Research Article

The effect of a probiotic mixture combined with high-intensity interval training on the intestinal expression of FXR and PPAR-γ genes in diabetic rats

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Abstract

This study investigated the effects of high-intensity interval training (HIIT) and a multi-strain probiotic mixture, on the intestinal expression of FXR and PPAR-γ in a rat model of type 2 diabetes mellitus (T2DM). Forty male Wistar rats were randomly assigned to five groups (n=8): Healthy Control (HC), Diabetic Control (DC), Diabetic+HIIT (DH), Diabetic+Probiotic (DP), and Diabetic+HIIT+Probiotic (DHP). T2DM was induced via a single intraperitoneal injection of nicotinamide (95 mg/kg) followed by streptozotocin (STZ, 55 mg/kg). The HIIT protocol was performed on a rodent treadmill for 8 weeks (5 sessions/week). The probiotic mixture (Lactobacillus rhamnosus GG, Lactobacillus casei, Lactobacillus reuteri; 1×10^{10} CFU/mL each) was administered daily via oral gavage. Diabetes induction significantly downregulated the intestinal expression of both FXR and PPAR-γ compared to healthy controls ($p<0.001$). HIIT and probiotic interventions, individually, significantly increased the expression of both nuclear receptors compared to the diabetic control group ($p<0.001$). Notably, the combined HIIT and probiotic intervention (DHP) produced the highest expression levels of FXR and PPAR-γ, which were significantly greater than either intervention alone ($p<0.01$) and restored FXR expression to levels comparable to healthy controls. Both HIIT and multi-strain probiotic supplementation effectively upregulate the intestinal expression of FXR and PPAR-γ in diabetic rats, with the combination exerting a synergistic effect. These findings identify a novel mechanism by which lifestyle interventions may restore intestinal metabolic function and inter-organ communication in T2DM, highlighting the therapeutic potential of targeting the gut through combined exercise and probiotic strategies.

Key Words: Type 2 diabetes mellitus, High-intensity interval training, Probiotics, Gut-muscle axis, Organ crosstalk

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Introduction

Type 2 diabetes mellitus (T2DM) remains a global health challenge characterized by insulin resistance (Rana et al., 2026), chronic low-grade inflammation, and progressive pancreatic β-cell dysfunction (Zheng et al., 2018). While pharmacological interventions effectively manage hyperglycemia, they often fail to address the underlying multi-organ dysregulation that perpetuates the disease. This limitation has driven growing interest in non-pharmacological strategies capable of restoring metabolic homeostasis through integrated, multi-tissue mechanisms. The concept of organ crosstalk has emerged as a central paradigm in understanding both the pathophysiology of metabolic diseases and the therapeutic effects of lifestyle interventions. Exercise, in particular, induces the release of bioactive molecules from multiple organs–including myokines from skeletal muscle, adipokines from adipose tissue, and hepatokines from the liver–that collectively contribute to systemic metabolic regulation. Within this framework, the "gut-muscle axis" represents a critical bidirectional communication pathway linking intestinal health to skeletal muscle metabolism and systemic insulin sensitivity (Bindels & Delzenne, 2013; Przewłócka et al., 2020).

At the molecular level, intestinal nuclear receptors serve as key sensors of metabolic and microbial signals, translating them into transcriptional programs that regulate lipid metabolism, inflammation, and energy homeostasis. Two such receptors with emerging importance in T2DM are the Farnesoid X Receptor (FXR) and Peroxisome Proliferator-Activated Receptor Gamma (PPAR-γ). FXR, a bile acid-activated nuclear receptor highly expressed in the intestine and liver, plays a central role in maintaining metabolic homeostasis (Ding et al., 2015). Intestinal FXR activation regulates bile acid enterohepatic circulation, lipid metabolism, and glucose homeostasis. Emerging evidence indicates that FXR signaling in the gut influences insulin sensitivity in peripheral tissues, positioning as a critical node in organ crosstalk (Fang et al., 2015)

Dysregulation of FXR expression and signaling has been implicated in obesity, insulin resistance, and T2DM (González et al., 2019). PPAR-γ, a master regulator of adipogenesis and insulin sensitivity, is also expressed in intestinal epithelial cells where it modulates lipid metabolism, inflammation, and gut barrier function (Dubuquoy et al., 2006). Intestinal PPAR-γ activation has been shown to ameliorate insulin resistance and reduce systemic inflammation, partly through its effects on gut microbiota composition and intestinal permeability (Manoharan et al., 2021). The expression of PPAR-γ is typically reduced in metabolic disorders, contributing to impaired metabolic signaling.

High-intensity interval training (HIIT) has emerged as a time efficient exercise modality with potent effects on insulin sensitivity and metabolic health (Shirvani & Aslani, 2017; Lee et al., 2020). Beyond its well-characterized effects on skeletal muscle, HIIT exerts profound effects on intestinal health, including modulation of gut microbiota composition, enhancement of intestinal barrier function, and regulation of gut-derived signaling molecules (Mailing et al., 2019; Motiani et al., 2019). However, the effects of HIIT on intestinal nuclear receptor expression remain poorly characterized. Concurrently, probiotic supplementation has garnered attention for its ability to modulate the gut ecosystem and improve metabolic parameters in T2DM (Koutnikova et al., 2019). Multi-strain probiotics, in particular, may offer complementary benefits through what has been termed the "probiotic team effect," where different strains exert distinct yet synergistic effects on gut barrier integrity, immune modulation, and metabolite production. The selected strains—Lactobacillus rhamnosus GG, Lactobacillus casei, and Lactobacillus reuteri—are known to produce short-chain fatty acids (SCFAs) and modulate host gene expression, potentially influencing nuclear receptor signaling (Koh et al., 2016).

Despite growing recognition of the gut as a therapeutic target in metabolic disease, the combined effects of exercise and probiotics on intestinal FXR and PPAR-γ expression have not been investigated. Given the central roles of these nuclear receptors in integrating metabolic and microbial signals, understanding their modulation by lifestyle interventions may reveal novel mechanisms underlying the gut-muscle axis and organ crosstalk in T2DM.

Therefore, this study was designed to test the hypothesis that an 8-week HIIT program and multi-strain probiotic supplementation, individually and in combination, would upregulate intestinal FXR and PPAR-γ expression in a rat model of T2DM. We further hypothesized that the combined intervention would produce synergistic effects, reflecting enhanced restoration of intestinal metabolic signaling.

Materials and methods

Experimental design and animals

Forty 8-week-old male Wistar rats, weighing 200-220 grams at study commencement, were obtained and housed in the animal facility under standardized conditions (temperature: 22±2°C; humidity: 55%; 12-hour light/dark cycle). Animals had free access to standard pellet food and water throughout the experimental period. Following a one-week acclimatization period, rats were randomly assigned to five experimental groups (n=8 per group):

1. Healthy Control (HC): Non-diabetic, no intervention

2. Diabetic Control (DC): Diabetic, no intervention

3. Diabetic+HIIT (DH): Diabetic, subjected to HIIT protocol

4. Diabetic+Probiotic (DP): Diabetic, received probiotic mixture

5. Diabetic+HIIT+Probiotic (DHP): Diabetic, received both HIIT and probiotic interventions

All experimental procedures were approved by the institutional animal ethics committee and conducted in accordance with international guidelines for the care and use of laboratory animals.

Induction of type 2 diabetes

Type 2 diabetes was induced using a well-established nicotinamide-streptozotocin (STZ) model. Following an overnight fast, nicotinamide (95 mg/kg body weight) dissolved in normal saline was administered via intraperitoneal injection. Fifteen minutes later, freshly prepared STZ (55 mg/kg body weight) dissolved in 0.1 M citrate buffer (pH4.5) was injected intraperitoneally. One-week post-induction, diabetes was confirmed by measuring non-fasting blood glucose levels from the tail vein. Animals with blood glucose levels exceeding 200 mg/dL were classified as diabetic and included in the study.

High-intensity interval training protocol

Following a one-week familiarization period to treadmill running, an incremental exercise test to volitional exhaustion was conducted to establish baseline running capacity. This test was repeated bi-weekly to adjust training intensities according to the principle of progressive overload. The test protocol consisted of a 5-minute warm-up at 5 m/min, followed by incremental speed increases of 2 m/min every 2 minutes starting from 12 m/min until exhaustion (defined as the inability to resume running despite 10 seconds of gentle encouragement).

The 8-week HIIT program was performed five days per week during the afternoon (16:00-18:00) as previously described. Each 30-minute session comprised:

• 5-minute warm-up

• Five 2-minute high-intensity intervals (75-100% of estimated VO_2max)

• Five 1-minute active recovery intervals between high-intensity bouts

• 5-minute cool-down

Running speeds were progressively increased across the 8 weeks while maintaining constant session duration, as detailed in Table 1.

Probiotic preparation and administration

The probiotic strains—Lactobacillus rhamnosus GG (PTCC1637), Lactobacillus casei, and Lactobacillus reuteri—were obtained from the Microbial Sciences Research Center at Baqiyatallah University of Medical Sciences. Each strain was cultured anaerobically in de Man, Rogosa, and Sharpe (MRS) broth supplemented with 0.05% L-cysteine hydrochloride and incubated at 37°C for 24 hours. Bacterial cells were harvested by centrifugation, washed, and resuspended in sterile saline. A fresh multi-strain probiotic suspension was prepared daily at a final concentration of 1×10^9 CFU/mL per strain. The designated groups received 1 mL of this suspension via oral gavage daily, five days per week for eight weeks, administered between 08:00 and 10:00.

Tissue collection

Forty-eight hours after the final intervention session (to eliminate acute effects of the last exercise bout), rats were anesthetized following an overnight fast. The jejunum segment of the small intestine was rapidly excised, flushed with ice-cold sterile saline to remove luminal contents, snap-frozen in liquid nitrogen, and stored at -80°C until RNA extraction.

Gene expression analysis by qRT-PCR

Total RNA was isolated from jejunal tissue samples using a commercial extraction kit (Qiagen, Germany) according to the manufacturer's instructions. RNA concentration and purity were assessed spectrophotometrically (A260/A280 ratio between 1.8-2.0). Complementary DNA (cDNA) was synthesized from 1 µg of total RNA using a reverse transcription kit.

Quantitative real-time PCR (qRT-PCR) was performed using SYBR Green master mix on a real-time PCR detection system. Primer sequences for target genes (FXR/Nr1h4 and PPAR-γ) and the reference gene (GAPDH) were designed and validated (Table 2). Reactions were performed in duplicate for each sample. Relative gene expression levels were calculated using the 2^(-ΔΔCT) method, with GAPDH as the endogenous control and the Healthy Control group as the calibrator.

Statistical analysis

Data were analyzed using SPSS software (version 23, IBM Corp., Armonk, NY, USA). Normality of distribution was verified using the Shapiro-Wilk test, and homogeneity of variances was assessed using Levene's test. Group comparisons were performed using one-way analysis of variance (ANOVA), followed by Bonferroni-adjusted post hoc tests for multiple comparisons. Statistical significance was defined as $p<0.05$. Data are presented as mean ± standard deviation (SD).

Results

Intestinal FXR gene expression

Diabetes induction profoundly downregulated intestinal FXR expression. As shown in Figure 1A and Table 3, the Diabetic Control group exhibited approximately 72% lower FXR mRNA levels compared to the Healthy Control group (DC: 0.283 ± 0.062 vs. HC: 1.000±0.310; $p<0.001$).

Both HIIT and probiotic interventions individually significantly upregulated FXR expression compared to the diabetic control. The Diabetic+HIIT group showed a 7.6-fold increase relative to DC (DH: 2.166±0.634; $p<0.001$), while the Diabetic+Probiotic group demonstrated a 10.1-fold increase (DP: 2.853±0.775; $p< 0.001$). The difference between DH and DP groups did not reach statistical significance ($p=0.262$).

Remarkably, the combined HIIT and probiotic intervention (DHP) produced the highest FXR expression levels, with a 14.4-fold increase compared to DC (DHP: 4.087±0.807; $p<0.001$ vs. DC). The DHP group exhibited significantly higher FXR expression than both the DH ($p<0.001$) and DP ($p=0.002$) groups, indicating a synergistic effect of the combined intervention.

Table 1. High-intensity interval training (HIIT) protocol

Week	Warm-up (5 min)	High-Intensity Intervals (5 × 2 min)	Active Recovery (5 × 1 min)	Cool-down (5 min)
1	10 m/min	24-27 m/min	12 m/min	5 m/min
2	10 m/min	32-36 m/min	12 m/min	5 m/min
3	12 m/min	36-40 m/min	15 m/min	10 m/min
4	12 m/min	41-45 m/min	15 m/min	10 m/min
5	15 m/min	46-50 m/min	20 m/min	12 m/min
6	15 m/min	51-55 m/min	20 m/min	12 m/min
7	20 m/min	56-60 m/min	25 m/min	15 m/min
8	20 m/min	61-65 m/min	25 m/min	15 m/min

Table 2. Primer sequences used for qRT-PCR analysis.

Gene Name	Primer Sequence (5'⟶3')	Accession Number
FXR (Nr1h4)		NM_021745.2
Forward	CCACCATCCCAGAAGCACAT	
Reverse	AAATGGGAGGGCCCAATCAG	
PPAR-γ		NM_013124.3
Forward	TCGCTGATGCACTGCCTATG	
Reverse	ATAATAAGGCGGGGACGCAG	
GAPDH		NM_017008.4
Forward	CAAGTTCAACGGCACAGTCA	
Reverse	CCCCATTTGATGTTAGCGGG	

Table 3. Intestinal FXR and PPAR-γ mRNA Expression Across Experimental Groups.

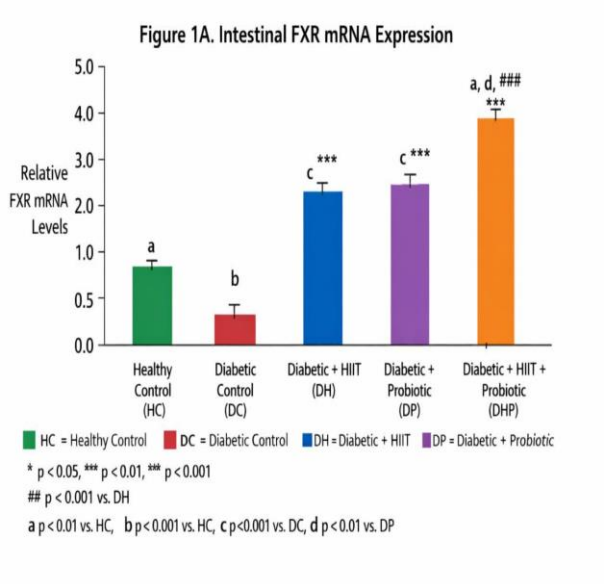
Group	FXR (Mean ± SD)	PPAR-γ (Mean ± SD)
Healthy Control (HC)	1.000 ± 0.310	1.000 ± 0.310
Diabetic Control (DC)	0.283 ± 0.062†††	0.358 ± 0.120†††
Diabetic+HIIT (DH)	2.166 ± 0.634***,††	2.237 ± 0.462***,††
Diabetic+Probiotic (DP)	2.853 ± 0.775***,††	2.997 ± 0.378***,††
Diabetic+HIIT+Probiotic (DHP)	4.087 ± 0.807***,###,§§	3.310 ± 0.938***,††,‡

intervention. Notably, FXR expression in the DHP group was not significantly different from the Healthy Control group (p=0.209), suggesting near-complete restoration.

Intestinal PPAR-γ gene expression

A similar pattern was observed for PPAR-γ expression. Diabetes significantly reduced PPAR-γ mRNA levels by approximately 64% compared to healthy controls (DC: 0.358±0.120 vs. HC: 1.000±0.310; p<0.001; Figure 1B).

HIIT and probiotic interventions each significantly upregulated PPAR-γ expression. The DH group exhibited a 6.3-fold increase relative to DC (DH: 2.237±0.462; p<0.001), while the DP group showed an 8.4-fold increase (DP: 2.997±0.378; p<0.001). The difference between DH and DP approached but did not reach statistical significance (p=0.060). The combined intervention (DHP) again produced the highest PPAR-γ expression levels, with a 9.3-fold increase compared to DC (DHP: 3.310±0.938; p <0.001). The DHP group showed significantly higher PPAR-γ expression than the DH group (p=0.002), but the difference compared to the DP group did not reach statistical significance (p=1.000). PPAR-γ expression in the DHP group remained significantly lower than the Healthy Control group (p<0.01), indicating partial but substantial restoration.



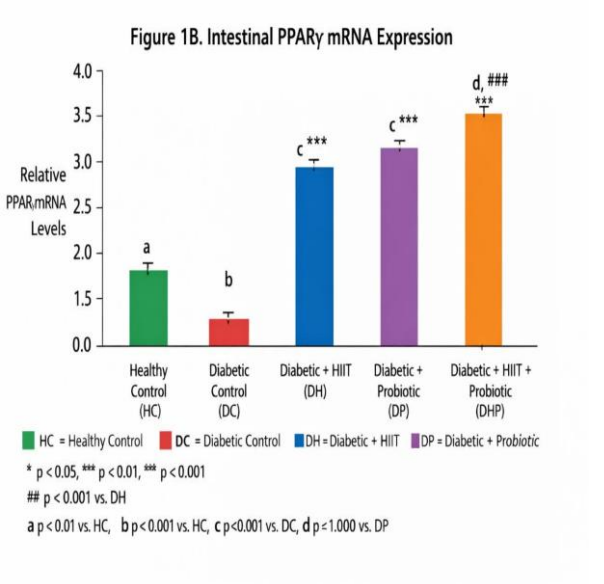


Figure 1. Relative mRNA expression of FXR (A) and PPAR-γ (B) in jejunal tissue across experimental groups. Gene expression was quantified using the 2^ΔΔCt method with GAPDH as the reference gene. Data are presented as mean ± SD. Statistical comparisons were performed using one-way ANOVA with Bonferroni-adjusted post hoc tests. *p < 0.05, **p < 0.01, ***p < 0.001.

Table 4. Summary of key statistical comparisons (p-values)

Comparison	FXR	PPAR-γ
HC vs. DC	< 0.001	< 0.001
DC vs. DH	< 0.001	< 0.001
DC vs. DP	< 0.001	< 0.001
DC vs. DHP	< 0.001	< 0.001
DH vs. DP	0.262	0.060
DH vs. DHP	< 0.001	0.002
DP vs. DHP	0.002	1.000
HC vs. DHP	0.209	0.002

Summary of statistical comparisons

One-way ANOVA revealed highly significant differences among groups for both FXR ($F(4,35)=51.467$, $p<0.001$) and PPAR-γ ($F(4,35)=47.998$, $p<0.001$). Post hoc comparisons with Bonferroni correction are summarized in Table 4.

Discussion

This study provides the first evidence that HIIT and multi-strain probiotic supplementation, both individually and particularly in combination, significantly upregulate the intestinal expression of FXR and PPAR-γ in a rat model of T2DM. These findings identify a novel molecular mechanism through which lifestyle interventions may restore intestinal metabolic function and enhance organ crosstalk in diabetes. The synergistic effect observed with combined intervention aligns with the growing understanding of how exercise-induced signals from multiple organs integrate to regulate metabolic health, contributing to the conceptual framework of exercise-mediated organ crosstalk.

Diabetes-induced downregulation of intestinal nuclear receptors

The marked downregulation of both FXR and PPAR-γ in the diabetic control group extends our understanding of intestinal pathophysiology in T2DM. FXR, as a bile acid sensor, plays a crucial role in maintaining metabolic homeostasis through regulation of lipid metabolism, glucose homeostasis, and inflammatory signaling (Ding et al., 2015). Reduced intestinal FXR expression in diabetes likely contributes to dysregulated bile acid signaling, impaired lipid absorption, and disrupted enterohepatic circulation, ultimately affecting systemic metabolism (González et al., 2019). Similarly, the observed reduction in PPAR-γ expression aligns with previous reports of decreased PPAR-γ signaling in metabolic disorders (Dubuquoy et al., 2006). Intestinal PPAR-γ deficiency has been associated with enhanced inflammatory responses, impaired barrier function, and altered gut microbiota composition (Manoharan et al., 2021). The concurrent downregulation of both nuclear receptors in diabetic rats suggests a coordinated disruption of intestinal metabolic sensing that may perpetuate systemic insulin resistance through impaired gut-liver and gut-muscle communication.

HIIT upregulates intestinal nuclear receptor expression

The significant upregulation of both FXR and PPAR-γ following HIIT training demonstrates that exercise exerts profound effects on intestinal gene expression beyond its well-characterized effects on skeletal muscle. This finding contributes to the growing recognition of exercise as a modulator of intestinal health and the gut-muscle axis (Mailing et al., 2019; Ticinesi et al., 2019).

Several mechanisms may underlie HIIT-induced upregulation of intestinal nuclear receptors. First, exercise increases splanchnic blood flow and reduces intestinal permeability, potentially creating a more favorable environment for nuclear receptor signaling (Keirns et al., 2020). Second, exercise-induced changes in gut microbiota composition and metabolic activity may generate microbial metabolites, including SCFAs and secondary bile acids, that serve as ligands or regulators of FXR and PPAR-γ (Koh et al., 2016). Third, exercise reduces systemic inflammation, which may relieve inflammatory suppression of nuclear receptor expression (Allen et al., 2017).

The magnitude of HIIT-induced FXR upregulation (7.6-fold) was particularly striking and exceeded that observed for PPAR-γ (6.3-fold). This differential response may reflect the sensitivity of FXR to exercise-induced changes in bile acid metabolism, as physical activity has been shown to alter bile acid pool size and composition (Meissner et al., 2011).

Probiotic supplementation enhances nuclear receptor expression

Probiotic supplementation alone produced robust upregulation of both FXR (10.1-fold) and PPAR-γ (8.4-fold), exceeding the effects of HIIT alone. This finding provides molecular evidence supporting the role of probiotics in modulating host gene expression through gut microbiota-host signaling. The selected probiotic strains—Lactobacillus rhamnosus GG, Lactobacillus casei, and Lactobacillus reuteri—are known to produce SCFAs, particularly butyrate and acetate, through fermentation of dietary substrates. SCFAs act as signaling molecules through G-protein coupled receptors (GPR41/43) on intestinal epithelial cells and can influence nuclear receptor expression and activity (Koh et al., 2016). Additionally, probiotics may modulate bile acid metabolism through bacterial bile salt hydrolase activity, altering the composition of the bile acid pool and consequently affecting FXR signaling (Wahlström et al., 2016).

The observed upregulation of PPAR-γ by probiotics is consistent with its role as a sensor of microbial signals and regulator of intestinal homeostasis. PPAR-γ activation by probiotics or their

metabolites have been shown to enhance tight junction protein expression, reduce inflammatory signaling, and improve barrier function. These effects may contribute to the systemic metabolic improvements associated with probiotic supplementation in T2DM.

Synergistic effects of combined HIIT and probiotic intervention

The most significant finding of this study is the synergistic interaction between HIIT and probiotics, most evident in FXR expression, where the combined intervention produced levels significantly exceeding either intervention alone and restored expression to values comparable to healthy controls. This synergy has important implications for understanding organ crosstalk and designing optimized lifestyle interventions for T2DM.The synergistic effect may arise from complementary and mutually reinforcing mechanisms. HIIT may "prime" the intestinal environment for enhanced probiotic efficacy by increasing gut perfusion, reducing oxidative stress, and modulating immune function (Mailing et al., 2019). Conversely, probiotics may enhance the intestinal adaptations to exercise by improving nutrient absorption, reducing endotoxemia, and providing SCFAs that support epithelial health (Moludi et al., 2020). The resulting improvement in intestinal barrier function and reduction in systemic inflammation may create a positive feedback loop that amplifies the effects of both interventions.

For FXR specifically, the synergy may involve enhanced production of secondary bile acids by probiotic bacteria, combined with exercise-induced changes in bile acid enterohepatic circulation. The near-complete normalization of FXR expression in the combined group suggests that this intervention effectively reversed the diabetes-induced suppression of this critical metabolic regulator. For PPAR-γ, although the combined intervention produced the highest expression levels, the difference from probiotic alone did not reach statistical significance. This may indicate that PPAR-γ expression reaches a physiological ceiling with probiotic supplementation alone, or that the synergistic mechanisms preferentially target FXR signaling. Nevertheless, the significant improvement over HIIT alone supports the value of combined intervention.

Implications for organ crosstalk and the gut-muscle axis

These findings extend our understanding of the gut-muscle axis by identifying intestinal nuclear receptors as molecular targets through which lifestyle interventions may influence systemic metabolism. FXR and PPAR-γ integrate signals from diet, microbiota, and host metabolism to regulate intestinal function, and their expression levels likely influence the production of gut-derived factors that affect skeletal muscle insulin sensitivity.

The upregulation of these nuclear receptors by HIIT and probiotics may enhance the gut's capacity to produce beneficial signaling molecules, including GLP-1, peptide YY, and fibroblast growth factor 19 (FGF19), which communicate with distant organs including muscle, liver, and adipose tissue (Przewłócka et al., 2020). This aligns with the emerging paradigm of exercise-mediated organ crosstalk, where physical activity induces the release of peptides and proteins from multiple organs that collectively contribute to systemic metabolic regulation.

Furthermore, the synergistic effects observed here complement previous findings on GLP-1 and DPP-4 modulation in similar experimental models, suggesting that combined HIIT and probiotics broadly restore intestinal endocrine function and metabolic signaling. Together, these studies support the concept that targeting the gut through integrated lifestyle interventions may produce multi-level benefits for T2DM management.

Limitations and future directions

Several limitations of this study should be acknowledged. First, gene expression analysis was limited to mRNA levels; confirmation at the protein level and assessment of receptor activity would strengthen the conclusions. Second, the jejunum was selected for analysis, but regional differences along the intestinal tract may exist. Third, the study did not directly assess bile acid composition, SCFA levels, or microbiota composition, which would provide mechanistic insight into the observed gene expression changes. Fourth, while the sample size was adequate for detecting large effects, smaller but potentially meaningful differences (e.g., PPAR-γ between DP and DHP) may have been missed due to limited statistical power.

Future studies should investigate the functional consequences of FXR and PPAR-γ upregulation, including effects on intestinal permeability, inflammatory markers, and metabolic outcomes. Direct measurement of bile acid profiles and microbial metabolites would clarify the mechanisms underlying nuclear receptor modulation. Finally, translational studies in humans with T2DM are needed to determine whether similar effects occur with combined exercise and probiotic interventions.

Conclusion

This study demonstrates that both HIIT and multi-strain probiotic supplementation effectively upregulate the intestinal expression of the nuclear receptors FXR and PPAR-γ in a rat model of T2DM. The combination of HIIT and probiotics produces synergistic effects, particularly for FXR, restoring expression to levels comparable to healthy controls. These findings identify a novel mechanism by which lifestyle interventions may restore intestinal metabolic function and enhance organ crosstalk in diabetes. The results support the therapeutic potential of

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What is already known on this subject?

The intestinal nuclear receptors FXR and PPAR-γ are critical regulators of metabolic homeostasis, and their expression is often downregulated in metabolic disorders like type 2 diabetes mellitus (T2DM).

High-intensity interval training (HIIT) and multi-strain probiotic supplementation are known to independently improve metabolic health and insulin sensitivity.

The "gut-muscle axis" is a key pathway for organ crosstalk, where intestinal health influences skeletal muscle metabolism and systemic insulin sensitivity.

What this study adds?

This study provides the first evidence that both HIIT and multi-strain probiotic supplementation individually upregulate the intestinal expression of FXR and PPAR-γ in a rat model of T2DM.

It demonstrates that a combined intervention of HIIT and probiotics produces a synergistic effect, leading to significantly greater upregulation of these nuclear receptors than either intervention alone.

The findings reveal a novel molecular mechanism—restoration of intestinal nuclear receptor signaling—through which combined exercise and probiotic strategies may enhance organ crosstalk and improve metabolic function in T2DM.

Organ Cross-Talk Tips:

- Combining HIIT and probiotics synergistically restores intestinal FXR and PPAR-γ expression in diabetic rats, revealing a novel mechanism for enhancing gut-muscle crosstalk and metabolic homeostasis.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Compliance with ethical standards

Conflict of interest the authors declare that there is no conflict of interest in the present research.

Ethical approval All experimental procedures were approved by the institutional animal ethics committee and conducted in accordance with international guidelines for the care and use of laboratory animals.

Informed consent Animal study.

Author contributions

Conceptualization: T.J., Methodology: H.P., Software: T.J, H.P., Validation: T.J, H.P.,; Formal analysis: T.J, H.P.,; Investigation: T.J, H.P.,; Resources: T.J, H.P.,; Data curation: T.J, H.P.,; Writing - original draft: T.J, H.P.,; Writing–review & editing T.J, H.P, H.P.,; Visualization: T.J, H.P.,; Supervision: H.P.; Project administration: T.J, H.P.,.; Funding acquisition: T.J.

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