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Tuna Bone Collagen Peptides Modulate Insulin Sensitivity via PI3K/AKT/GLUT4 Pathway in HepG2 Cells

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Abstract: ***Background*: Insulin resistance is a key metabolic disorder associated with type 2 diabetes and cardiovascular diseases, presenting significant challenges in clinical management. This study explores the potential of tuna bone collagen peptides (TBCP) to enhance insulin sensitivity in HepG2 cells through modulation of the PI3K/AKT/GLUT4 signaling pathway. *Methods*: HepG2 cells were treated with varying concentrations of TBCP, and cell viability, glucose uptake, and the expression levels of key proteins and genes related to glucose metabolism were assessed. *Results*: TBCP significantly enhanced insulin sensitivity by improving cell viability, promoting glucose uptake, and modulating the expression of PI3K, AKT, and GLUT4. Notably, TBCP upregulated the PI3K/AKT/GLUT4 pathway, indicating its role in enhancing insulin signaling. *Conclusion*: The findings suggest that TBCP could serve as a novel therapeutic agent for managing insulin resistance. This study provides valuable insights into the potential of marine-derived collagen peptides for improving metabolic health and supports the development of functional foods or therapeutics targeting insulin resistance.***

Keywords: Tuna Bone Collagen Peptides, HepG2 Cells, PI3K/AKT/GLUT4 Pathway, Insulin Resistance, Type 2 Diabetes.

1. Introduction

Insulin resistance, a critical metabolic disorder characterized by reduced sensitivity to insulin, is a significant contributor to the development of type 2 diabetes (T2DM). This condition not only impedes glucose uptake and utilization but also exacerbates cardiovascular risks [1-3]. Current treatments for insulin resistance include lifestyle modifications, oral hypoglycemic agents, and insulin therapy. However, these approaches often face limitations such as side effects, cost, and patient compliance issues [4, 5]. There is, therefore, a pressing need for novel therapeutic strategies that are safer, more effective, and better tolerated.

Traditional Chinese Medicine (TCM) has been utilized for centuries to treat various ailments, including metabolic disorders. The use of herbal medicines in TCM often involves complex formulations aimed at restoring balance and enhancing bodily functions [6]. Recent studies have shown promising results in using TCM to treat insulin resistance, leveraging its multitargeted and holistic approach [7-9]. Collagen peptides, derived from the partial hydrolysis of collagen, are known for their diverse biological activities, including anti-inflammatory, antioxidative, and anti-aging properties [10]. Their potential in managing metabolic disorders, including insulin resistance, has been increasingly recognized. Collagen peptides have been shown to modulate glucose and lipid metabolism, thereby improving insulin sensitivity [11]. This makes them promising candidates for the development of functional foods or therapeutic agents targeting metabolic syndromes.

Tuna, a widely consumed fish, offers not only a rich source of protein and omega-3 fatty acids but also a potential source of collagen peptides through its bones [12]. The recycling of tuna bones presents an environmentally sustainable and economically viable approach to obtaining bioactive compounds [13]. By extracting collagen peptides from these bones, the seafood industry can add value to an otherwise underutilized byproduct while contributing to the development of health-promoting products. The PI3K/AKT/GLUT4 pathway is a central regulator of glucose homeostasis, playing a pivotal role in insulin signaling and glucose uptake [14]. Activation of this pathway leads to the translocation of GLUT4 to the cell membrane, enhancing glucose entry and utilization [15]. Targeting this pathway has emerged as a key strategy in the treatment of diabetes, with several pharmacological agents being developed to modulate its components [16]. Given the potential of collagen peptides in managing insulin resistance and the importance of the PI3K/AKT/GLUT4 pathway, this study aimed to investigate the effects of tuna bone collagen peptides (TBCP) on insulin sensitivity in HepG2 cells. Specifically, we sought to explore how TBCP modulates the PI3K/AKT/GLUT4 signaling pathway to enhance insulin sensitivity. Our findings could provide valuable insights into the mechanisms by which collagen peptides exert their hypoglycemic effects and contribute to the development of novel therapeutic strategies for insulin resistance. This introduction sets the stage for a detailed exploration of TBCP's effects on insulin sensitivity, drawing on established knowledge of insulin resistance, traditional Chinese medicine, and the role of the PI3K/AKT/GLUT4 pathway in glucose metabolism. By connecting these elements, we aim to provide a comprehensive understanding of TBCP's potential as a therapeutic agent for insulin resistance.

2. Material and Methods

2.1 Materials and Reagents

Tuna bone collagen peptides (TBCP) were obtained from [Ningbo Meishan International Cold Chain Co., LTD, Ningbo, China]. Human hepatocellular carcinoma HepG2 cells were purchased from [Shanghai Institute of Cell Biology, Shanghai, China]. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), and penicillin–streptomycin were purchased from Gibco (Thermo Fisher Scientific, USA). Insulin, MTT, and dimethyl sulfoxide (DMSO) were obtained from Solarbio Life Science (Solarbio, Beijing, China). Commercial assay kits for glucose, total cholesterol (TC), triglycerides (TG), pyruvate kinase (PK), hexokinase (HK), superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), reduced glutathione (GSH), glycogen, and α-glucosidase (α-GC) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Antibodies against PI3K, phosphorylated AKT (p-AKT), GLUT4, and β-actin were purchased from ABclonal Technology (Wuhan, China).

2.2 Cell Culture and Induction of Insulin Resistance

HepG2 cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin–streptomycin at 37 ℃ in a humidified atmosphere containing 5% CO_2. To establish insulin resistance, cells at approximately 80% confluence were exposed to 10 mM insulin for 18 h. After induction, the culture medium was replaced with fresh medium containing various concentrations of TBCP as described in the following assays

2.3 Assessment of TBCP Cytotoxicity in HepG2 Cells Using the MTT Assay

HepG2 cells in the logarithmic growth phase were seeded into 96-well plates and cultured under standard conditions. After cell attachment, TBCP was added at final concentrations ranging from 0 to 250 μg/mL, and cells were incubated for 12, 24, 36, or 48 h. Subsequently, 10 μL of MTT solution was added to each well and incubated for an additional 4 h. The medium was removed, and the resulting formazan crystals were dissolved in 100 μL of DMSO. Absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated relative to the untreated control group to assess the cytotoxicity of TBCP toward HepG2 cells.

2.4 Evaluation of Glucose Utilization in Insulin-resistant (IR) HepG2 Cells Treated with TBCP

HepG2 cells were induced to develop insulin resistance by exposure to 10 mM insulin for 18 h. After induction, the culture medium was replaced, and cells were treated with TBCP at final concentrations of 0, 50, 100, 150, and 200 μg/mL for 48 h. The supernatant was collected, and glucose concentrations were determined using a commercial glucose assay kit. Glucose consumption was calculated by subtracting the residual glucose concentration from that of the blank control. Data were expressed relative to the insulin-resistant control group to evaluate the effects of TBCP on glucose utilization in insulin-resistant HepG2 cells.

2.5 Determination of Intracellular Lipid Levels in IR-HepG2 Cells Treated with TBCP

Following insulin-resistance induction, HepG2 cells were treated with TBCP at 0, 50, 100, and 150 μg/mL for 48 h. Cells were collected and lysed by ultrasonic disruption. Intracellular total cholesterol (TC) and triglyceride (TG) levels were determined using commercial assay kits according to the manufacturer's protocols. Absorbance was measured at 500 nm using a microplate reader. TC and TG contents were calculated from standard curves and expressed relative to the insulin-resistant control group to assess the effects of TBCP on lipid accumulation.

2.6 Determination of Glycolytic Enzyme Activities in IR-HepG2 Cells Treated with TBCP

After insulin-resistance induction, HepG2 cells were treated with TBCP at 0, 50, 100, and 150 μg/mL for 48 h. Cells were collected, lysed by ultrasonic disruption, and the activities of pyruvate kinase (PK) and hexokinase (HK) were determined using commercial kits according to the manufacturer's instructions. Absorbance was recorded using a microplate reader, and enzyme activities were calculated from standard curves. Results were expressed relative to the insulin-resistant control group to evaluate the regulatory effects of TBCP on glycolytic enzymes.

2.7 Determination of Oxidative Stress Markers in IR-HepG2 Cells Treated with TBCP

Insulin-resistant HepG2 cells were treated with TBCP at 0, 50, 100, and 150 μg/mL for 48 h. After treatment, cells were collected and lysed by ultrasonic disruption. The activities of superoxide dismutase (SOD) and catalase (CAT), and the levels of malondialdehyde (MDA) and reduced glutathione (GSH), were determined using commercial assay kits. Absorbance was measured at the appropriate wavelengths using a microplate reader. Data were normalized to total protein content and expressed relative to the insulin-resistant control group to evaluate the antioxidant effects of TBCP.

2.8 Determination of Glycogen Content and α-glucosidase Activity in IR-HepG2 Cells Treated with TBCP

HepG2 cells were induced to develop insulin resistance and subsequently treated with TBCP at 0, 50, 100, and 150 μg/mL for 48 h. After incubation, cells were collected and lysed by ultrasonic disruption. Intracellular glycogen content and α-glucosidase (α-GC) activity were determined using commercial kits. Absorbance was measured at the specified wavelength with a microplate reader. Results were normalized to total protein and expressed relative to the insulin-resistant control group to assess the influence of TBCP on glucose metabolism.

2.9 Quantitative Analysis of PI3K, AKT, and GLUT4 Gene Expression in IR-HepG2 Cells Treated with TBCP

Following induction of insulin resistance, HepG2 cells were treated with TBCP (0–150 μg/mL) for 48 h. Total RNA was extracted using a commercial RNA isolation kit, and cDNA was synthesized by reverse transcription. The relative mRNA

expression levels of phosphoinositide 3-kinase (PI3K), protein kinase B (AKT), and glucose transporter 4 (GLUT4) were quantified by real-time quantitative PCR (qPCR). β-actin was used as the internal control, and relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method to assess the effects of TBCP on insulin signaling.

2.10 Western Blot Analysis of PI3K, p-AKT, and GLUT4 Protein Expression in Insulin-resistant HepG2 Cells Treated with TBCP

After insulin-resistance induction, HepG2 cells were treated with TBCP (0–150 μg/mL) for 48 h. Cells were collected and lysed in RIPA buffer, and total proteins were separated by SDS–PAGE and transferred onto nitrocellulose (NC) membranes. The membranes were blocked and incubated overnight at 4 °C with primary antibodies against PI3K, phosphorylated AKT (p-AKT), GLUT4, and β-actin (ABclonal, 1:2000). After washing with TBST, membranes were incubated with HRP-conjugated secondary antibodies (ABclonal, 1:2000) for 2 h at room temperature. Protein bands were visualized using an enhanced chemiluminescence (ECL) detection system, and band intensities were quantified to determine the effects of TBCP on insulin signaling proteins.

2.11 Statistical Analysis

Data are presented as the mean ± standard deviation (SD) from at least three independent experiments. Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for comparisons between multiple groups. Differences were considered significant at $p < 0.05$. All statistical analyses were performed using GraphPad Prism (version X, GraphPad Software, USA).

3. Result

3.1 Time- and Concentration-Dependent Cytotoxicity of TBCP in HepG2 Cells

The cytotoxicity of TBCP in HepG2 hepatocellular carcinoma cells was assessed at 12, 24, 36, and 48 hours following treatment with increasing concentrations (0–250 μg/mL). Cell viability was expressed as a percentage of untreated controls. At 12, 24, and 36 hours (Figure 1A–C), no significant differences in viability were observed across all concentrations, indicating minimal cytotoxicity within the first 36 hours of exposure. In contrast, after 48 hours (Figure 1D), cell viability significantly decreased at 250 μg/mL ($p < 0.05$), suggesting a concentration- and time-dependent cytotoxic effect. Overall, TBCP displayed negligible short-term toxicity but induced measurable cell damage upon prolonged, high-dose exposure, defining a temporal threshold for its hepatocellular safety profile.

3.2 Time- and Dose-Dependent Hypoglycemic Effects of TBCP in IR-HepG2 Cells

The hypoglycemic activity of TBCP was assessed in IR-HepG2 cells. Cells were treated with TBCP at 0–200 μg/mL, and glucose consumption was measured at 12, 24, 36, and 48 hours. At 12 and 24 hours (Figure 2A–B), TBCP had no significant effect on glucose utilization compared with the IR group. After 36 hours (Figure 2C), glucose consumption significantly increased at 150 and 200 μg/mL (*$p < 0.05$), indicating the onset of a dose-dependent effect. At 48 hours (Figure 2D), TBCP markedly enhanced glucose uptake in a concentration-dependent manner, with significant increases observed at ≥50 μg/mL (*$p < 0.05$, **$p < 0.01$). These findings demonstrate that TBCP improves glucose metabolism in IR-HepG2 cells in a time- and dose-dependent manner, suggesting its potential to enhance insulin sensitivity and support glucose homeostasis under insulin-resistant conditions.

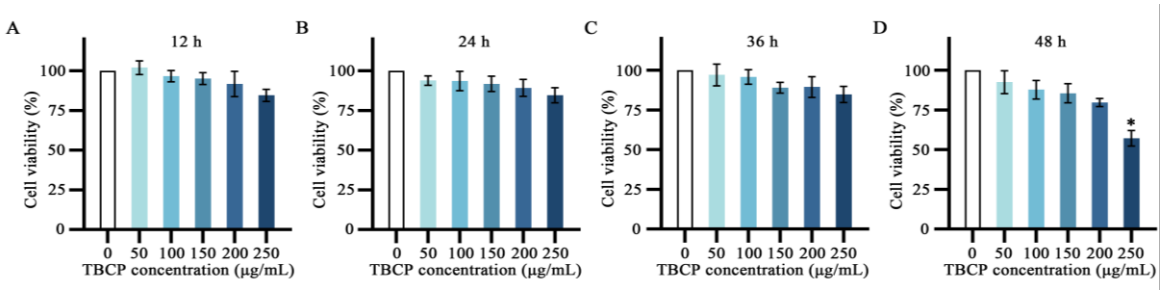

Figure 1: Cytotoxic effects of tuna bone collagen peptides (TBCP) on HepG2 cells at different exposure times. (A) 12 h, (B) 24 h, (C) 36 h, and (D) 48 h after treatment. Cell viability was measured using the control (0 μg/mL) group as reference. *$p < 0.05$, **$p < 0.01$ vs. 0 group.

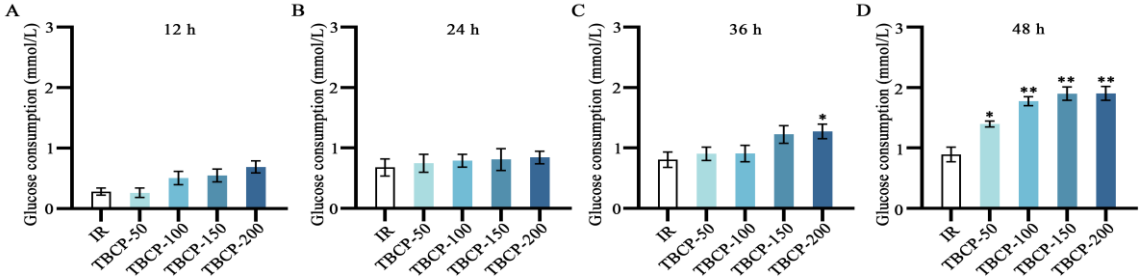

Figure 2: Effects of tuna bone collagen peptides (TBCP) on glucose consumption in insulin-resistant HepG2 (IR-HepG2) cells at different exposure times. (A) 12 h, (B) 24 h, (C) 36 h, and (D) 48 h after treatment with TBCP (0–200 μg/mL). Data are presented as mean ± SD (n = 3). *$p < 0.05$, **$p < 0.01$ vs. IR group.

3.3 TBCP Improves Lipid Metabolism in IR-HepG2 Cells

The effects of TBCP on lipid metabolism were evaluated by measuring TC and TG levels in IR-HepG2 cells treated with 0–150 μg/mL TBCP. As shown in Figure 3A, TC levels were significantly reduced at 100 and 150 μg/mL (**$p < 0.01$), indicating that TBCP effectively lowers cholesterol under insulin-resistant conditions. Similarly, TG levels (Figure 3B) decreased significantly at ≥50 μg/mL (*$p < 0.05$, **$p < 0.01$), demonstrating a concentration-dependent improvement in lipid metabolism. Collectively, these results suggest that TBCP ameliorates dyslipidemia by reducing both TC and TG levels in IR-HepG2 cells, highlighting its potential as a functional ingredient or therapeutic agent for managing lipid abnormalities associated with insulin resistance.

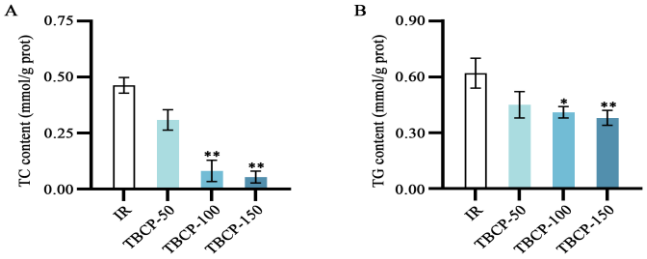

Figure 3: Effects of tuna bone collagen peptides (TBCP) on lipid metabolism in insulin-resistant HepG2 (IR-HepG2) cells. (A) Total cholesterol (TC) and (B) triglyceride (TG) levels after treatment with TBCP (0–150 μg/mL). Data are presented as mean ± SD (n = 3). *$p < 0.05$, **$p < 0.01$ vs. IR group.

3.4 TBCP Enhances Glycolytic Enzyme Activities in IR-HepG2 Cells

The effects of TBCP on key glycolytic enzymes were IR-HepG2 cells. Cells were treated with TBCP (0–150 μg/mL), and the activities of PK and HK were measured. As shown in Figure 4A, PK activity significantly increased at 100 and 150 μg/mL (**$p < 0.01$), indicating enhanced glycolytic flux. Similarly, HK activity (Figure 4B) was elevated in a concentration-dependent manner, with significant increases at 50 μg/mL (*$p < 0.05$) and greater effects at 100 and 150 μg/mL (**$p < 0.01$). These results suggest that TBCP enhances glucose utilization by upregulating PK and HK activities, thereby improving glycolytic efficiency under insulin-resistant conditions. Collectively, TBCP may exert its hypoglycemic effect through activation of key enzymes involved in glucose metabolism, supporting its potential as a natural modulator of insulin sensitivity.

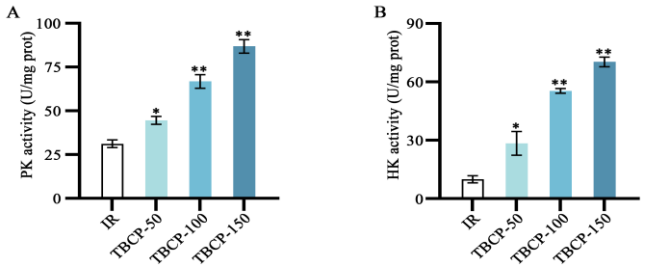

Figure 4: Effects of tuna bone collagen peptides (TBCP) on glycolytic enzyme activities in insulin-resistant HepG2 (IR-HepG2) cells. (A) Pyruvate kinase (PK) and (B) hexokinase (HK) activities after treatment with TBCP (0–150 μg/mL). Data are presented as mean ± SD (n = 3). *$p < 0.05$, **$p < 0.01$ vs. IR group.

3.5 TBCP Alleviates Oxidative Stress in IR-HepG2 Cells

The effects of TBCP on oxidative stress were evaluated in IR-HepG2 cells. Cells were treated with TBCP (0–150 μg/mL), and the activities of SOD, CAT, GSH content, and MDA levels were measured. As shown in Figure 5A–C, TBCP significantly increased SOD, CAT, and GSH levels at 100 and 150 μg/mL (**$p < 0.01$), indicating enhanced antioxidant capacity. Conversely, MDA content was markedly reduced at the same concentrations (**$p < 0.01$; Figure 5D), suggesting decreased lipid peroxidation. Collectively, these results demonstrate that TBCP alleviates oxidative stress by strengthening cellular antioxidant defenses and reducing oxidative damage in IR-HepG2 cells, thereby contributing to improved insulin sensitivity under oxidative stress conditions.

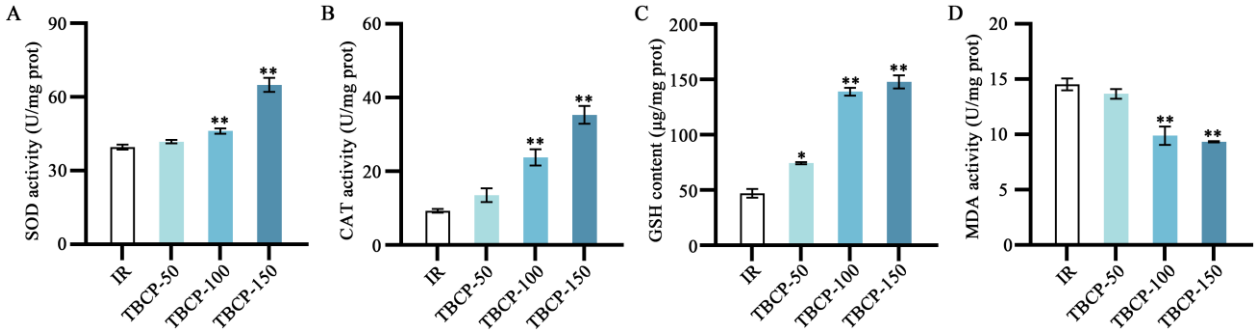

Figure 5: Effects of tuna bone collagen peptides (TBCP) on oxidative stress markers in insulin-resistant HepG2 (IR-HepG2) cells. (A) Superoxide dismutase (SOD) activity, (B) catalase (CAT) activity, (C) glutathione (GSH) content, and (D) malondialdehyde (MDA) levels after treatment with TBCP (0–150 μg/mL). Data are presented as mean ± SD (n = 3). *$p < 0.05$, **$p < 0.01$ vs. IR group.

3.6 TBCP Regulates Glucose Metabolism in IR-HepG2 Cells

The effects of TBCP on glucose metabolism were evaluated in IR-HepG2 cells. Cells were treated with TBCP (0–150 μg/mL), and glycogen content and α-glucosidase (α-GC) activity were measured. As shown in Figure 6A, TBCP significantly increased glycogen content at 100 and 150 μg/mL (**$p < 0.01$), indicating enhanced glucose storage. Conversely, α-GC activity was markedly reduced at the same concentrations (**$p < 0.01$; Figure 6B), suggesting inhibition of glycogen breakdown. These results demonstrate that TBCP improves glucose homeostasis in IR-HepG2 cells by promoting glycogen synthesis and suppressing its degradation.

Collectively, TBCP may enhance insulin sensitivity and exert hypoglycemic effects through dual modulation of glucose synthesis and breakdown pathways.

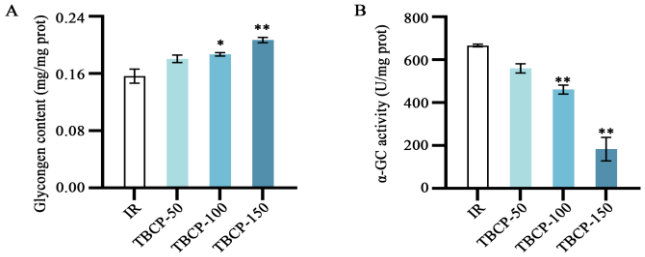

Figure 6: Effects of tuna bone collagen peptides (TBCP) on glucose metabolism in insulin-resistant HepG2 (IR-HepG2) cells. (A) Glycogen content and (B) α-glucosidase (α-GC) activity after treatment with TBCP (0–150 μg/mL). Data are presented as mean ± SD (n = 3). *$p < 0.05$, **$p < 0.01$ vs. IR group.

3.7 TBCP Activates the PI3K/AKT/GLUT4 Signaling Pathway in IR-HepG2 Cells

The effects of TBCP on the expression of key genes involved in glucose metabolism were examined in IR-HepG2 cells. The study focused on PI3K, AKT, and GLUT4, which are critical components of the insulin signaling pathway and glucose uptake. Cells were treated with TBCP at 0, 50, 100, and 150 μg/mL, and mRNA levels were quantified relative to the insulin-resistant control group. As shown in Figure 7A, TBCP significantly increased PI3K expression at 100 and 150 μg/mL (**$p < 0.01$), suggesting activation of the upstream insulin signaling cascade. Similarly, AKT mRNA levels (Figure 7B) were markedly elevated at the same concentrations (**$p < 0.01$), indicating enhanced downstream signal transduction within the PI3K/AKT pathway. These findings imply that TBCP facilitates insulin-mediated glucose metabolism by promoting the transcriptional activation of key signaling molecules. Furthermore, TBCP significantly upregulated GLUT4 expression (Figure 7C) at all tested concentrations (**$p < 0.01$). Given that GLUT4 mediates glucose transport across the cell membrane, its increased expression reflects an improved cellular capacity for glucose uptake under insulin-resistant conditions. This effect highlights a direct role of TBCP in restoring glucose utilization, which is typically impaired in metabolic disorders associated with insulin resistance. Collectively, these results demonstrate that TBCP enhances the expression of genes essential for insulin signaling and glucose transport in IR-HepG2 cells. By activating the PI3K/AKT/GLUT4 axis, TBCP improves glucose homeostasis and may alleviate hyperglycemia associated with insulin resistance. These findings provide a molecular basis for the hypoglycemic activity of TBCP and support its potential as a natural modulator of insulin sensitivity and glucose metabolism.

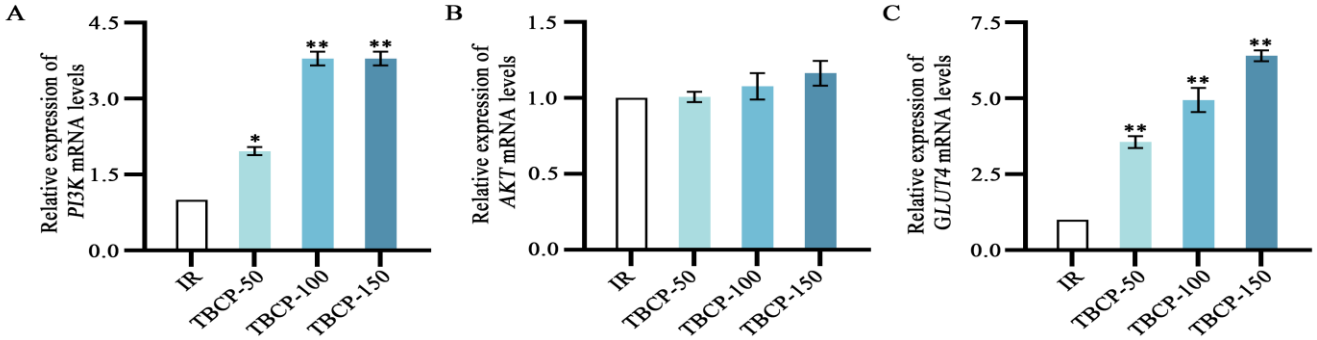

Figure 7: Effects of tuna bone collagen peptides (TBCP) on the expression of glucose metabolism–related genes in insulin-resistant HepG2 (IR-HepG2) cells. (A) PI3K, (B) AKT, and (C) GLUT4 mRNA expression after treatment with TBCP (0–150 μg/mL). Data are presented as mean ± SD (n = 3). *$p < 0.05$, **$p < 0.01$ vs. IR group.

3.8: Activation of Insulin Signaling Proteins by TBCP in IR-HepG2 Cells

The effects of TBCP on the expression of key proteins involved in glucose metabolism were investigated in insulin-resistant HepG2 (IR-HepG2) cells. The study focused on PI3K, p-AKT, and GLUT4, critical regulators of insulin signaling and glucose uptake. Cells were treated with TBCP at 0, 50, 100, and 150 μg/mL, and protein expression levels were analyzed by Western blotting. As shown in Figure 8A and B, TBCP significantly increased PI3K protein expression at 100 and 150 μg/mL (**$p<0.01$), indicating activation of the upstream insulin signaling cascade. Similarly, p-AKT levels (Figure 8A and C) were markedly elevated at the same concentrations (**$p<0.01$), suggesting enhanced downstream signaling that promotes glucose utilization. These results indicate that TBCP enhances insulin-mediated signal transduction through the PI3K/AKT pathway, a key axis regulating glucose homeostasis. Moreover, TBCP significantly upregulated GLUT4 expression (Figure 8A and D) at 50, 100, and 150 μg/mL (**$p<0.01$). As GLUT4 mediates glucose transport across the cell membrane, its increased expression suggests improved cellular glucose uptake under insulin-resistant conditions. This enhanced GLUT4 expression provides further evidence of TBCP's capacity to restore glucose metabolism impaired by insulin resistance. Collectively, these findings demonstrate that TBCP activates the PI3K/AKT/GLUT4 signaling pathway at both transcriptional and translational levels, thereby enhancing insulin signaling and glucose uptake in IR-HepG2 cells. This regulatory effect on key metabolic proteins supports the molecular mechanism underlying TBCP's hypoglycemic potential and highlights its promise as a natural therapeutic agent for improving insulin sensitivity and glucose regulation in metabolic disorders.

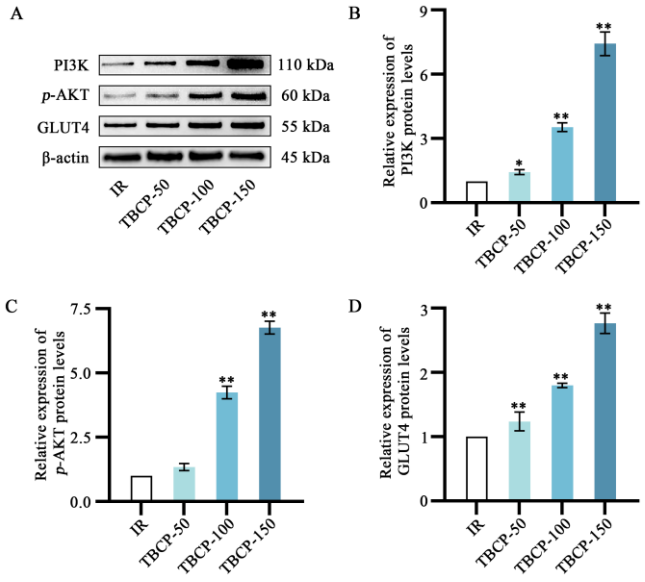

Figure 8: Effects of tuna bone collagen peptides (TBCP) on the expression of glucose metabolism–related proteins in insulin-resistant HepG2 (IR-HepG2) cells. (A) Representative Western blot images of PI3K, p-AKT, and GLUT4; (B) relative PI3K protein levels; (C) relative p-AKT protein levels; (D) relative GLUT4 protein levels after treatment with TBCP (0–150 μg/mL). Data are presented as mean ± SD (n = 3). $*p < 0.05$, $**p < 0.01$ vs. IR group.

4. Discussion

The present study investigated the effects of TBCP on insulin sensitivity in IR-HepG2 cells and elucidated the underlying mechanisms focusing on the PI3K/AKT/GLUT4 signaling pathway. The major findings demonstrated that TBCP improved glucose and lipid metabolism, enhanced glycolytic and antioxidant enzyme activities, and upregulated the expression of PI3K, AKT, and GLUT4 at both mRNA and protein levels. These results suggest that TBCP enhance insulin sensitivity by restoring impaired insulin signaling and promoting glucose utilization, indicating their potential as natural bioactive agents for managing insulin resistance and T2DM.

Prior to functional evaluation, we confirmed that TBCP exhibited minimal cytotoxicity in HepG2 cells within the tested concentration range. Cell viability remained stable up to 150 μg/mL, with a minor reduction observed only at 250 μg/mL after 48 h exposure. This finding confirms that TBCP are biocompatible at effective concentrations and that subsequent metabolic effects are not attributable to cytotoxicity. The safety profile of TBCP supports their suitability for long-term nutritional or therapeutic application. Considering that hepatocytes play a pivotal role in glucose and lipid metabolism, maintaining hepatic viability is critical for interpreting metabolic effects and for the translational potential of TBCP in managing insulin resistance.

Impaired glucose uptake is a defining feature of insulin resistance [17-19]. Our study revealed that TBCP significantly increased glucose consumption in IR-HepG2 cells after 36 h, with more pronounced effects at 48 h and higher concentrations (≥150 μg/mL). This time- and dose-dependent pattern suggests that TBCP require sustained exposure to exert their effects, likely through modulation of intracellular signaling and gene expression rather than direct insulin mimetic activity. By restoring glucose utilization in insulin-resistant cells, TBCP may contribute to improved hepatic glucose disposal and overall glycemic control. This finding aligns with therapeutic goals for insulin resistance management, which emphasize restoration of insulin responsiveness over transient glucose lowering. Such improvements at the cellular level may have significant implications for systemic glucose homeostasis in vivo.

Dyslipidemia is frequently associated with insulin resistance, characterized by elevated triglycerides, increased total cholesterol, and lipid accumulation in hepatocytes [20, 21]. TBCP significantly reduced total cholesterol at 100 and 150 μg/mL and decreased triglycerides at concentrations ≥50 μg/mL in IR-HepG2 cells. These results suggest that TBCP improve hepatic lipid metabolism and may help mitigate lipotoxicity, a major contributor to impaired insulin signaling. Excess lipid accumulation interferes with the PI3K/AKT signaling pathway through increased diacylglycerol and ceramide levels [22]. Therefore, the lipid-lowering effect of TBCP may secondarily enhance insulin sensitivity by alleviating inhibitory lipid signals. The dual regulation of glucose and lipid metabolism positions TBCP as a multifunctional modulator of metabolic homeostasis, addressing two interconnected hallmarks of insulin resistance.

TBCP treatment significantly increased the activities of key glycolytic enzymes—HK and PK—at concentrations of 100 and 150 μg/mL, indicating improved glycolytic flux and glucose utilization. Enhanced HK activity facilitates glucose phosphorylation, a rate-limiting step in glycolysis, while increased PK activity promotes pyruvate formation, facilitating ATP production [23]. Together, these effects reflect enhanced intracellular glucose metabolism in insulin-resistant hepatocytes. Moreover, TBCP significantly enhanced antioxidant capacity, elevating SOD, CAT, and GSH levels while reducing MDA. Oxidative stress is a well-recognized contributor to insulin resistance by impairing insulin receptor substrate (IRS) phosphorylation and downstream PI3K activation [24-26]. By mitigating oxidative stress, TBCP may preserve insulin signaling integrity. This dual modulation of metabolic and oxidative parameters suggests that TBCP not only restore insulin sensitivity but also protect cells from oxidative damage—a critical advantage in chronic metabolic disorders.

At the molecular level, TBCP markedly upregulated PI3K, AKT, and GLUT4 at both mRNA and protein levels in IR-HepG2 cells. The PI3K/AKT pathway mediates the major insulin signaling cascade, linking receptor activation to glucose transporter translocation and uptake [27, 28]. Activation of PI3K generates phosphatidylinositol (3,4,5)-trisphosphate (PIP_3), which recruits and activates AKT, leading to phosphorylation of downstream targets that promote GLUT4 translocation [29]. GLUT4 serves as the primary transporter responsible for insulin-stimulated glucose uptake [30]. In insulin-resistant states, this pathway is blunted, with decreased PI3K activity, reduced AKT phosphorylation, and diminished GLUT4 translocation [31]. Our data show that TBCP restore this signaling axis, enhancing both upstream (PI3K, AKT) and downstream (GLUT4) components. These effects likely explain the observed improvements in glucose

consumption and glycogen accumulation. Targeting the PI3K/AKT/GLUT4 pathway thus represents a mechanistically coherent strategy to restore insulin sensitivity. The fact that TBCP enhance this pathway suggests their potential as natural insulin sensitizers.

Our findings indicate that TBCP exert multifaceted effects on hepatic insulin resistance through both direct and indirect mechanisms. Directly, TBCP activate insulin signaling via the PI3K/AKT/GLUT4 pathway, leading to enhanced glucose uptake and utilization. Indirectly, TBCP reduce lipid accumulation and oxidative stress, both of which are key negative regulators of insulin signaling. The combination of these effects likely contributes to the observed improvement in insulin sensitivity. Unlike pharmacological insulin sensitizers such as metformin, which act primarily on AMP-activated protein kinase (AMPK) pathways [32, 33], TBCP appear to engage the PI3K/AKT cascade directly, suggesting complementary or synergistic potential. Moreover, their natural origin and low cytotoxicity profile support their suitability as functional food ingredients or nutraceuticals for long-term metabolic health management.

5. Conclusion

In conclusion, this study demonstrates that TBCP enhance insulin sensitivity in insulin-resistant HepG2 cells by activating the PI3K/AKT/GLUT4 signaling pathway. TBCP improved glucose and lipid metabolism, enhanced glycolytic enzyme activities, and strengthened antioxidant defense, collectively contributing to restored insulin responsiveness. These findings provide a molecular basis for the hypoglycemic activity of TBCP and highlight their potential as functional food ingredients or therapeutic candidates for the prevention and management of insulin resistance and type 2 diabetes mellitus.

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Author Contributions

Ying Xu and Haomiao Ding contributed to the conception of the study. Zhuoran Li, Chaoqing Cao, Yuhan Ge and Nan Xu performed the experiment. Wei Chen contributed significantly to the analysis. Zhuoran Li and Yu pan performed the data analysis and wrote the manuscript. Ying Xu and Haomiao Ding helped perform the analysis with constructive discussion.

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References

[1] Oh SY, Lee MS, and Lee BC. Geniposide mitigates insulin resistance and hepatic fibrosis via insulin signaling pathway [J]. International Journal of Molecular Sciences, 2025, 26: 8079.

[2] Gonzalez GL and Manrique-Acevedo C. Vascular insulin resistance as a potential mechanism of increased cardiovascular risk in gestational diabetes [J]. American Journal of Physiology-Heart and Circulatory Physiology, 2025, 327(4): H828-H829.

[3] Kanagalingam T, Yu C, Ke C. Early-onset type 2 diabetes [J]. Canadian Medical Association Journal, 2024, 196(37): e1274-e1275.

[4] Ke ZZ, Wen H, Huang RH, et al. Long-term insulin resistance is associated with frailty, frailty progression, and cardiovascular disease [J]. Journal of Cachexia Sarcopenia and Muscle, 2024, 15(4): 1578-1586.

[5] Bielka W, Przezak A, Moleda P, et al. Double diabetes-when type 1 diabetes meets type 2 diabetes: definition, pathogenesis and recognition [J]. Cardiovascular Diabetology, 2024, 23(1): 62.

[6] Song ZL, Chen GX, and Chen CYC. AI empowering traditional Chinese medicine? [J]. Chemical Science, 2024, 15(41): 16844-16886.

[7] Wang F, Neumann D, Kapsokalyvas D, et al. Specific compounds derived from traditional Chinese medicine ameliorate lipid-induced contractile dysfunction in cardiomyocytes [J]. International Journal of Molecular Sciences, 2024, 25(15): 8131.

[8] Liu J, Li FX, Yang L, et al. Gut microbiota and its metabolites regulate insulin resistance: traditional Chinese medicine insights for T2DM [J]. Frontiers in Microbiology, 2025, 16: 1554189.

[9] Wu JL, Pan YY, Lu YY, et al. Exploring the mechanisms of Chaige Kangyi Recipe in treating recurrent pregnancy loss with insulin resistance [J]. Scientific Reports, 2025, 15(1): 13866.

[10] Cadar E, Pesterau AM, Prasacu I, et al. Marine antioxidants from marine collagen and collagen peptides with nutraceuticals applications: A review [J]. Antioxidants, 2024, 13(8): 919.

[11] He L, Gao YF, Ju CQ, et al. Collagen peptides alleviate hyperglycemia in mice by modulating insulin resistance, glucose metabolism and gut microbiota [J]. International Journal of Biological Macromolecules, 2025, 301: 140498.

[12] Tan YL, Juhari NH, Jambari NN, et al. Tuna and tuna products: a review of the nutrition, processing, safety, and future prospects [J]. Fisheries Science, 2025, 91(4): 657-678.

[13] Yin QH, Shi HH, Zhao YQ, et al. Physiochemical and functional properties of gelatin obtained from frigate mackerel (Auxis thazard), skipjack tuna (Katsuwonus pelamis), Longtail tuna (Thunnus tonggol) and yellowfin tuna (Thunnus albacares) skin [J]. Food Chemistry-X, 2025, 27:102360.

[14] Chen JL, Zhang XM, Chang YT, et al. Selenium-enriched Cordyceps militaris polysaccharides alleviate insulin resistance in HepG2 cells by regulating the PI3K/AKT/GLUT4 signaling pathway [J]. Food Science and Nutrition, 2025, 13(5): e70246.

[15] Long XS, Liu SC, Yang XQ, et al. The hypoglycemic activity of Gracilaria lemaneiformis polysaccharide gels based on IR/IRS-2/PI3k/Akt/Glut4 and glycometabolism signaling pathways in HepG2 cells [J]. Gels, 2025, 11(5): 366.
[16] Issa, SM, Thabet EH, Dief AE, et al. Metformin-loaded Chitosan nanoparticles alleviate insulin resistance in the polycystic ovarian syndrome rat model through modulation of PI3K/AKT/ GLUT4 in ovarian tissue [J]. Reproductive Biology, 2025, 25(4): 101081.
[17] Ding CY, Jia YH, Liu L, et al. Glucose-responsive PAGR1-regulated skeletal muscle gene program controls systemic glucose homeostasis and hepatic metabolism [J]. Advanced Science, 2025, 12(39): 2763.
[18] Hopkins M, Beaulieu K and Finlayson G. Psychobiology of appetite and food reward in adults with type 1 and type 2 diabetes: Is there a role for exercise? [J]. Canadian Journal of Diabetes, 2020, 44(8): 768-774.
[19] Xie YF, Gao RF, Gao Y, et al. The proteasome activator REGg promotes diabetic endothelial impairment by inhibiting HMGA2-GLUT1 pathway [J]. Translational Research, 2022, 246: 33-48.
[20] Jahdkaran M and Sistanizad M. From lipids to glucose: Investigating the role of dyslipidemia in the risk of insulin resistance [J]. Journal of Steroid Biochemistry And Molecular Biology, 2025, 250: 106744.
[21] Wilson DP and Shah AS. Metabolic syndrome in youth - A fresh look at an old problem [J]. Journal of Clinical Lipidology, 2025, 19(4): 4-14.
[22] Song C, Wang ZX, Cao J, et al. Hesperetin alleviates aflatoxin B1 induced liver toxicity in mice: Modulating lipid peroxidation and ferritin autophagy [J]. Ecotoxicology and Environmental Safety, 2024, 284: 116854.
[23] van Dijk MJ, de Wilde JRA, Bartels M, et al. Activation of pyruvate kinase as therapeutic option for rare hemolytic anemias: Shedding new light on an old enzyme [J]. Blood Reviews, 2023, 61: 101130.
[24] Taha EFS, Mohamed HE, Anees LM, et al. The potential protective effects of intermittent fasting against radiation-induced brain damage in a rat model: Suggested involvement of IRS-1/PI3 K/AKT and BDNF/TrkB signaling pathways [J]. Molecular Neurobiology, 2025, 62(11): 13807-13826.
[25] Roy JR, Janaki CS, Jayaraman S, et al. Hypoglycemic potential of carica papaya in liver is mediated through IRS-2/PI3K/SREBP-1c/GLUT2 signaling in high-fat-diet-induced type-2 diabetic male rats [J]. Toxics, 2023, 11(3): 240.
[26] Liu J, Wang X, Zhu YQ, et al, Theabrownin from dark tea ameliorates insulin resistance via attenuating oxidative stress and modulating IRS-1/PI3K/Akt pathway in HepG2 cells [J]. Nutrients, 2023, 15(18): 3862.
[27] Feng YH, Ren Y, Zhang X, et al. Metabolites of traditional Chinese medicine targeting PI3K/AKT signaling pathway for hypoglycemic effect in type 2 diabetes [J]. Frontiers in Pharmacology, 2024, 15: 1373711.
[28] Chen HJ, Liu PX, Pan X, et al. Downregulation of collagen IV deposition and ITGB1-FAK signaling pathway to inhibit adipogenesis: A novel mechanism of swertiamarin in treating type 2 diabetes mellitus [J]. International Journal of Biological Macromolecules, 2025, 299: 140048.
[29] Wang, J, Luo, YH, Liu, YT, et al. Network pharmacology and experimental validation to elucidate the mechanism of the treatment of polycystic ovary syndrome with insulin resistance by Resina Draconis [J]. Journal of Ovarian Research, 2025, 18(1): 108.
[30] Fan L, Li LP, Zhao Y, et al. Antagonizing effects of chromium against iron-decreased glucose uptake by regulating ROS-mediated PI3K/Akt/GLUT4 signaling pathway in C2C12 [J]. Biological Trace Element Research, 2023, 202(2): 701-712.
[31] Liu N, Cui X, Guo TL, et al. Baicalein ameliorates insulin resistance of HFD/STZ mice through activating PI3K/AKT signal pathway of liver and skeletal muscle in a GLP-1R-dependent manner [J]. Antioxidants, 2024, 13(10): 1246.
[32] Li JT, Jiang Q, Wang X, et al. Metformin preserves insulin secretion in pancreatic β-cells through FGF21/Akt pathway in vitro and in vivo [J]. Combinatorial Chemistry and High Throughput Screening, 2024, 27(18): 2691-2698.
[33] Pan Q, Ai WQ and Guo SD. TGF-β1 Signaling impairs metformin action on glycemic control [J]. International Journal of Molecular Sciences, 2024, 25(4): 2424.