findings of the current research firmly confirm the modulating action of multimodal probiotics in the management of nocturnal hypertension. The 9.3 mmHg decrease in nocturnal systolic blood pressure in the probiotic group, eightfold greater than in the placebo group, is important on two grounds: firstly, it was highly statistically significant and secondly, it is clinically significant to a 10–15% reduction in risk of cardiovascular events. This change was also accompanied by a threefold reduction in the prevalence of the non-dipping pattern, demonstrating the effectiveness of the intervention to standardize the physiological rhythm of night-time blood pressure. This change in the dipping pattern has important clinical implications, as the non-dipping pattern is a predictor of cardiac death.

The mechanism underlying such effects can be looked for in structural and functional alterations in gut microbiota. Stool sample analysis showed that probiotic intake significantly enhanced microbial diversity and by 2.7% raised the abundance of the genus Lactobacillus

and by 1.9% the abundance of butyrate-producing bacteria. These microbial alterations are largely accountable for reducing blood pressure through two primary mechanisms: the production of protective metabolites such as short-chain fatty acids (SCFAs), which, by activating specific receptors (GPR41/43), cause vasodilation, downregulation of the renin-angiotensin system, and increased sodium excretion; and the enhancement of intestinal barrier integrity, which prevents the flow of inflammatory endotoxins such as LPS into the systemic circulation. Consistent with these results, the concurrent reduction of inflammatory markers like hs-CRP, IL-6, and TNF-α in the probiotic group, as well as the robust negative correlation of these results with nocturnal blood pressure improvement, confirm the etiologic role of inflammation in this pathophysiology.

The novel contribution of this research is the specific effectiveness of the intervention on nocturnal blood pressure, which is not usually responsive to conventional therapy. These findings affirm the hypothesis of the gut-brain axis effect on circadian regulation of blood pressure, with the probiotics seeming to achieve this through modulation of autonomic nervous system (ANS) activity, i.e., enhanced parasympathetic tone during night. Such a mechanism could explain why the reduction in blood pressure at night was so much larger in the present study than in previous studies of diurnal blood pressure.

Despite the robust study design of the trial, several limitations are to be considered. The 12-week intervention period may be too brief to measure the long-term maintenance of microbiota changes. In addition, measurement of microbial metabolites in feces rather than in systemic circulation is not an honest report of the bioavailability of such substances. In contrast, the inability to separate the specific effect of each individual probiotic strain used in the multi-faceted combination used is one of the issues setting the best factor. It is advisable that factorial experimental designs, direct measurement of circulating metabolites, and long-term follow-up studies be conducted to establish optimal strains. Additionally, examination of gender differences in response to the intervention and concurrent measurements of change in metabolomics and autonomic nervous system activity can reveal new features of this phenomenon.

Conclusions

This trial provides strong evidence that a multifaceted probiotic intervention can specifically affect the pathological nocturnal blood pressure pattern through multiple mechanisms. The clinically relevant reduction of nocturnal systolic blood pressure (mean 9.3 mmHg) and the normalization of the non-dipping pattern in three-quarters of subjects are evidence for the therapeutic effectiveness of this approach. The findings support the working hypothesis that probiotic therapy of the gut-vascular axis can potentially selectively augment non-responsiveness nocturnal blood pressure control disorders that are not responsive to standard treatments. On the basis of the intervention's beneficial safety and comparative cost-effectiveness, the therapeutic intervention could then be considered as an add-on in a Personalized Hypertension Management Protocol, especially in the case of those with a continued non-dipping phenomenon. The therapeutic potential of these findings may lead to the development of new paradigms for the prevention of cardiovascular complications secondary to resistant hypertension.

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