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Interferon beta overexpression attenuates adipose tissue inflammation and high fat diet-induced obesity and maintains glucose homeostasis

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Abstract

The worldwide prevalence of obesity is increasing, raising health concerns regarding obesity-related complications. Chronic inflammation has been characterized as a major contributor to the development of obesity and obesity-associated metabolic disorders. The purpose of the current study is to assess whether overexpression of interferon beta (IFNβ1), an immune-modulating cytokine, will attenuate high fat diet-induced adipose inflammation and protect animals against obesity development. Using hydrodynamic gene transfer to elevate and sustain blood concentration of IFNβ1 in mice fed a high fat diet, we showed that overexpression of *Ifnβ1* gene markedly suppressed immune cell infiltration into adipose tissue, and attenuated production of pro-inflammatory cytokines. Systemically, IFNβ1 blocked adipose tissue expansion and body weight gain, independent of food intake. Possible browning of white adipose tissue might also contribute to blockade of weight gain. More importantly, IFNβ1 improved insulin sensitivity and glucose homeostasis. These results suggest that targeting inflammation represents a practical strategy to block the development of obesity and its related pathologies. In addition, IFNβ1-based therapies have promising potential for clinical applications for the prevention and treatment of various inflammation-driven pathologies.

INTRODUCTION

The increasing prevalence of obesity represents a global health concern, largely due to its related co-morbidities. Obesity is closely associated to many metabolic disorders, including cardiovascular diseases, type-2 diabetes, and fatty liver; and to various neoplasms such as colon carcinoma[1]. Increasing number of studies suggests that low grade chronic inflammation is a driving force for obesity-related pathologies.[2] It has been shown that inflammation in adipose tissue is accompanied by enhanced immune cell infiltration, particularly macrophages, due to prolonged nutrient overload.[3] Induced inflammation

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CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

appears essential for adipose tissue remodeling and modulation of adipocyte functions, and responsible for development of insulin resistance, inhibition of adiponectin secretion, and alteration of local and systemic cytokine profiles. As such, more research is being focused on further investigating inflammation's contribution to obesity pathogenesis, and developing strategies to target inflammation for treatment of the related diseases.

Interferon beta (IFNβ1) is a cytokine with pleiotropic activities, including antiviral and antitumor activities, as well as immunomodulatory effects.[4] Recombinant IFNβ1 is used in clinic for treatment of multiple sclerosis[5] owing to its disease-modifying anti-inflammatory properties. Mechanistically, IFNβ1 effects are attributed to increasing production of anti-inflammatory cytokines such as IL-10, decreasing production of inflammatory mediators, such as IL-17, osteopontin and TNFα, and impairment of inflammatory cell migration across BBB.[6] Therefore, IFNβ1 is increasingly being investigated for the therapeutic potential to treat inflammation-driven pathologies.

In this study, we explored the therapeutic potential of IFNβ1 to suppress adipose tissue inflammation and to block obesity development in mice. We showed that IFNβ1 overexpression attenuates obesity-induced adipose inflammation, while modulating adipose tissue hypertrophy. These effects were associated with blockade of weight gain, and restoration of glucose homeostasis. Together, our findings suggest that IFNβ1 has beneficial effects on lipid metabolism in an obesity model, and that IFNβ1 is a novel therapeutic target for prevention and treatment of obesity and insulin resistance.

RESULTS

Hydrodynamic injection resulted in efficient gene transfer in mice

We first assessed the efficiency of hydrodynamic injection to transfer the *Ifnβ1* gene into mouse hepatocytes. Hydrodynamic injection of pLIVE-IFNβ1 plasmid resulted in a successful delivery, and subsequent expression of the *Ifnβ1* gene into the liver but not into white (WAT) and brown (BAT) adipose tissue (Figure 1a). IFNβ1 signaling was induced and sustained in all three tissues for more than nine weeks post plasmid injection, evidenced by induction of *Mx1* gene (Figure 1b). There was no change in the serum levels of the liver enzymes ALT and AST, suggesting that neither the hydrodynamic injection nor IFNβ1 activity causes liver damage (Figures 1c, 1d). These results prove hydrodynamic injection as an efficient and safe method for delivery and expression of *Ifnβ1* gene in the liver.

IFNβ1 attenuates HFD-induced adipose hypertrophy and inflammation

HFD-induced obesity is often accompanied by WAT hypertrophy, fat accumulation, and induction of inflammation of adipose tissue as evidenced by an increased expression of inflammatory cell marker genes such as *F4/80*, *Cd11c*, and *Mcp1*. IFNβ1 overexpression efficiently blocked hypertrophy and expansion of WAT tissues (Figures 2a, 2b). Suppressed fat accumulation in adipose tissue, upon treatment, was demonstrated with H&E examination of WAT and BAT tissue sections (Figure 2c). More importantly, IFNβ1 decreased adipose expression of inflammatory cell marker genes (Figure 2d), suggesting attenuated trafficking of inflammatory cells into adipose tissue. In parallel, IFNβ1 reversed

cytokine profiles toward anti-inflammatory phenotype by downregulating common pro-inflammatory signals *Tnf-α*, *Il-1β* and *Il-6* (Figure 2e), and upregulating the anti-inflammatory cytokine *Il-10* (Figure 2f). Given the impacts of inflammation on adipokine production, we also assessed expression levels of leptin and adiponectin upon HFD feeding. While the leptin level was increased, suggesting leptin resistance, adiponectin expression was significantly decreased (Figure 2g). IFNβ1 restored the expression of these adipokines to normal levels. Overall, IFNβ1 suppressed HFD-induced adipose inflammation, hypertrophy, and ameliorated the dysregulated adipokines back to normal.

IFNβ1 blocked HFD-induced weight gain without impacting food intake

IFNβ1 not only generates attenuation of adipose tissue inflammation, but also blocks development of obesity in spite of HFD feeding (Figure 3a). While control HFD-fed animals gained approximately 20 g in nine weeks, *Ifnβ1* gene transfer completely blocked body weight gain (Figure 3b). Analysis of body composition showed IFNβ1 overexpression had no significant impact on lean mass, confirming that the difference in body weight between the treated and control animals was primarily due to the increase in fat mass, and precluding toxicity-related weight loss (Figure 3c). The anti-obesity effects of IFNβ1 were independent of food intake since both groups of animals had comparable food intake rates over nine weeks of HFD feeding (Figure 3d).

IFNβ1 altered gene expression in adipose tissues toward thermogenic phenotype

Given the critical role of adipose tissue, particularly BAT in thermogenesis and overall energy balance, we further examined the anti-obesity effects of IFNβ1 by the assessment of expression levels of thermogenic genes in WAT and BAT. IFNβ1 significantly upregulated the expression of various isoforms of uncoupling protein gene (*Ucp*) in both WAT (Figure 4a) and BAT (Figure 4b), suggesting browning of white adipose tissue and activation of BAT, and possibly enhanced energy expenditure in these tissues, which further contributes to anti-obesity effects. In addition, IFNβ1 upregulates the expression of genes (*Dio2, Pgc1a, Cidea, Elovl3*) that are involved in mitochondrial biogenesis and energy utilization in BAT (Figure 4c), increasing the overall energy expenditure in these tissues.

IFNβ1 didn't reverse HFD-induced fatty liver

Fatty liver is a common manifestation of diet-induced obesity. We examined the effects of IFNβ1 on liver *de novo* lipogenesis and ectopic fat accumulation. Liver analysis showed increased liver weight upon HFD feeding, in spite of IFNβ1 treatment (Figure 5a). Assessment of triglyceride content showed comparable levels of liver triglycerides in treatment and control animals (Figure 5b) suggesting that IFNβ1 did not protect against fatty liver development. These results were confirmed by H&E staining of liver sections, which showed vacuole structures in both HFD-fed groups, but not chow-fed animals (Figure 5c). To explore the underlying mechanisms of fat accumulation in the liver, we assessed the expression of genes involved in lipogenesis and lipid uptake. While control animals possessed an increased expression of lipogenic genes *Srebp1c*, *Fas*, and *Scd1*, suggesting increased *de novo* lipid biosynthesis, IFNβ1-treated animals showed lower levels of these genes, albeit higher than the normal levels (Figure 5d). On the other hand, the expression level of *Cd36*, the major fatty acid transporter in the liver, was significantly increased in

IFNβ1-treated animals, even higher than that of control animals. These results suggest that while IFNβ1 downregulated the expression of lipogenic genes and attenuated lipid biosynthesis, these effects were counterbalanced by an increased uptake of ectopic fat, resulting in fat accumulation in the liver.

IFNβ1 restores insulin sensitivity and improves glucose homeostasis

It has been well established that obesity is a risk factor for diabetes since obese individuals often display a decreased sensitivity to insulin-stimulated glucose uptake. To assess IFNβ1 effects on glucose homeostasis, we conducted a glucose tolerance test to examine systemic insulin sensitivity upon a glucose challenge. Results showed impaired tolerance to glucose in the control group; whereas IFNβ1-treated animals demonstrated the same efficient glucose clearance as did chow-fed animals (Figure 6a). These results were verified by calculation of the area under the curve (Figure 6b). Insulin sensitivity was also assessed by an insulin tolerance test (ITT) showing similar results, in which HFD-fed IFNβ1-treated animals demonstrated improved insulin sensitivity compared to HFD-fed control animals (Figure 6c). Assessment of fasting glucose and insulin levels showed consistent results as in IPGTT and ITT. IFNβ1-treated animals remained within normal ranges of glucose (Figure 6d) and insulin (Figure 6e), compared to hyperglycemic, hyperinsulinemic HFD-fed control animals.

DISCUSSION

It is evident that adipose tissue inflammation is a hallmark for obesity development, and a critical contributor to obesity-related pathologies. Targeting adipose tissue inflammation, therefore, is a potential therapeutic approach that could block development of obesity and its related disorders. Results presented here demonstrated that efficient IFNβ1 overexpression (Figure 1) attenuated adipose tissue inflammation (Figure 2), blocked the development of HFD-induced obesity (Figure 3), and alleviated insulin resistance (Figure 6). Anti-obesity effects of IFNβ1 were also linked to increased adipose tissue thermogenesis (Figure 4). However, IFNβ1 overexpression did not protect animals from developing fatty liver, which is attributed to an increased lipid uptake rather than lipid biosynthesis (Figure 5).

Earlier studies reporting increased TNF-α expression in adipose tissue of obese mice, and its role in insulin resistance provide the first evidence of the contributing factor of chronic inflammation in obesity and its complications,[7] which was later supported by numerous studies identifying elevated levels of various inflammatory mediators in different obesity models.[8] It has been proposed that fat accumulation and adipose hypertrophy induces a hypoxia response, which accounts for the various oxidative and inflammatory stress events caused by chronic activation of several inflammatory pathways, particularly NF-kB.[9–11] The local inflammation is translated into systemic events, such as low-grade systemic inflammation, insulin resistance, adiponectin suppression, and ectopic fat accumulation through cytokines and free fatty acids released from adipose tissue. In agreement with these theories, we observed a significant increase in adipose tissue inflammation (Figure 2) accompanied by ectopic fat accumulation in the liver (Figure 5) and exacerbated insulin resistance (Figure 6) upon HFD feeding. Thus, our results present new evidence supporting

the use of anti-inflammatory therapies to prevent obesity and its related pathologies, such as insulin resistance.

Type I interferons, including IFNβ1, are widely expressed cytokines with profound antiviral and immune modulating effects. IFNβ1, in particular, is well recognized for its anti-inflammatory activity, and has been proven effective in treating inflammatory diseases, such as multiple sclerosis[5] and ulcerative colitis.[12] IFNβ1 signals through a heterodimeric and ubiquitously expressed IFNα/β receptor (IFNAR), and mediates downstream events through several STAT family members. While the pro-inflammatory IFNγ acts through STAT1 to promote production of pro-inflammatory mediators and enhances antigen processing, IFNβ1 acts through STAT3 which suppresses pro-inflammatory responses, and directly inhibits STAT1 activation.[13] In addition, IFNβ1 enhances the production of IL-10 independent of STAT3, by activating the PI3K signaling pathway.[14] Consistent with these mechanisms, we have demonstrated that IFNβ1 overexpression attenuated HFD-induced inflammation in adipose tissue (Figure 2), and thus blocked obesity development (Figure 3). IFNβ1 likely acts directly through suppression of inflammatory cell infiltration into adipose tissue and subsequent production of inflammatory mediators, and indirectly through promotion of IL-10 in adipose tissue, which in turn represses various TNF-α and IL-1β-mediated inflammatory events.[15] Beneficial effects of IFNβ1 may also be attributed to the inhibition of the NF-kB pathway, a major pathway underlying inflammation-driven metabolic disorders.[16,17]

The anti-inflammatory effects of IFNβ1 were translated systemically into the blockage of adipose hypertrophy and weight gain, without affecting food intake (Figure 3). Adipose tissue has the capacity to expand under conditions of energy surplus. Macrophage infiltration is critical for adipose tissue expansion due to the tissue remodeling properties of macrophages, such as stimulation of angiogenesis,[18] and production of growth factors essential for adipose tissue growth.[19] Thus, the observed anti-obesity effects of IFNβ1 are likely mediated through inhibition of macrophage infiltration and/or activation, and blockage of adipose tissue remodeling. Increased *Ucp1* expression (Figure 4) suggests browning of WAT which might be linked to increased energy expenditure in this adipose tissue (Figure 4) also contributes to anti-obesity effects of IFNβ1. These effects may also be related to the restoration of adiponectin expression, which acts centrally and peripherally to increase thermogenic hormones, lipid oxidation, and glucose utilization.[20]

Insulin resistance in obesity is a consequence of fatty acid release from adipocytes and accumulation in insulin target organs. This fatty acid release and accumulation inhibits glucose uptake and activates pro-inflammatory pathways in these organs by adipose-derived cytokines. Together, this leads to inhibition of the insulin pathway and impaired glucose homeostasis. Mounting evidence suggests that targeting inflammatory pathways in obesity efficiently restores insulin sensitivity and improves glucose tolerance.[21] Moreover, restoring adiponectin levels boosts insulin signaling and ameliorates glycemic control.[22] Here, we have shown similar findings, in which IFNβ1 overexpression resulted in improvement of insulin sensitivity and glucose homeostasis (Figure 6) indirectly, through suppression of local and systemic inflammation and restoration of adiponectin expression. IFNβ1 can also

improve glucose homeostasis directly, via activation of the PI3K/Akt pathway leading to enhanced glucose uptake.[23]

Fuel mobilization from adipose tissue in the form of free fatty acids, deposited in non-adipose cells, often results in various pathologies, such as fatty liver and atherosclerosis. Despite reversing several obesity-related pathologies, IFNβ1 failed to protect animals from developing fatty liver (Figure 5). While fatty liver may result from *de novo* lipogenesis or lipids mobilization, fatty liver in IFNβ1-treated animals appears to be linked to increased lipid uptake into hepatocytes, rather than increased hepatic lipogenesis, as evidenced by substantial increase of *Cd36* expression, the major fatty acid transporter, along with downregulation of lipogenic genes, such as *Srebp1c*, *Fas* and *Scd1*.

In summary, we demonstrated in this study that targeting adipose tissue inflammation by IFNβ1 overexpression is a promising therapeutic approach to protect against obesity and its related complications. Our data provide additional evidence to support the rationale to use IFNβ1 as an immune modulator to treat various inflammatory diseases.

MATERIALS AND METHODS

Materials

The pLIVE plasmid vector was purchased from Mirus Bio (Madison, WI). Mouse *Ifnβ1* gene was sub-cloned into pLIVE plasmid using complementary DNA sequences. DNA sequencing was used to confirm the sequence of the constructed plasmid. Plasmid DNA was prepared using the method of cesium chloride-ethidium bromide gradient centrifugation, and kept in saline at −80 °C until use. The purity of the plasmid preparation was examined by absorbency ratio at 260 and 280 nm and 1% agarose gel electrophoresis.

Mice and treatments

Male C57BL/6 mice purchased from Charles River Laboratories (Wilmington, MA) were randomly assigned to the treatment (n=5) or vector control group (n=5) and housed under standard conditions with a 12-h light–dark cycle. All animal procedures used were approved by the Institutional Animal Care and Use Committee at the University of Georgia, Athens, Georgia (protocol number, A2011 07-Y2-A3). HFD (60% kJ/fat, 20% kJ/carbohydrate, 20% kJ/protein) used in this study was purchased from Bio-Serv (Frenchtown, NJ). The procedure of hydrodynamic gene delivery has been previously reported.[24,25] Briefly, 10 μg of plasmid DNA carrying mouse *Ifnβ1* gene in saline solution with a volume equal to 9% body weight was injected into a mouse tail vein over 5–8 s. Plasmid carrying mouse *Seap* (secreted alkaline phosphatase) gene was used as a control. Immediately after plasmid injection, animals were put on HFD for nine weeks, during which body weight and food intake were measured weekly, and body composition analysis was performed at the end of the experiment using EchoMRI-100 (Echo Medical Systems, Houston, TX). Mice were euthanized nine weeks after plasmid injection, and organs were immediately collected. Throughout analytical assays, epididymal fat was used as white adipose tissue. All measurements were performed by an investigator in blinded fashion.

Evaluation of glucose homeostasis

Intraperitoneal glucose tolerance test (IPGTT) was carried out in mice that fasted for 6 h. Glucose solubilized in phosphate-buffered saline was injected (*i.p.*) at 2 g/kg, and the time-point was set as 0 min. Blood glucose was measured at predetermined time-points (0, 30, 60, and 120 min) using glucose test strips and glucose meters. Insulin tolerance test (ITT) was performed in mice that fasted for 4 h. Insulin (Humulin, 0.75 U/kg) purchased from Eli Lilly (Indianapolis, IN) was injected (*i.p.*) and blood glucose was measured at predetermined time-points identical to IPGTT. Blood insulin was measured using an ELISA kit (#10-1113-01) purchased from Mercodia Developing Diagnostics (Winston Salem, NC). HOMA-IR was calculated by using the formula: HOMA-IR = [fasting insulin (ng/ml) × fasting plasma glucose (mg/dl)/405].

H&E staining

Tissue samples were collected, fixed in 10% neutrally buffered formalin, and dehydrated using increasing ratios of ethanol/water (v/v). Tissue samples were embedded into paraffin for 16 h. Paraffin-embedded tissue samples were cut into sections at 6 μm in thickness and dried at 37 °C for 1 h before incubation in xylene, followed by a standard H&E staining using a commercial kit (BBC Biochemical, Atlanta, GA).

Oil-red O staining

Freshly collected liver samples were immediately frozen in liquid nitrogen. Tissue sections were cut at 8 μm in thickness using a Cryostat. Sections were placed on slides and fixed using neutrally buffered formalin for 30 min. The sections were washed with 60% isopropanol before being stained with freshly prepared Oil-red O working solution (#26079-05, Electron Microscopy Sciences) and counterstained with haematoxylin.

Determination of liver triglyceride

Freshly collected liver samples (200–300 mg) were homogenized in 1 ml of phosphate buffered saline, and protein concentration was determined. Total lipids in homogenate were extracted by addition of 5 ml of chloroform-methanol (2:1, vol/vol) mixture and incubated overnight at 4 °C. The tissue homogenates were then centrifuged at 12,000 rpm for 20 min, and the supernatants were dried and the contents re-dissolved in 2% Triton X-100. Hepatic triglyceride level was determined by using a commercial kit from Thermo-Scientific (Waltham, MA).

Gene expression analysis

Total mRNA was isolated from the collected tissues using TRIZOL reagent purchased from Invitrogen (Carlsbad, CA). One μg of total RNA was used for first strand cDNA synthesis using a Superscript RT III enzyme kit from Invitrogen (Carlsbad, CA). Quantitative real-time PCR (qPCR) was performed using SYBR Green as the detection reagent on the ABI StepOnePlus Real-Time PCR system. The data were analyzed using the ΔΔCt method, and normalized to internal control of GAPDH mRNA. Primers employed were synthesized in Sigma (St. Louis, MO) and their sequences are summarized in Supplementary Table 1.

Statistics

All results are expressed as means ± SD, and statistical difference was determined using student t-test and analysis of variance. A value of $P < 0.05$ was considered significant difference.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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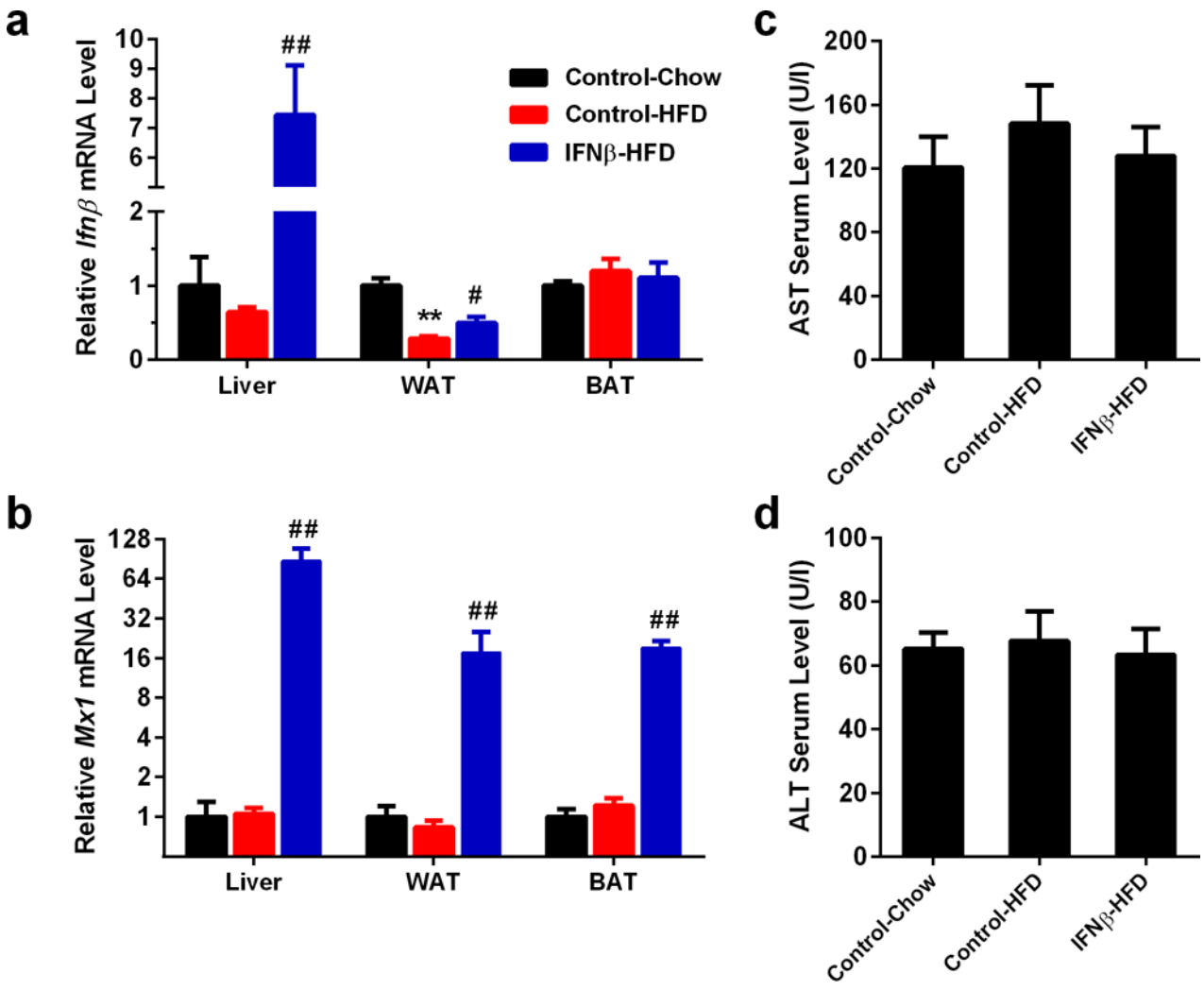

Figure 1.

Efficiency and safety of *IFNβ1* gene transfer using hydrodynamic delivery method. Mice received hydrodynamic injection of plasmid DNA carrying *Seap* (control) or *Ifnβ1* and fed with HFD for 9 weeks. Chow-fed mice received hydrodynamic injection of plasmid DNA carrying *Seap.* (**a**) Relative mRNA levels of *Ifnβ1* in liver, WAT and BAT. (**b**) Relative mRNA levels of *Mx1* in liver, WAT and BAT. (**c**) Serum level of aspartate aminotransferase (AST). (**d**) Serum level of alanine aminotransferase (ALT). Values represent average ± SD (n=5). ** $P < 0.01$ compared with chow-fed *Seap*-injected mice. $^{\#}$ $P < 0.05$, $^{\#\#}$ $P < 0.01$ compared with HFD-fed and *Seap*-injected mice.

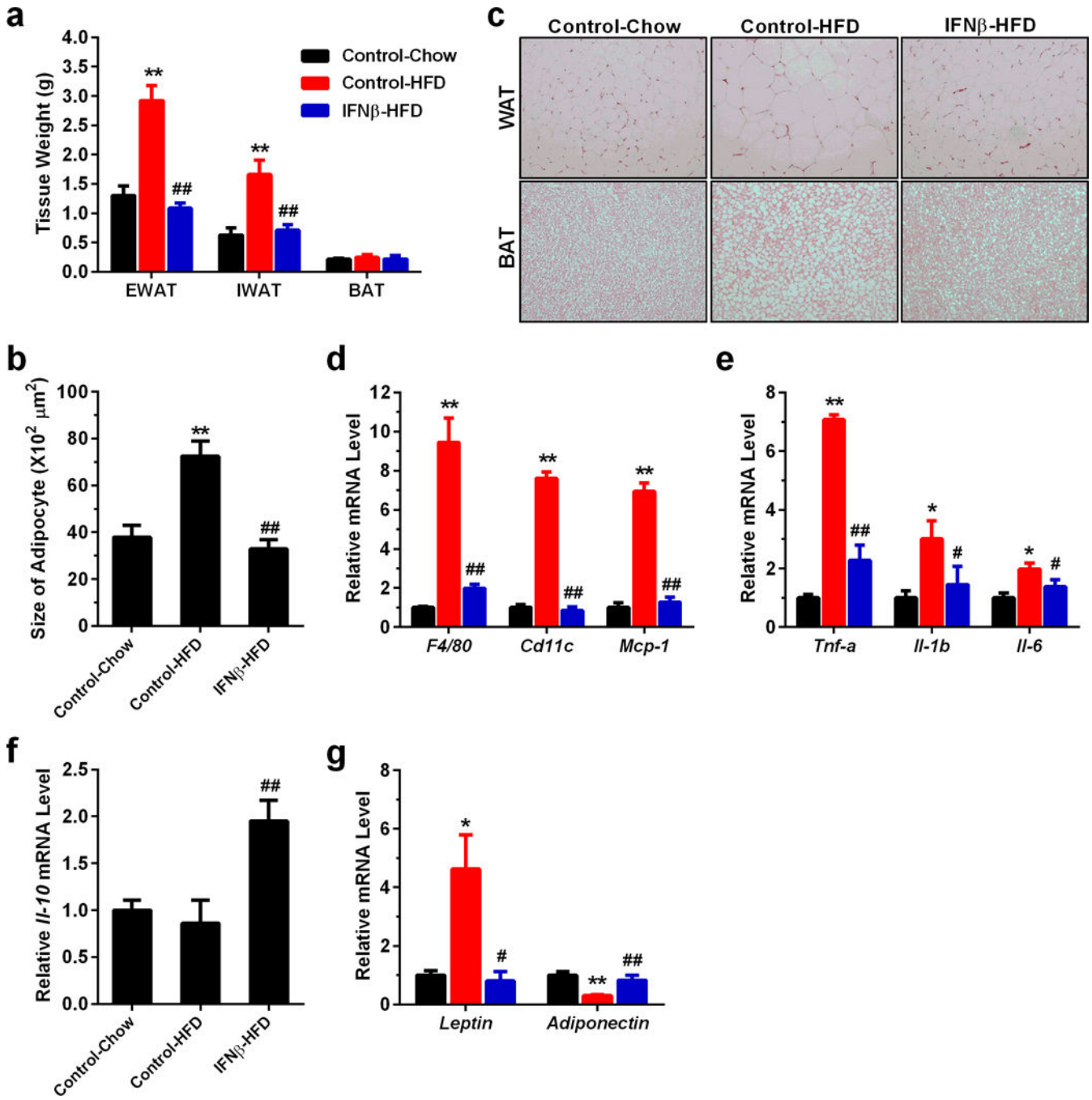

Figure 2.

IFNβ1 effects on HFD-induced adipose tissue inflammation and adipocyte hypertrophy**.** (**a**) Average weights of epididymal WAT, inguinal WAT, and BAT. (**b**) Average areas of adipocytes in epididymal WAT. (**c**) Representative images of H&E staining of WAT and BAT (100x). (**d**) Relative mRNA levels of *F4/80*, *Cd11c*, and *Mcp1* in WAT. (**e**) Relative mRNA levels of *Tnf-α*, *Il-β*, and *Il-6* in WAT. (**f**) Relative mRNA levels of *Il-10* in WAT. (**g**) Relative mRNA levels of *Leptin* and *Adiponectin* in WAT. Values represent average ± SD

(n=5). * $P < 0.05$, ** $P < 0.01$ compared with chow-fed *Seap*-injected mice. $^{\#}$ $P < 0.05$, $^{\#\#}$ $P < 0.01$ compared with HFD-fed and *Seap*-injected mice.

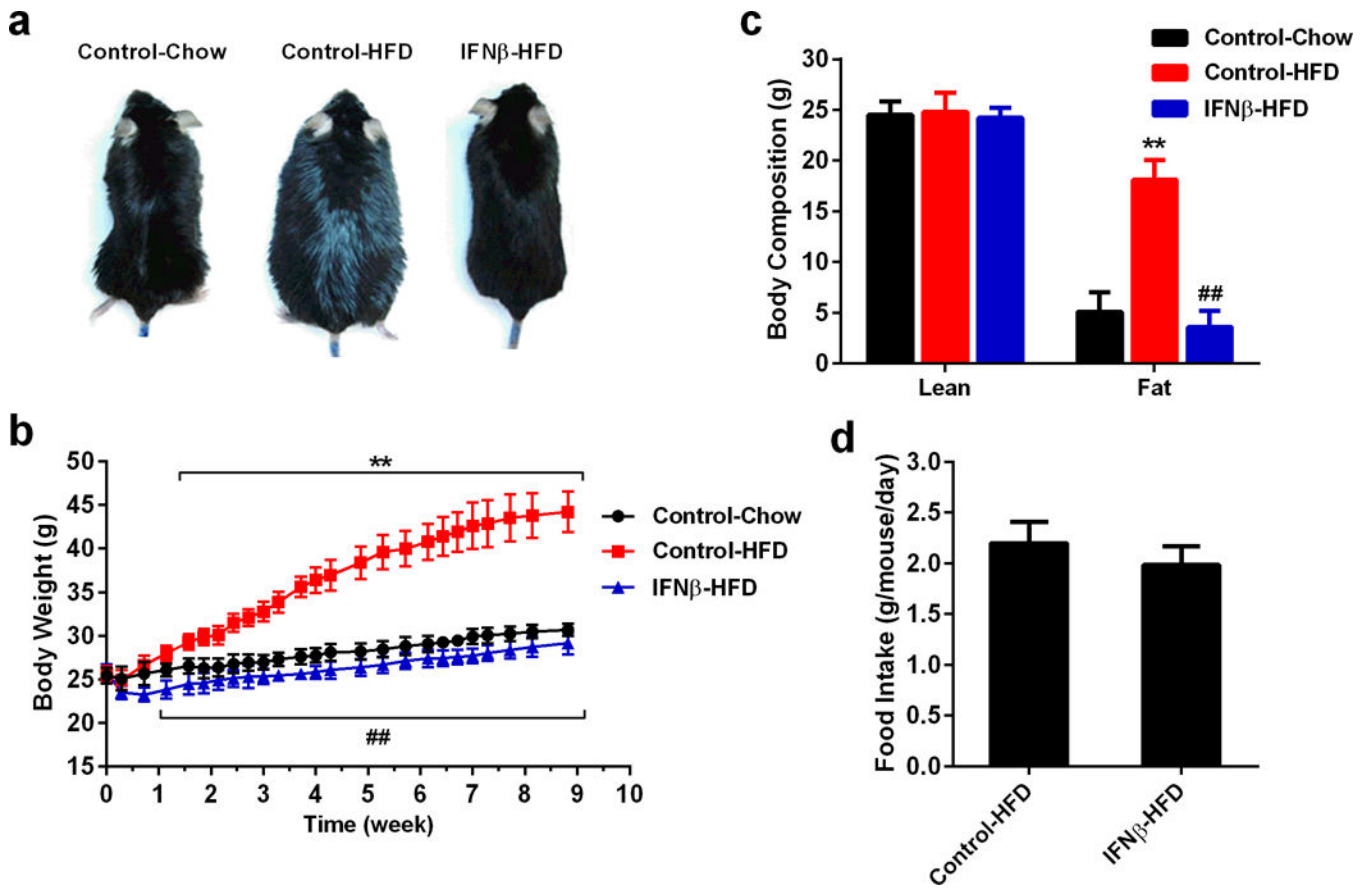

Figure 3.

IFNβ1 effects on body weight and composition, and food intake. (**a**) Representative images of mice at the end of 9-week experiment. (**b**) Growth curves of control and IFNβ1-treated mice over a 9-week period. (**c**) Body composition of mice from the three groups. (**d**) Average food intake over the 9-week period. Values represent average ± SD (n=5). ** $P <$ 0.01 compared with chow-fed *Seap*-injected mice. $^{\#\#}$ $P < 0.01$ compared with HFD-fed and *Seap*-injected mice.

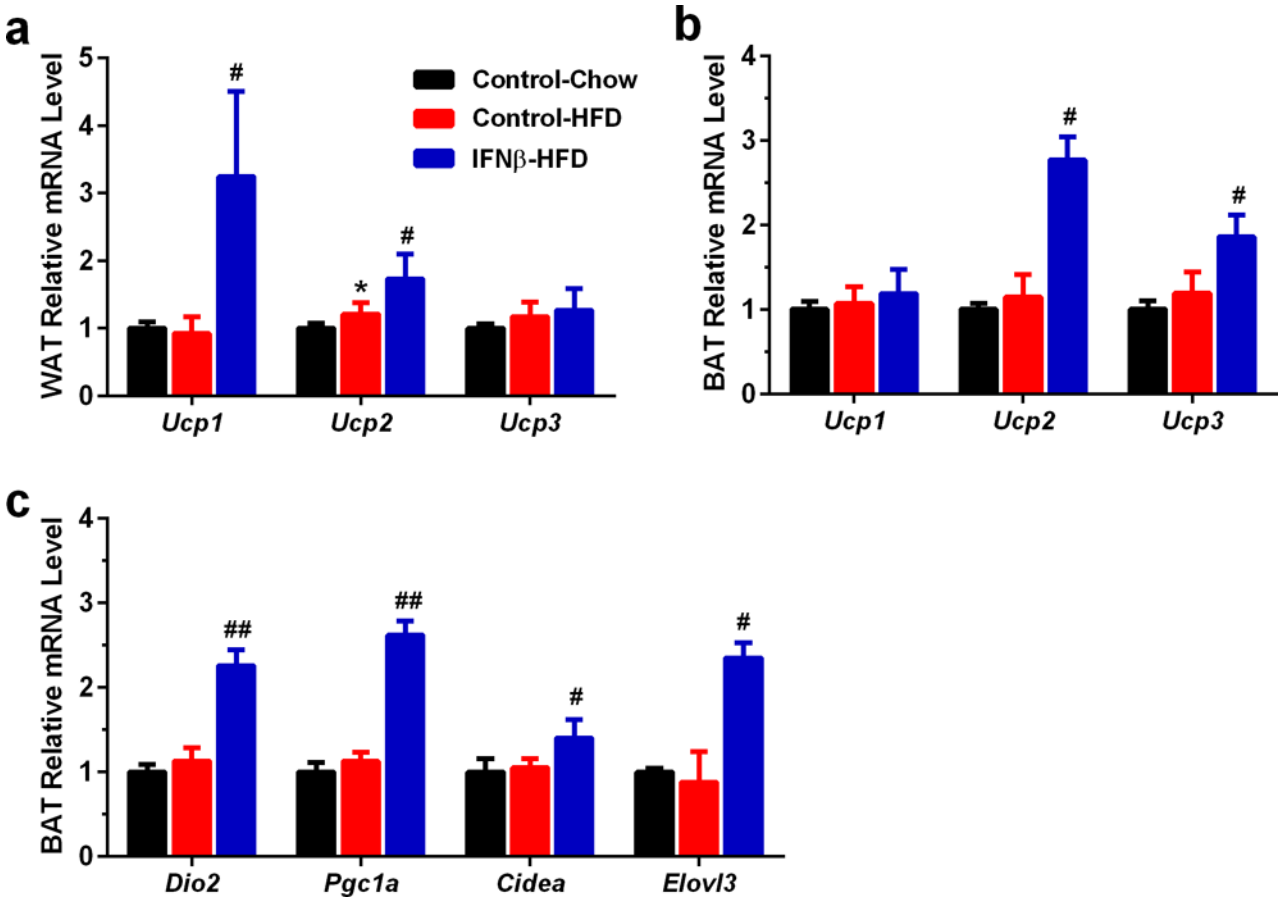

Figure 4.

IFNβ1 effects on thermogenic genes in WAT and BAT. (**a**) Relative mRNA levels of *Ucp1*, *Ucp2* and *Ucp3* in WAT. (**b**) Relative mRNA levels of *Ucp1*, *Ucp2* and *Ucp3* in BAT. (**c**) Relative mRNA levels of *Dio2*, *Pgc1-α*, *Cidea* and *Elovl3* in BAT. Values represent average ± SD (n=5). * $P < 0.05$ compared with chow-fed *Seap*-injected mice. $^{\#}$ $P < 0.05$, $^{\#\#}$ $P < 0.01$ compared with HFD-fed *Seap*-injected mice.

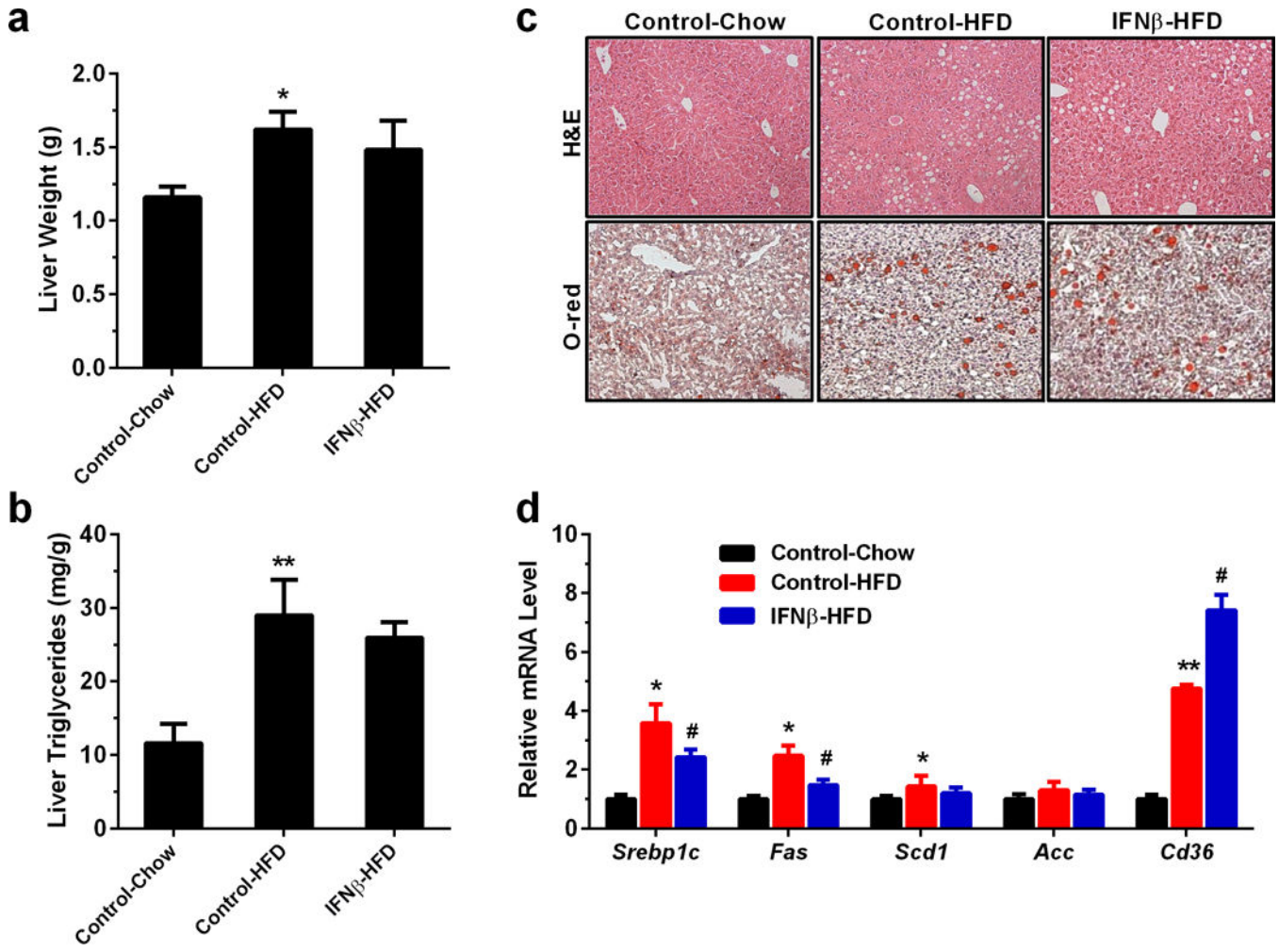

Figure 5.

IFNβ1 effects on HFD-induced fatty liver and hepatic lipogenesis. (**a**) Average liver weight in chow- and HFD-fed mice. (**b**) Average liver triglyceride content in these mice. (**c**) Representative images of H&E staining (upper panel) and O-red oil staining (lower panel) of liver sections (100x). (**d**) Relative mRNA levels of *Srebp1c*, *Fas*, *Scd1*, *Acc*, and *Cd36* in liver. Values represent average ± SD (n=5). * $P < 0.05$, ** $P < 0.01$ compared with chow-fed *Seap*-injected mice. # $P < 0.05$ compared with HFD-fed and *Seap*-injected mice.

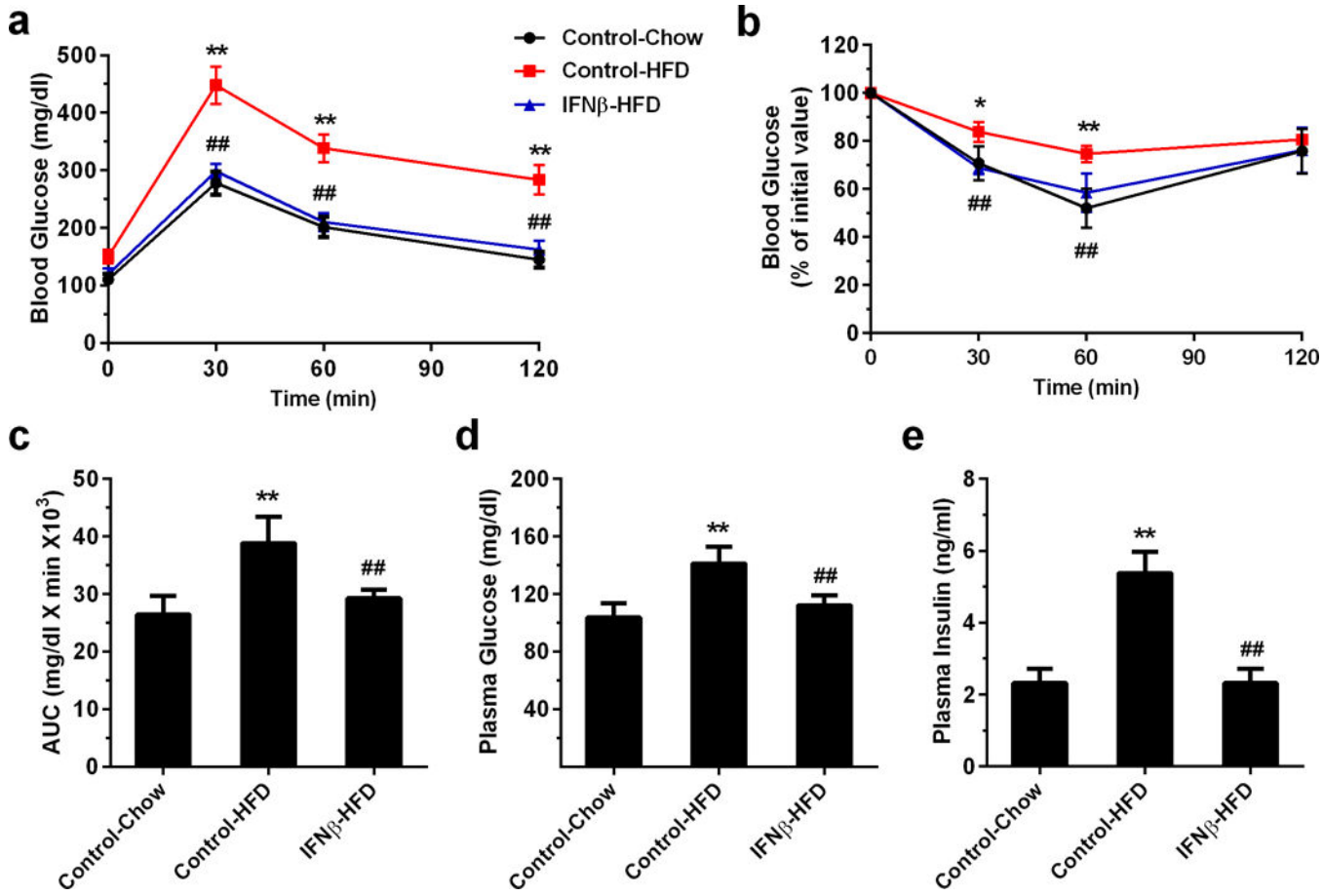

Figure 6.

IFNβ1 effects on insulin sensitivity and glucose homeostasis. (**a**) Blood glucose level as a function of time in intraperitoneal glucose tolerance test (IPGTT). (**b**) Blood glucose level as a function of time in insulin tolerance test (ITT). (**c**) Calculated area under the curve for IPGTT. (**d**) Blood levels of fasting glucose. (**e**) Blood levels of fasting insulin. Values represent average ± SD (n=5). * $P < 0.05$, ** $P < 0.01$ compared with chow-fed *Seap*-injected mice. ## $P < 0.01$ compared with HFD-fed *Seap*-injected mice.