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Obesity

Interleukin-32β Ameliorates Metabolic Disorder and Liver Damage in Mice Fed High-Fat Diet

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Objective: Chronic excessive food intake leads to energy imbalance, resulting in hepatic steatosis and inflammation. Interleukin-32 (IL-32) is known to be a pro-inflammatory cytokine associated with chronic inflammation and cancer. Therefore, the relationship between IL-32 and chronic excessive food intake-induced liver disease was investigated.

Methods: Male IL-32β transgenic and wild-type mice were fed a high-fat diet (HFD) for 15 weeks. They were compared with wild-type mice on a standard chow diet. Daily food intake, body and liver weight, serum biochemistry, histopathological analysis of the liver, and hepatic immune response were determined.

Results: IL-32β mice on HFD showed lower lipid accumulation, reduced infiltration of immune cells, and lower production of pro-inflammatory cytokines in the liver. The expression of the peroxisome proliferator-activated receptor γ (PPARγ) was downregulated and the adenosine 50-monophosphate (AMP)-activated protein kinase (AMPK) was activated in the liver of IL-32β mice compared to wild-type mice. Furthermore, IL-32β over-expression activated the AMPK pathway and IL-32β downregulation inactivated the AMPK pathway in HepG2 cells under high-glucose conditions.

Conclusions: These data suggest that IL-32β modulates lipid accumulation through inhibition of PPARγ expression and AMPK activation.

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Introduction

Fatty liver disease is associated with abnormal accumulation of triglyceride fats in the liver via the process of steatosis, and it is commonly associated with alcohol overconsumption or metabolic syndrome (1). Non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease, and viral hepatitis (2) show identical biopsies (3). Although the liver damage caused by NAFLD is indistinguishable from that caused by alcohol abuse, the diseases can be distinguished by liver biopsy features such as steatosis, mixed inflammatory cell infiltration, hepatocyte necrosis, and fibrosis (1). High-fat diet (HFD)-induced obesity leads to chronic inflammation (4), and chronic HFD exposure causes accumulation of lipids in the liver, a process leading to NAFLD and NASH (5). Therefore, obesity is closely correlated with NAFLD and the prevalence of NAFLD increases by 4.6-fold in obese people (6).

Peroxisome proliferator-activated receptor γ (PPARγ) is expressed in various tissues and plays a role in adipocyte differentiation and lipid storage (7). Although PPARγ is predominantly expressed in adipose tissue and is normally expressed at very low levels in the liver, PPARγ expression is significantly increased in animal models with insulin resistance and fatty liver (8). Gavrilova et al. reported that liver-specific PPARγ knockout mice reduced lipid accumulation in the liver (9). AMP-activated protein kinase (AMPK) is a major cellular energy sensor and a key player in regulating glucose and lipid homeostasis (10). According to recent studies, metformin, an oral biguanide antidiabetic drug, has a lipid-lowering effect through AMPK activation in insulin-resistant HepG2 cells caused by high glucose concentration (11) and has been reported to play a role in the management of NAFLD (12). In addition, Sirt 1 has been reported to protect against HFD-induced metabolic liver damage (13) and to regulate hepatocyte lipid metabolism through AMPK activation (14).

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Interleukin-32 (IL-32) was first reported as a novel molecule that is highly expressed in activated natural killer or T cell (15). Of the six described isoforms of IL-32 (16), IL-32β is expressed in various cell types including human hepatocytes (17) and seems to be most abundant among other isoforms (16). Following the first report on the biological function of IL-32 in 2005 by Kim et al., a large number of reports have been published in which IL-32 has been shown to be associated with infectious diseases, chronic inflammation, and cancer (18). However, although IL-32 is defined as a pro-inflammatory cytokine, it differs from other cytokines in several respects; the structure of IL-32 lacks the sequence homology seen in most of the known cytokines and an unambiguous IL-32 receptor has not yet been identified (19). Therefore, the question arises whether IL-32 signaling proceeds via an extra- or intra-cellular pathway. The majority of reports on IL-32 indicate that IL-32 is an intracellularly expressed protein, because the secreted form of IL-32 is seldom detected alone, and IL-32 signaling is only achieved when IL-32 is released by cell death (19).

In this study, we investigated the function of endogenous IL-32β in fatty liver disease. Abnormal lipid accumulation in the liver causes metabolic disorder as well as induces inflammation. IL-32 is closely associated with chronic inflammation and autoimmune diseases. However, no reports to date have described the function of IL-32 in fatty liver disease. Therefore, we explored the regulatory roles and mechanisms of IL-32 in IL-32 transgenic mice on HFD and IL-32-expressing human hepatic cells.

Methods

Transgenesis and animal experimentation

We previously generated transgenic mice for IL-32β and identified IL-32β ubiquitously expressed in various tissues such as liver, kidney, and intestine, spleen, and thymus (20). Briefly, to generate IL-32β transgenic mice, a 705-bp fragment of the human IL-32β gene was subcloned into the EcoRI sites of the pCAGGs expression vector. The IL-32β gene-containing genomic fragment was released from the vector and the fragment was purified and microinjected at a concentration of 4 ng/ml into the embryos of BDF1 mice. The experimental treatments were carried out according to the guidelines for animal experiments of the Faculty of Disease Animal Model Research Center, Korea Research Institute of Bioscience and Biotechnology (Daejeon, Korea), as well as the Guidelines for the welfare and use of animals in cancer research (21). Male, IL-32β transgenic (IL-32β mice) and wild-type (WT) mice were randomly divided into three groups ($n = 8$ per group), and were fed either with HFD (60% kJ from fat) (IL-32β-HFD and WT-HFD) or with a standard chow diet (IL-32β-SD and WT-SD) (Central Lab. Animal, Inc., Seoul, Korea) starting when the mice were 6–7 weeks old and for a total of 15 additional weeks. Mice were weighed three times per week, and mice were sacrificed after 15 weeks of feeding. All studies were approved by and performed according to the ethical guidelines by the Chungbuk National University Animal Care Committee (CBNU-523-13-01).

Serum chemistry measurements

Mice were anesthetized with an overdose of pentobarbital (100 mg/kg) and blood was taken by heart puncture. Serum levels of glutamate oxaloacetate transaminase (GOT), glutamic pyruvic transaminase (GPT), and triglycerides were measured at laboratory animal research center in Chungbuk National University.

Reagents and cell culture

The HepG2 human hepatic cells were obtained from the American Type Culture Collection (Manassas, VA). HepG2 cells were grown at 37°C in 5% CO_2-humidified air in Dulbecco's modified Eagle (DMEM) medium that contained 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 mg/ml streptomycin. DMEM, penicillin, streptomycin, and FBS were purchased from Gibco Life Technologies (Grand Island, NY). To develop a model of high-glucose-induced lipid accumulation, HepG2 cells were incubated in serum-free DMEM containing either normal concentrations of glucose (5.5 mM D-glucose) or high concentrations of glucose (30 mM D-glucose) for 24 h.

Transfection

Human IL-32β-6xmyc sequences were PCR-amplified from cDNA and subcloned into the *Eco*RI and *Xho*I sites of pcDNA3.1+. Transiently IL-32β over-expressing cell lines were constructed by transfecting into HepG2 cells with plasmid of pcDNA3.1+-6xmyc or pcDNA3.1+IL-32β-6xmyc and Lipofectamine 2000 transfection reagents (Invitrogen, Carlsbad, CA). To create HepG2 cells that transiently inhibit IL-32 protein expression by small interfering RNA technology, plasmids of IL-32 small hairpin RNA (shRNA) were kindly obtained from Soo-Hyun Kim, Konkuk University (22).

Oil Red O staining

Cells were washed twice with phosphate-buffered saline (PBS), fixed with 0.5% glutaraldehyde for 3 h at room temperature, washed again with PBS, and allowed to dry completely. Next, fixed cells were stained with a 0.2% Oil Red O solution in isopropanol diluted in distilled water (6:4) for 1 h at room temperature, and subsequently washed twice with PBS. Stained lipid droplets were observed with a light microscope (Nikon, Tokyo, Japan). Stained oil droplets were dissolved with isopropanol and quantified by spectrophotometrical analysis at 500 nm

RNA isolation and quantitative real-time RT-PCR

Total RNA was isolated from gastrocnemius muscle using Trizol (Invitrogen, Carlsbad, CA). Samples were reverse-transcribed using ProSTAR^{TM} (Stratagene, La Jolla, CA). Gene expression analysis was performed by RT-PCR using QuantiNova SYBR green PCR kit (Qiagen, Valencia, CA).

Western blot analysis

Homogenized liver tissues and HepG2 cells were lysed by protein extraction solution (PRO-PREP, iNtRONBiotechnology, Korea) containing protease inhibitor cocktail (Calbiochem, Germany) and phosphatase inhibitor cocktail (Roche, Germany). Total proteins (50 μg) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA). The membrane was blocked with 5% skim milk overnight and then incubated with primary antibodies (diluted 1:1000) for 1 h at room temperature. The membranes were immunoblotted with the following primary antibodies: mouse monoclonal antibodies directed against PPARγ,

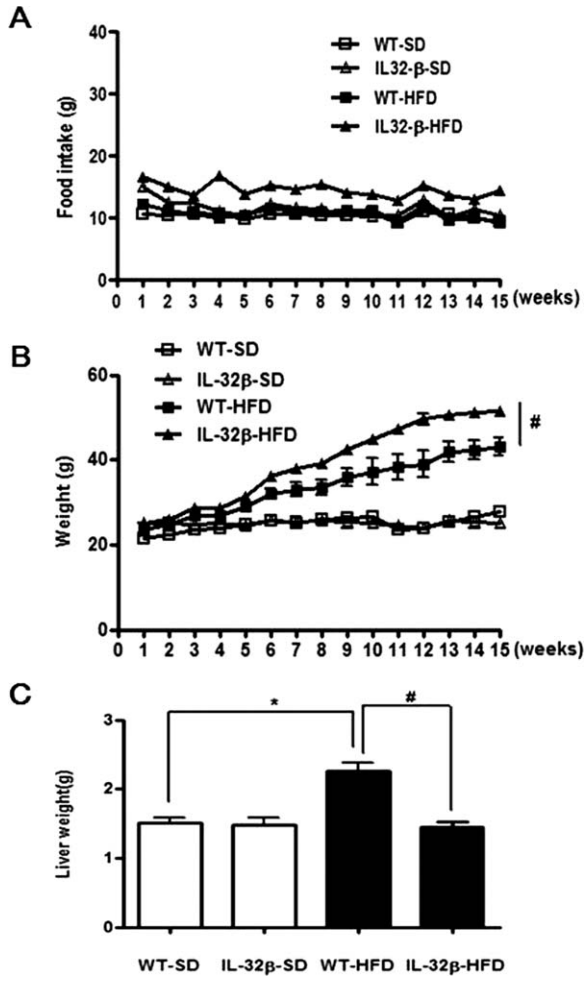

Figure 1 The body weight, liver weight, and daily food intake in transgenic IL-32β mice. (**A**) Daily food intake and (**B**) body weight of WT or during exposure to SD or HFD. (**C**) Liver weight of WT and IL-32β mice after 15 weeks of exposure to SD or HFD. $n = 8$ per group; means ± SEM. $^{*}P < 0.05$, WT-SD mice versus WT-HFD mice. $^{\#}P < 0.05$, WT-HFD mice versus IL-32β-HFD mice.

CD3, and β-actin (Santa Cruz Biotechnology) and against myc-tag (Millipore, Billerica, MA), rabbit polyclonal antibodies directed against CD14, inducible nitric oxide synthase (iNOS), Akt, Phospho-Akt, GSK3α/β, and Phospho-GSK3α/β (Santa Cruz Biotechnology), and against AMPK, Phospho-AMPK, acetyl-CoA carboxylase (ACC), Phospho-ACC, and cyclooxyganase-2 (COX-2) (Cell Signaling Technology, Beverly, MA). The monoclonal anti-hIL-32 antibody KU32–52 was used (23). After washing with Tris-buffered saline containing 0.05% Tween-20 (TBST), the membrane was incubated with horseradish peroxidase-conjugated secondary antibodies (diluted 1:3,000) for 1 h at room temperature. Binding of antibodies to the PVDF membrane was detected with enhanced chemiluminescence solution (Amersham Bioscience, Buckinghamshire, UK) and X-ray film (AGFA, Belgium).

Immunohistochemistry

All specimens were fixed in formalin and embedded in paraffin for examination. Sections (4-mm thickness) were stained with H&E and analyzed by immunohistochemistry using primary mouse monoclonal antibodies directed against CD3 and iNOS (1:100 dilution), primary rabbit polyclonal antibody directed against COX-2 (1:100), primary rat monoclonal antibody directed against F4/80 (1:100), and secondary biotinylated anti-mouse, anti-rabbit, and anti-rat antibodies.

Cytokine assay

Liver tissues homogenized with protein extraction solution (PRO-PREP, iNtRONBiotechnology, Korea) and measured quantity of IL-6, IL-10, and TNF-α in total proteins (1 mg) using mouse cytokine assay kit (R&D systems, Minneapolis).

Results

Weight and daily food intake of IL-32 transgenic mice

To examine the effects of IL-32β over-expression on HFD mice, we measured body weight, daily food intake, and liver weight. Body weight of WT-SD mice was not significantly different from IL-32β-SD mice, but it was lower in IL-32β-HFD mice than in WT-HFD mice (Figure 1A). Daily food intake weakly increased in IL-32β-HFD mice compared to the other groups (Figure 1B). The liver weight of WT-HFD mice was higher than in WT-SD mice, but that of IL-32β-HFD mice did not significantly differ from that of WT-SD mice and IL-32β-SD mice (Figure 1C).

IL-32β transgenic mice are protected from HFD-induced hepatic steatosis

To examine the degree of lipid accumulation in the liver, we performed a histopathological analysis of the liver. H&E staining of the liver revealed that fatty infiltration in the liver of IL-32β-HFD mice was lower than in the liver of WT-HFD mice (Figure 2A). Levels of triglycerides, GPT, and GOT in the serum of IL-32β-HFD mice were lower than in WT-HFD mice (Figure 2B and 2C). In order to understand the defects underlying diabetic dyslipidemia, we analyzed the expression of the principal factors related fatty acid uptake or synthesis in the liver. Levels of mRNA expression of the fatty acid transporters such as fatty acid transport protein 5 (FATP5) and fatty acid binding protein (FABP) in the liver of IL-32β-HFD mice were greatly lower than the liver of WT-HFD mice (Figure 2D). Moreover, we observed that levels of mRNA expression related fatty acid synthesis such as sterol regulatory element binding protein 1c (SREBP-1c) and PPAR-γ coactivator 1 α (PGC-1α) in the liver of IL-32β-HFD mice were also significantly reduced compared with the liver of WT-HFD mice (Figure 2E).

IL-32β modulates lipid accumulation and insulin signaling pathway in HepG2 cells

To examine the effect of IL-32β on lipid accumulation in human hepatic cells induced by high-glucose conditions, we evaluated the amount of the lipid accumulation in IL-32β over-expressed or IL-32 shRNA transfected HepG2 cells under high-glucose conditions through Oil Red O staining. These experiments revealed that IL-32β over-expressed HepG2 cells exhibited more reduced lipid accumulation than control vector HepG2 cells (Figure 3A), whereas IL-32 shRNA transfected HepG2 cells exhibited more increased lipid

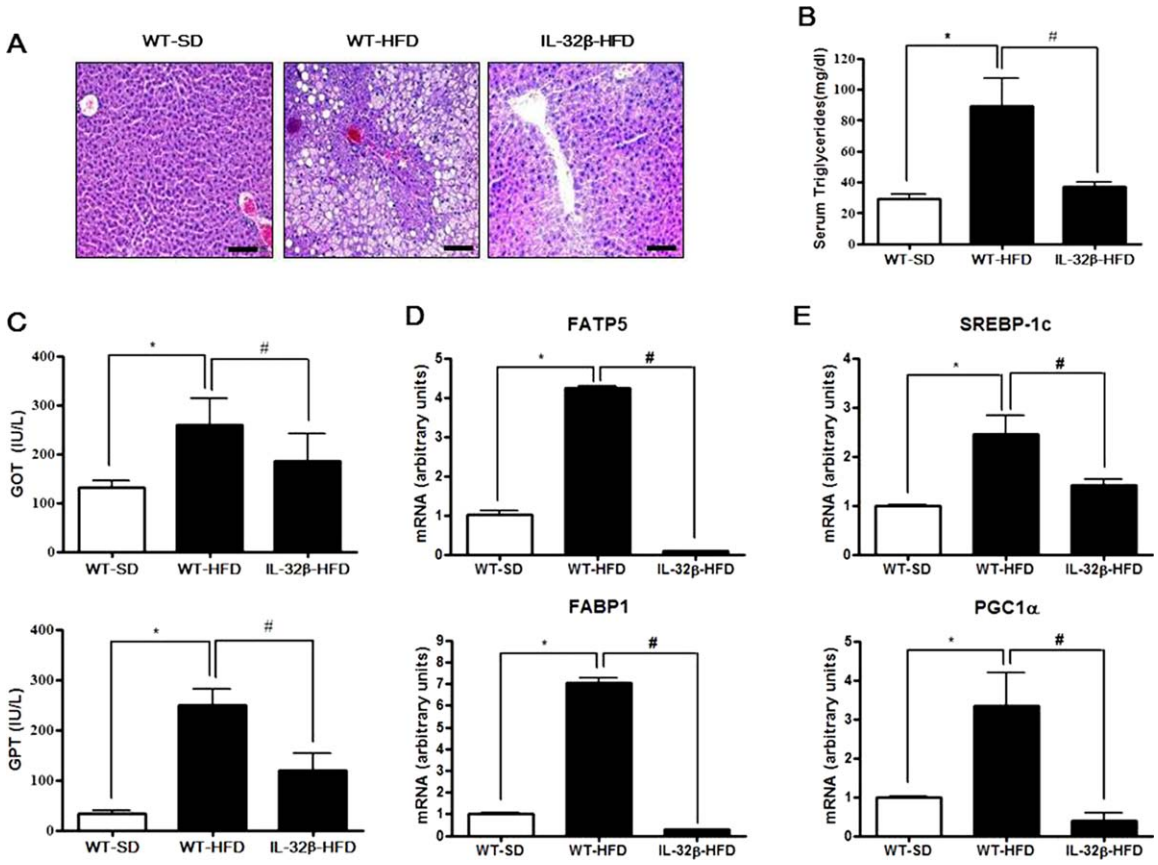

Figure 2 Transgenic IL-32β mice are protected from HFD-induced hepatosteatosis. (**A**) Liver sections of WT mice and IL-32β mice on SD or HFD exposure for 15 weeks were stained with hematoxylin and eosin (H&E) (scale bars, 100 μm). Serum (**B**) triglycerides, (**C**) GPT, and GOT levels in WT mice and IL-32β mice on SD or HFD exposure for 15 weeks. The mRNA expression involved in (**D**) fatty acid uptake and (**E**) lipogenesis in WT mice and IL-32β mice on SD or HFD exposure for 15 weeks. $n = 8$ per group; means ± SEM. $^{*}P < 0.05$, WT-SD mice versus WT-HFD mice. $^{\#}P < 0.05$, WT-HFD mice versus IL-32β-HFD mice. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

accumulation than scrambled shRNA transcfected HepG2 cells (Figure 3B). And, to examine the effect of IL-32β on model of insulin resistance in HepG2 cells (11), the phosphorylation of Akt and its down-stream target, GSK3α/β, was determined by Western blotting in IL-32β over-expressed or IL-32 shRNA transfected HepG2 cells under high-glucose conditions. Insulin-stimulated phosphorylation of Akt and GSK3α/β were increased in IL-32β over-expressed HepG2 cells (Figure 3C), whereas those were decreased in IL-32 shRNA transfected HepG2 cells (Figure 3D) compared with control HepG2 cells.

IL-32β reduced PPARγ expression in the liver of HFD mice and HepG2 human hepatic cells

PPARγ is a key factor in adipogenesis and triglyceride metabolism in the liver. The expression of PPARγ was lower in the liver of IL-32β-HFD mice than in WT-HFD mice (Figure 4A). To investigate the effect of IL-32β on PPARγ expression in human hepatic cells, we performed transient transfection with constructs driving the expression of C-terminally 6xmyc-tagged IL-32β in HepG2 human hepatic cells. Under high-glucose conditions, the expression of PPARγ decreased in IL-32β-over-expressed HepG2 cells compared to control cells (Figure 4B). Because HepG2 human hepatic cells express IL-32 (17), to inhibit endogenous IL-32 production, we performed transient transfection with scrambled shRNA or IL-32 shRNA (clone 5) (22) in HepG2 human hepatic cells. As shown in Figure 4C, HepG2 cells transfected with IL-32 shRNA (clone 5) exhibited reduced IL-32 levels, and the expression of PPARγ was significantly higher under high-glucose conditions in IL-32 shRNA transfected cells compared to HepG2 cells transfected with scrambled shRNA.

IL-32β reduced lipogenesis through activation of AMPK in the liver of HFD mice and HepG2 human hepatic cells

Because HFD-induced lipid accumulation was attenuated in the liver of IL-32β mice, we examined the effect of IL-32β on lipogenesis via the AMPK pathway in the liver of HFD mice and HepG2 human hepatic cells. As shown in Figure 5A, the phosphorylation of AMPK in the liver of WT-HFD mice was significantly lower than in WT mice-SD, but that of IL-32β-HFD mice was higher. In addition, the phosphorylation of ACC in the liver of IL-32β-HFD mice was

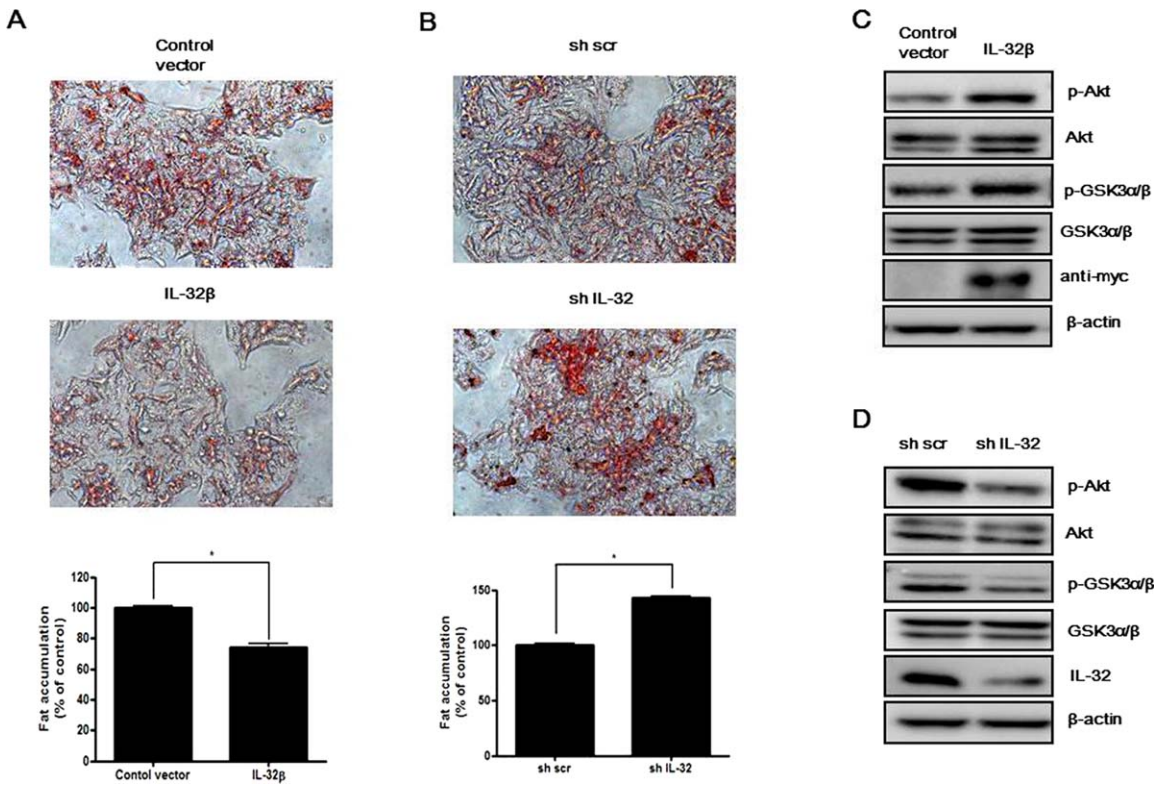

Figure 3 IL-32β attenuates lipid accumulation and improves insulin signaling pathway in human hepatic HepG2 cells under high-glucose conditions. Accumulated lipids in (**A**) control and IL-32β over-expressed cells and (**B**) scrambled shRNA and IL-32 shRNA transfected cells were stained with Oil Red O and photographed, then eluted and quantitated by spectrophotometrical analysis at 500 nm. Three independent experiments were performed in triplicate; means ±SEM. $^{*}P < 0.05$, control vector versus IL-32 over-expression. $^{\#}P < 0.05$, scrambled shRNA versus IL-32 shRNA. Western blotting analysis of insulin signaling pathway in (**C**) control and IL-32β over-expressed cells and (**D**) scrambled shRNA and IL-32 shRNA transfected cells. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

slightly higher than in WT mice-HFD (Figure 5A). Next, we investigated the effect of IL-32β on lipogenesis in human hepatic HepG2 cells. Under high-glucose conditions, phosphorylation of AMPK and ACC increased in IL-32β-over-expressed HepG2 cells compared to control cells (Figure 5B). In the HepG2 cells exhibiting reduced IL-32 expression levels due to transfection with IL-32 shRNA (clone 5), inhibition of AMPK activation was observed under normal glucose conditions in IL-32 shRNA transfected cells compared to HepG2 cells transfected with scrambled shRNA (Figure 5C). Under high-glucose conditions, the phosphorylation of ACC weakly decreased in HepG2 cells transfected with IL-32 shRNA IL-32 compared to HepG2 cells transfected with scrambled shRNA (Figure 5C).

IL-32β transgenic mice are protected from HFD-induced hepatic inflammation

Chronic exposure to HFD and diet-induced obesity lead to chronic inflammation, and HFD-induced obesity increases lymphocyte migration to the liver. Thus, we examined immune cell infiltration and pro-inflammatory cytokine levels in the liver of WT-HFD mice and IL-32β-HFD mice. The expression of F4/80, a macrophage marker, and CD3, a T-cell marker, were higher in the liver of WT-HFD mice than in the liver of IL-32β-HFD mice (Figure 6A and 6B). Furthermore, the expression of factors involved in inflammation, such as COX-2 and iNOS-reactive cells, significantly increased in the liver of WT-HFD mice than in IL-32β-HFD mice (Figure 6A and 6B). HFD-induced IL-6 and TNF-α production, which are two major pro-inflammatory cytokines, were decreased in the liver of IL-32β-HFD mice compared to the liver of WT-HFD mice (Figure 6C). IL-10 production, which is an anti-inflammatory cytokine, did not show significant change between the liver of WT-SD and WT-HFD mice, but it was more increased in the liver of IL-32β-HFD mice than WT-SD and WT-HFD mice (Figure 6C).

Discussion

We previously generated IL-32β transgenic mice and reported that IL-32β is ubiquitously expressed in various tissues, such as the liver, kidney, intestine, spleen, and thymus; IL-32β was not expressed in the tissues of nontransgenic mice (20). Human and other mammals such as pigs, cows, and horses express IL32 gene, but IL-32 is still not found in rodents such as mice and rats (19). Many efforts show that *IL32* gene is located between *MMP25* and *ZSCAN10* gene in rodents, however a large part of IL32 gene is missing and its functional transcription is still unknown (19). Therefore, in our data, the IL-32β transgenic mice were used in investigation of the function of

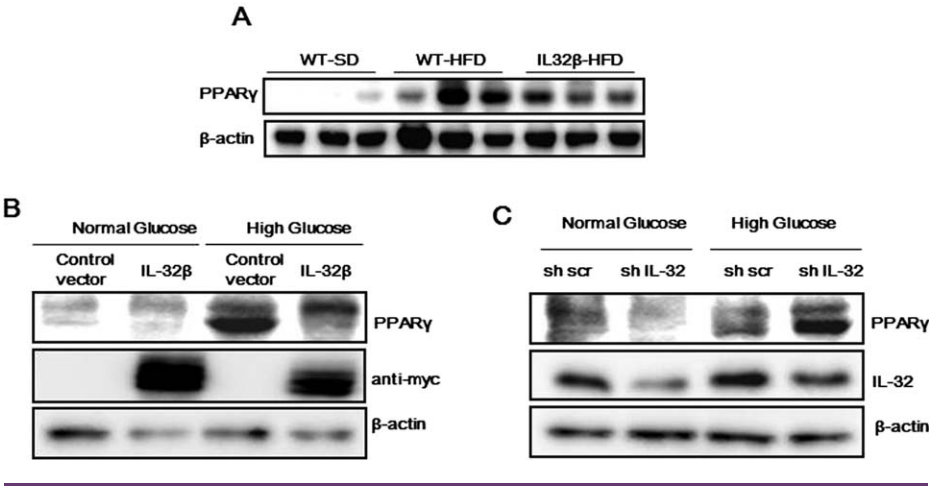

Figure 4 Effects of IL-32β on PPARγ expression in the liver of HFD-induced mice and high-glucose-induced HepG2 human hepatic cells. (**A**) The expression of PPARγ in the liver of WT mice and IL-32β mice on SD or HFD exposure for 15 weeks. The expression of PPARγ in human hepatic HepG2 cells transfected with (**B**) control or IL-32β and (**C**) scrambled shRNA or IL-32 shRNA.

IL-32β. Moreover, many studies including our studies previously published data have reported the effect of IL-32 using IL-32 transgenic mice (24,25). In the present study, we have shown, for the first time, that IL-32β protects hepatic lipid metabolism from the HFD-induced damage in mice, and that it regulates lipogenesis in high-glucose-induced HepG2 human hepatic cells. The body weight of IL-32β mice was lower than that of WT mice on HFD, and liver weight and serum triglycerides were lower in IL-32β mice than in WT mice on HFD. Fatty liver induced by metabolic disorder resulting from obesity and insulin resistance has been shown to increase triglyceride levels and liver weight (26). Our data showed that hepatosteatosis had alleviated and triglyceride levels were lower in the

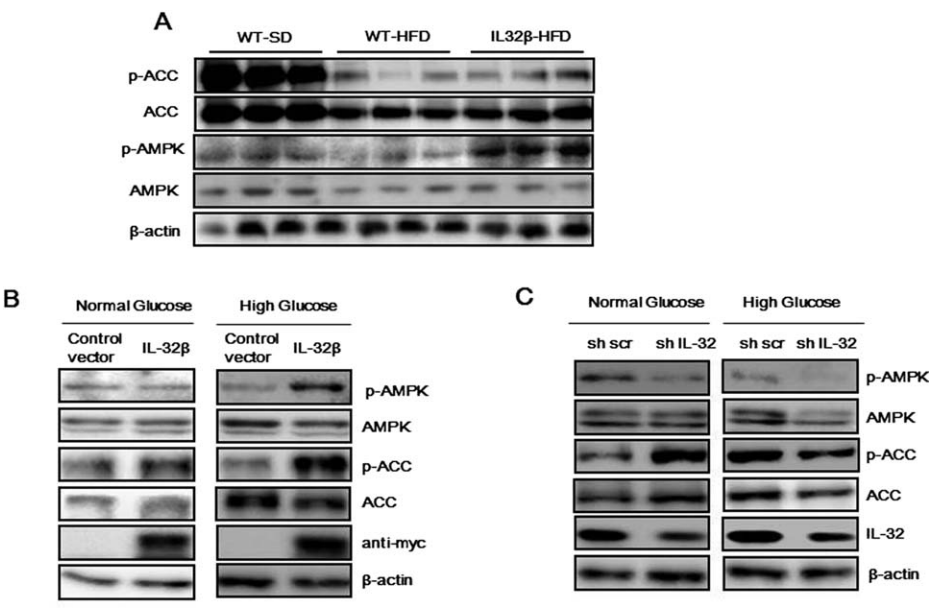

Figure 5 Effects of IL-32β on AMPK pathway in the liver of HFD-induced mice and high-glucose-induced HepG2 human hepatic cells. (**A**) Immunoblots of AMPK and ACC phosphorylation in the liver of WT mice and IL-32β mice on SD or HFD exposure for 15 weeks. (**B**) Immunoblots of AMPK and ACC phosphorylation in high glucose (30 mM D-glucose) or normal glucose (5 mM D-glucose) treated control and IL-32β over-expressed HepG2 cells. (**C**) Immunoblots of AMPK and ACC phosphorylation in high glucose (30 mM D-glucose) or normal glucose (5 mM D-glucose) treated scrambled shRNA and IL-32 shRNA transfected HepG2 cells.

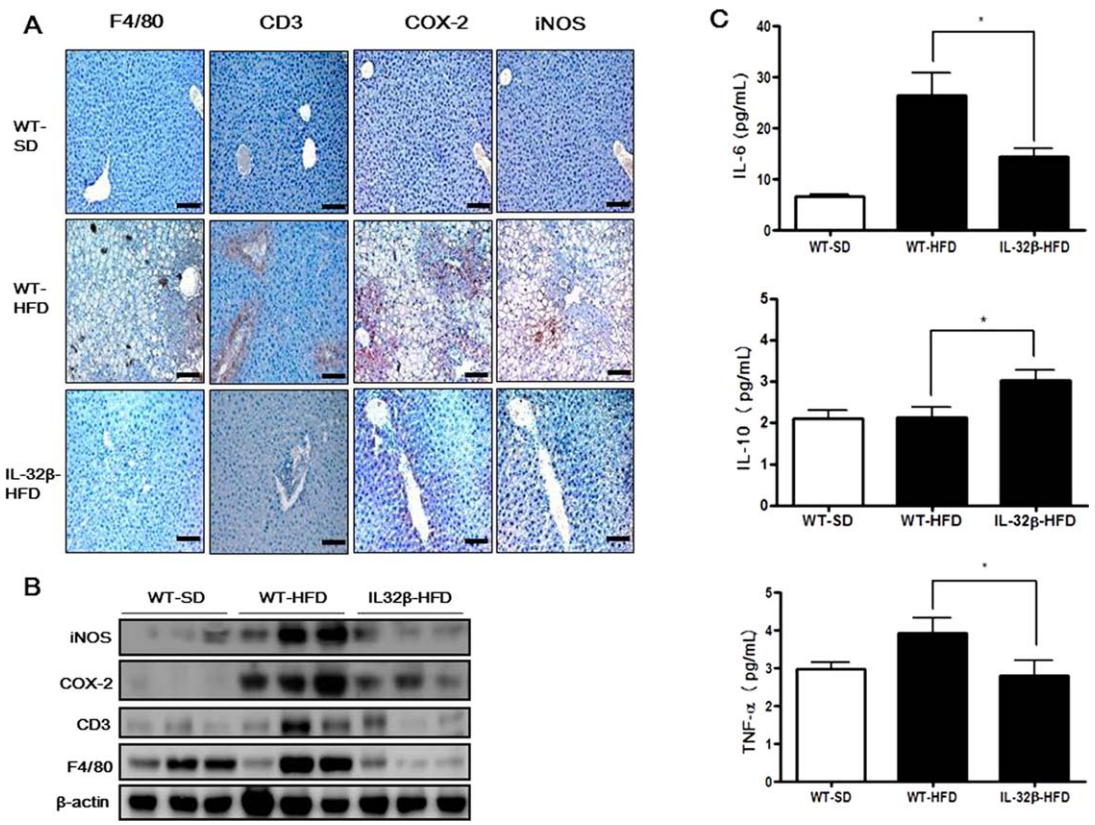

Figure 6 Transgenic IL-32β mice are protected from hepatic inflammation. (**A**) Immunohistochemistry of infiltrated immune cells such as macrophage (F4/80) and T-cells (CD3), and factors involved in inflammation such as iNOS and COX-2 in the livers of WT-SD, WT-HFD, and IL-32β-HFD mice (scale bars, 100 μm). (**B**) Western analysis of factors involved in inflammation such as iNOS and COX-2 and immune cells such as macrophage (F4/80) and T-cells (CD3) in the livers of WT-SD, WT-HFD, and IL-32β-HFD mice. (**C**) Cytokine assay of the pro-inflammatory cytokines IL-6, IL-10, and TNF-α in the livers of WT and IL-32β mice on SD or HFD (15 weeks). $n = 8$ per group; means ± SEM. $^*P < 0.05$, WT-HFD mice versus IL-32β-HFD mice. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

serum of IL-32β mice than in WT mice on HFD. Up-regulated expression of principle factors involved in fatty acid uptake and lipogenesis in the liver on HFD were also decreased in IL-32β mice. In human hepatic HepG2 cells, fat accumulation was decreased in IL-32β over-expressing cells, and it was increased in IL-32 shRNA transfected cells compared to control cells under high-glucose conditions. Insulin resistance is closely associated with NAFLD. Concomitant with hepatosteatosis, hepatic inflammation lead to insulin resistance (27), and hepatic insulin resistance, in turn, can stimulate the development of hepatosteatosis (28). In our insulin resistance model of human hepatic cell, IL-32β over-expressed cells have improved insulin sensitivity, otherwise IL-32 shRNA transfected cells was abrogated insulin signaling. Collectively, these results suggest that IL-32β may ameliorate the fatty liver and hepatic insulin resistance induced by a high-calorie diet.

Inoue et al. reported that PPARγ expression increased in HFD-induced liver steatosis in mice (29). Liver-specific disruption of PPARγ improves fatty liver but exacerbates the diabetic phenotype by reducing insulin sensitivity in muscle and fat (30). Therefore, hepatic PPARγ plays a critical role in the regulation of triglyceride and glucose homeostasis. Exposure to HFD has been shown to cause hypertriglyceridemia accompanied by reduced hepatic phospho-AMPK levels (31). According to recent research, metabolic syndrome in patients with NAFLD is associated with a diet containing more carbohydrates and less fat (32). In addition, AMPK activation leads to improved glucose tolerance and lipid accumulation (11). Our data showed that the expression of PPARγ was lower in the liver of IL-32β-HFD mice than in WT-HFD mice, and that the phosphorylation of AMPK and ACC was higher in the liver of IL-32β-HFD mice than in WT-HFD mice. In addition, in human hepatic HepG2 cells, decreased PPARγ expression and activated AMPK and ACC were observed in IL-32β over-expressed cells, and increased PPARγ expression and inactivated AMPK and ACC were observed in IL-32 shRNA transfected cells compared to control cells under high-glucose conditions. These data suggest that IL-32β protects against metabolic disorder induced by excess energy via downregulation of PPARγ expression and activation of AMPK signaling.

Obesity is closely associated with immune response and chronic inflammation (33). Many researchers have reported that HFD-induced fatty liver disease and liver inflammation are improved by amelioration of metabolic disorder and attenuation of lipid accumulation in the liver (4,13,30). Our data showed that increased serum

levels of GPT and GOT, markers of liver damage, induced by HFD were restored in IL-32β mice. Obesity-induced fatty liver has also been shown to increase lymphocyte and macrophage recruitment in the liver (34). We observed that HFD-induced CD3, a T-cell marker, decreased in the liver of IL-32β mice. Furthermore, f4/80, a macrophage marker, and iNos and COX-2, factors involved in inflammation, decreased in the liver of IL-32β mice compared to WT on HFD. Finally, HFD promoted pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α in mouse liver, but did not promote other cytokines such as IL-4, IL-10, IL-11, IL-13, IL-15, and IFN-γ (35). We previously reported that IL-32β showed antitumor activity in IL-32β mice and modulated the levels of cytokines such as IL-6, TNF-α, IL-1β, and IL-10 (20). Kang et al. reported that IL-32β promotes the production of IL-10, which is an anti-inflammatory cytokine in human myeloid cells (36). Similarly, in the liver of IL-32β-HFD mice, downregulated production of two major pro-inflammatory cytokines such as IL-6 and TNF-α, and upregulated production of IL-10, which is an anti-inflammatory cytokine in the liver were observed compared to liver of WT-HFD mice. Collectively, these results suggest that IL-32β attenuated HFD-induced hepatic inflammatory response in the liver.

In summary, our results suggest that IL-32β over-expression may prevent high-glucose- or HFD-induced NAFLD, and that this effect may result from downregulation of lipid accumulation by AMPK activation and inhibition of PPARγ expression in the liver. Finally, the attenuation of hepatosteatosis by IL-32β may lead to decreased chronic immune response. O

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