Fenretidine Ameliorates Insulin Resistance and Fatty Liver in Obese Mice

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Fenretidine (FEN), a ligand of retinol binding protein 4 (RBP4), has been suggested as a measure to reduce insulin resistance and its associated disorders such as obesity, and fatty liver by reducing serum RBP4. We investigated whether there is another possible mechanism by which fenretidine reduces insulin resistance and fatty liver in genetically obese (ob/ob) mice. Male obese mice fed a high-fat diet (45% of calories from fat) were divided into two groups (n=13 each). One (FEN) received fenretidine (20mg/kg body weight, intraperitoneally) and the other (O) received vehicle three times weekly for 24 d. C57BL/6J mice fed a normal-fat diet (16% of calories from fat) were used as a control (C; n=13). No changes in fat weight and serum leptin level could be observed in FEN mice. Lower plasma RBP4 was observed in FEN mice compared with O mice. Fenretidine improved whole-body insulin sensitivity based on glucose and insulin tolerance tests and the homeostasis model assessment of insulin resistance. Fenretidine decreased the plasma lipid (triglyceride, cholesterol, and free-fatty acid) levels, hepatic TG level, and histological steatosis score. The mechanism by which fenretidine prevents fatty liver may be explained by an increased plasma adiponectin level, increased activation of hepatic AMP-activated protein kinase, and the expression of peroxisome proliferator-activated protein-α and peroxisomal acyl-CoA oxidase, which promote fat oxidation. FEN alleviated insulin resistance and fatty liver in obese mice and thus may act as an anti-lipidemic and anti-diabetic drug.

Key words fenretidine; retinol binding protein-4; insulin resistance; fatty liver; fatty acid oxidation

Obesity is a worldwide public health problem and is associated with hypertension, dyslipidemia, nonalcoholic fatty liver disease (NAFLD), and type 2 diabetes, with insulin resistance being a common factor. The pathogenesis of these conditions occurs in insulin-acting peripheral tissues, including skeletal muscle, liver, and adipose tissue. In particular, insulin resistance-related adipose tissue dysfunction leads to the release of a high level of free-fatty acids and perturbs the levels of various adipokines, such as leptin, adiponectin, and retinol binding protein 4 (RBP4), which directly or indirectly affect insulin sensitivity by modulating insulin signaling and molecules involved in glucose and lipid metabolism in many tissues.

RBP4 is a compact, globular, 21-kDa protein and was known only as a retinol carrier synthesized and secreted by the liver, until recently. Although serum RBP4 originates largely from the liver under physiological conditions, it is secreted considerably from fat tissues in an insulin-resistant state. Yang et al. showed that adipose tissue-specific ablation of glucose transporter 4 increased RBP4 mRNA expression in adipocytes and serum RBP4 levels in rats, and focused on the link between the serum RBP4 level and insulin sensitivity. Despite some reports that serum RBP4 is not associated with insulin resistance, others have suggested that increasing RBP4 levels by transgenic overexpression of RBP4 or injection of purified RBP4 aggravate insulin resistance and related diseases. RBP4 gene knockout enhances insulin sensitivity, suggesting that the reduction of serum RBP4 may reduce insulin resistance. RBP4 is present in the serum as a complex with transthyretin (TTR), which effectively increases the molecular weight of RBP4 and protects it from glomerular filtration. Among the RBP4-lowering agents, fenretinide [(4-hydroxyphenyl)retinamide] (FEN), a synthetic retinoid and anticancer agent, reduces serum RBP4 levels by interfering with RBP4 binding to TTR, resulting in an improvement in insulin sensitivity, even showing an anti-obesity effect.

Unlike other retinoids, fenretinide at moderate doses in animal models is not toxic to the liver or other organs, as it is not stored in the liver. Although its actions on the serum RBP4 level and adiposity have been observed, the molecular mechanisms of fenretinide actions in tissues, especially the liver, are not clearly understood.

Due to the relationship between the levels of adipocytokine RBP4 and insulin resistance with related metabolic disorders, RBP4 lowering effect of fenretinide is noticeable as anti-lipidemic and anti-diabetic drug. Even though the direct relationship between fenretinide and fat oxidation has not been reported yet, the increase in plasma adiponectin by fenretinide in the present study was coincided with down-stream consequences of AMP-activated protein kinase (AMPK) activation which in turn would increase fat oxidation in the tissue. In this study, we demonstrate that fenretinide decreases weight gain and insulin resistance and fatty liver in genetically obese (ob/ob) mice fed a high-fat diet. Additionally, we investigated the decrease in fat accumulation in response to circulating RBP4-lowering synthetic retinoid fenretinide. We hypothesized that fenretinide reduces insulin resistance, and fatty liver possibly by increasing fat oxidation in a high fat-fed obese mouse model.

MATERIALS AND METHODS

Animals and Treatments All animal protocols were approved by the animal care and use committee of the

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National Institute of Health and the Korean Food and Drug Administration. Male C57BL/6J mice (6 weeks old; n = 13) and genetically obese ob/ob mice (6 weeks old; n = 26) were purchased from Japan SLC (Shizuoka, Japan). After 1 week of adaptation to a normal-fat diet (a purified diet based on the AIN-93 rodent diet; 4.0 kcal/g with 16% from fat, 64% from carbohydrates, and 20% from protein), ob/ob mice were fed a high-fat diet (Research Diets, New Brunswick, NJ, U.S.A.; 4.78 kcal/g with 45% from fat, 35% from carbohydrates, and 20% from protein) and divided into two groups: a fenretinide-treated group (FEN, n = 13) and a vehicle-treated group (O, n = 13). Fenretinide (dissolved in sterile 0.9% NaCl solution with 5% ethanol and 1.65 mg/mL bovine serum albumin) or vehicle was intraperitoneally (i.p.) injected three times weekly for 24 d (20 mg/kg body weight). Fenretinide (≥95% purity), bovine serum albumin (fraction V), and reagent grades of all other chemicals were obtained from Sigma Chemical (St. Louis, MO, U.S.A.). Control C57BL/6J mice (C group, n = 13) were fed a normal-fat diet. Food intake was recorded daily and body weights were monitored every 3 d during the feeding period.

After overnight-fasting and anesthetization with carbon dioxide, blood samples were stored into a heparin-coated tube by heart puncture and plasma was obtained after centrifugation of the blood at 1500 × g for 15 min at 4°C. Collected samples of plasma, liver, and visceral fat pad were stored at −70°C until analyzed.

Glucose- and Insulin Tolerance Tests After 2 weeks of treatment, a glucose tolerance test (GGT) was performed by i.p. injection of glucose (1 g/kg body weight) after overnight fasting. After 3 weeks of treatment, an insulin tolerance test (ITT) was performed by i.p. injection of insulin (0.75 U/kg body weight, R-Insulin; Lilly Research Laboratories, Indianapolis, IN, U.S.A.) 4 h after food withdrawal. Glucose in a tail vein blood sample was measured at 15- or 30-min intervals for ≤2 h, using a glucometer (Arkray Global Business, Inc., Kyoto, Japan). Commercial kits were used according to the manufacturer’s instructions for measuring plasma glucose (Asan Pharmaceutical, Seoul, South Korea) and insulin (Linco Research, St. Charles, MO, U.S.A.) in plasma samples collected at sacrifice. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as an insulin sensitivity index.

Biochemical Analysis Plasma leptin and adiponectin were measured with commercial enzyme-linked immunosorbent assay (ELISA) kits (Linco Research) according to the manufacturer’s instructions. Plasma concentrations of triglyceride (TG), total cholesterol, high-density lipoprotein (HDL)-cholesterol, free-fatty acids (FFAs), and plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined enzymatically using commercial kits (Asan Pharmaceutical). Hepatic lipids were extracted as described previously, the dried lipid residue was dissolved in 1 mL of ethanol, and the same enzymatic kits were used to measure cholesterol and TG concentrations in the hepatic lipid extracts.

Western Blot Analysis Plasma RBP4 and TTR were measured by quantitative Western blot analysis using protein standards prepared from purified full-length mouse recombinant RBP4 and purified plasma TTR (Sigma). Forty-fold diluted plasma samples were separated by sodium dodecyl sulfate (SDS)/12% polyacrylamide gel electrophoresis (PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was immunoblotted with specific antibodies against RBP4 (Dako, Hamburg, Germany) or TTR (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.).

Equal amounts of whole-cell lysate proteins from liver or epididymal fat pads were separated by SDS-PAGE, transferred to PVDF membranes, and immunoblotted with primary antibodies against fatty acid synthase (FAS), sterol regulatory element-binding protein 1 (SREBP1), peroxisome proliferator-activated protein-α (PPARα), acetyl-coA carboxylase-α (ACCα), or phospho(Ser78/Ser80)-ACCα (all from Santa Cruz Biotechnology), and AMP-activated protein kinase (AMPK) or phospho(Thr172)-AMPK (Cell Signal, Danvers, MA, U.S.A.).

The proteins were detected by enhanced chemiluminescence detection system (Amersham Biosciences, Uppsala, Sweden) and quantified using Quantity One analysis software (Bio-Rad, Hercules, CA, U.S.A.). Each blot was also probed with antibodies against α-tubulin or β-actin to verify equal loading of samples.

Histological Analysis For hematoxylin and eosin (H&E) staining, a piece of liver or epididymal white adipose tissue of mice was fixed in 4% buffered formalin, embedded in paraffin, cut into 5-μm sections, mounted on glass slides, stained after dehydration. The sections were analyzed under a light microscope (Axioplan 2; Zeiss, Goettingen, Germany) and photographed at a final magnification of ×200. Hepatic steatosis was graded as 0 (0% of hepatocytes involved with macrovesicular steatosis), 1 (<33% involved), 2 (33—66% involved), and 3 (>66% involved), according to the percentage of hepatic fat droplets. The average size of adipocytes was measured using Image Analysis software (Image-Pro Plus 4.0; Media Cybernetics, Inc., Bethesda, MD, U.S.A.).

Statistical Analysis All values are expressed as mean ± standard error of the mean (S.E.M.). Statistical analysis was performed by one-way analysis of variance followed by Duncan’s multiple-range test, using SPSS 15.0 software for Windows (SPSS, Chicago, IL, U.S.A.). Means with different letters are significantly different at p < 0.05.

RESULTS

Reduction of Body Weight Gain and Food Efficiency Ratio by Fenretinide in Obese Mice The final body weight and body weight gain of O mice at 24 d (48.6 ± 0.8, 10.9 ± 0.5 g, respectively) were 91% and 331% greater, respectively, than the values for the C mice (25.4 ± 0.2, 2.5 ± 0.1 g, respectively). Body weight gain in FEN mice (9.4 ± 0.5 g) was ca. 13% less than that in O mice (10.9 ± 0.5 g) at 24 d (p < 0.05).

Fenretinide treatment did not alter food intake, but it reduced the food efficiency ratio (FER) in O mice by 13% (FEN vs. O: 0.075 vs. 0.085, p < 0.05), indicating an increased metabolic rate in FEN mice.

Adiposity, as assessed by total visceral fat depots, was higher in O mice than in C mice. Since ob/ob mice could not produce leptin genetically, plasma leptin levels in O mice were lower than that in C mice (0.98 ± 0.07, 3.82 ± 0.36 ng/mL), although O mice have higher fat weight. Fat weight was unaffected by fenretinide treatment (Fig. 1A). The plasma leptin level was not significantly changed by fenretinide.
(1.06±0.29 ng/mL). However, adipocytes in O mice had a larger surface area compared with adipocytes in C mice and fenretinide significantly reduced adipocyte surface area in ob/ob mice (Fig. 1B).

**Regulation of RBP4 and TTR Levels by Fenretinide in Obese Mice**

We measured plasma levels of RBP4 and transthyretin (TTR) in C, O, and FEN mice. As seen in Fig. 2A, the plasma RBP4 level in O mice was 1.3-fold that in C mice, and the plasma TTR level in O mice was 4.1-fold that in C mice. Relative to the levels in O mice, the plasma RBP4 and TTR levels decreased by 29% and 42%, respectively, with fenretinide treatment ($p<0.05$).

The RBP4 and TTR levels in adipose tissue were higher in obese mice than in C mice, and both levels decreased significantly (46, 42%, respectively; $p<0.05$) with fenretinide (Fig. 2B). However, this decrement was only observed in the hepatic TTR level (28%, $p<0.05$), and not in the hepatic RBP4 level (Fig. 2C).

**Improvement of Insulin Sensitivity by Fenretinide in Obese Mice**

Plasma levels of glucose and insulin were measured to investigate the consequences of fenretinide treatment on whole-body glucose and insulin tolerance, using GTT and
Fig. 3. Effect of Fenretinide on Insulin Sensitivity in Obese Mice

(A) and (B) Area-under-the-curve values calculated from oral glucose tolerance tests and insulin tolerance tests in the three groups (n=13/group). (C) and (D) Plasma levels of glucose (mg/dL) and insulin (ng/mL) measured in the three groups after 24 d (n=13/group). (E) Homeostasis model assessment of insulin resistance in the three groups (n=13/group). For graphs: white bars, C; black bars, O; and gray bars, FEN mice. Means with different letters are significantly different at p<0.05.

Fig. 4. Effect of Fenretinide on Plasma and Hepatic Lipid Levels in Obese Mice

White bars, control (C) mice; black bars, obese (O) mice; and gray bars, fenretinide (FEN) mice (n=13/group). (A) Plasma triglyceride (TG), total cholesterol, and HDL-cholesterol levels (mg/dL). (B) Plasma free fatty acid level, and (C) Hepatic TG level in the three groups of mice. (D) Histological changes in fatty liver states in the C, O, and FEN groups (E). Hepatic steatosis was graded according to Brunt et al. (for details, see Materials and Methods). Hematoxylin and eosin stain (scale bars, 100 μm) (n=5/group). Means with different letters are significantly different at p<0.05.
After 2 weeks of fenretinide treatment, systemic glucose clearance assessed with the GTT was improved compared with O mice, as demonstrated by a significant reduction (ca. 18%, \(p < 0.05\)) in the area under the curve (AUC) (Fig. 3A). After 3 weeks, FEN mice showed a smaller AUC (ca. 81%) on an ITT, indicating improved sensitivity to insulin (Fig. 3B; \(p < 0.05\)).

After 24 d, the fasting plasma glucose and insulin levels were measured. FEN mice showed significantly lower levels of fasting plasma glucose (by 27%, \(p < 0.05\)) and insulin (by 29%, \(p < 0.05\)) compared with O mice, indicating overall improvement in glucose homeostasis (Figs. 3C—E).

**Regulation of Plasma and Tissue Lipid Levels by Fenretinide in Obese Mice** The plasma levels of total cholesterol and TG in FEN mice were 18% and 17% lower, respectively, than those in O mice (Fig. 4A; \(p < 0.05\)). The plasma FFA concentration was higher in O mice than in C mice, and fenretinide decreased the level by 22% (Fig. 4B; \(p < 0.05\)). However, fenretinide did not affect the plasma HDL cholesterol level in ob/ob mice.

The hepatic TG concentration was higher in O mice than in C mice (\(p < 0.05\)), and fenretinide lowered the TG level in ob/ob mice by >30% (Fig. 4C; \(p < 0.05\)). Consistent with hepatic TG accumulation, histological examination with H&E staining (Fig. 4D) and hepatic steatosis grading (Fig. 4E) confirmed a significant improvement in liver steatosis in FEN mice, which exhibited smaller and fewer lipid droplets compared with O mice. While significantly more severe steatosis (\(p < 0.05\)) was observed in O mice compared with C mice, which showed little or no histological evidence of hepatic steatosis, fenretinide produced a marked reduction (\(p < 0.05\)) in the degree of steatosis in the ob/ob mice. Histological examination did not reveal any sign of fenretinide-related liver damage; instead, the alleviated steatosis was accompanied by improved liver function, as assessed by plasma ALT (O vs. FEN: 122.89±9.29 vs. 112.01±7.45, C: 15.84±0.82) and AST (O vs. FEN: 102.51±9.19 vs. 76.71±5.92, C: 15.13±0.84) activities (IU/L, \(p < 0.05\)).

**Hepatic Lipid Metabolism Alterations by Fenretinide in Obese Mice** The plasma adiponectin level was 53% lower in O mice than in C mice (4.80±0.32 μg/mL vs. 10.28±0.43 μg/mL, respectively), and the adiponectin level increased significantly with fenretinide (5.92±0.36 μg/mL, \(p < 0.05\)). Based on the above results of adiponectin increase by fenretinide, and previous reports showing the effect of adiponectin on hepatic lipid metabolism and AMPK regulation, \(^4\) we investigated the changes in activation of hepatic AMPK. Fenretinide treatment resulted in a significant improvement of ca. 21% (\(p < 0.05\)) in the level of phospho(Thr172)-AMPK protein (pAMPK/AMPK.

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**Fig. 5. Effect of Fenretinide on Hepatic Lipid Metabolism in Obese Mice**

(A) Western blot analysis of fatty acid oxidation-related hepatic proteins. Representative immunoblots of phospho(Thr172)-AMP-activated protein kinase (AMPK)-\(\alpha\), AMPK\(\alpha\), peroxisome proliferator-activated protein-\(\alpha\) (PPAR\(\alpha\)), phospho(Ser78/Ser80)-acetyl-coA carboxylase-\(\alpha\) (ACC\(\alpha\)), ACC\(\alpha\), peroxisomal acyl-CoA oxidase (AOX), and uncoupling protein 2 (UCP2) from liver are presented. Relative densitometric values (pAMPK/AMPK, pACC\(\alpha\)/ACC\(\alpha\), PPAR\(\alpha\), AOX, and UCP2; \(n=5\)) are expressed as means with standard errors indicated by vertical bars. (B) Western blot analysis of fatty acid synthesis-related hepatic proteins. Representative immunoblots of sterol regulatory element-binding protein 1 (SREBP1) and fatty acid synthase (FAS) are presented (\(n=5\)). For graphs: white bars, control (C) mice; black bars, obese (O) mice; and gray bars, fenretinide-treated (FEN) mice. Means with different letters are significantly different at \(p<0.05\)
ratio) in ob/ob mice (Fig. 5A). In addition, the phospho(Ser78/Ser80)-ACCα was significantly increased by fenretinide (Fig. 5A).

To further investigate the involvement of fenretinide in hepatic lipid homeostasis, we examined the effect of fenretinide treatment on lipid metabolism-related protein levels in ob/ob mice. Fenretinide increased the levels of proteins involved in fatty acid oxidation (Fig. 5A). The PPARα level, which was lower in O mice than in C mice, was significantly increased in FEN mice. Similarly, the lower level of AOX and UCP2 in O mice compared with C mice (−31% and −16%, respectively) was increased by fenretinide (+33% and +20%, respectively) (Fig. 5A).

The protein level of the lipogenesis-related transcription factor SREBP1 (p68, mature form) was not affected by fenretinide treatment (Fig. 5B). Furthermore, the protein level of FAS, a key lipogenic enzyme known as one of the SREBP1 targets, was significantly higher in O mice than in C mice. However FAS level was not affected by fenretinide.

Discussion

With the increased obesity prevalence, there was a trend toward a higher incidence of insulin resistance, hyperlipidemia, cardiovascular diseases, type 2 diabetes, and fatty liver. Adipocyte-derived cytokines have been proposed as diagnostic markers and therapeutic targets for obesity-linked insulin resistance, diabetes, and fatty liver. Adipocyte-specific increases in RBP4 mRNA expression and increases in serum RBP4 protein occur concurrently with disrupted glucose homeostasis, and genetically regulated or exogenously treated RBP4 modulates insulin sensitivity in skeletal muscle and liver. Among possible therapeutic interventions, fenretinide has been suggested as an effective drug to treat obesity and insulin resistance.

Originally developed as a chemotherapeutic agent, fenretinide can also lower serum RBP4 levels in rodents and humans by disrupting the ternary complex of retinol-RBP4-TTR, thereby promoting renal clearance of RBP4, with few side effects and with potential benefits for insulin sensitivity and glucose metabolism. Short-term treatment with fenretinide decreases circulating RBP4 levels only, whereas 16 weeks of treatment enhances insulin sensitivity. Long-term treatment (34 weeks) also decreases the severity of obesity.

While fenretinide showed different potentials depending on the duration and observed parameters, the mechanisms by which fenretinide can reverse insulin resistance and obesity were not completely clear. Here, we investigated the effects of fenretinide on weight gain, hepatic fat accumulation, and biochemical and molecular parameters related to lipid metabolism in genetically obese mice fed a high-fat diet, in order to explore the mechanisms by which fenretinide reduces fatty liver severity and enhances insulin sensitivity.

Preitner et al. observed that fenretinide reduced high-fat diet-induced adiposity and hyperleptinemia in C57BL/6J mice. Although we observed less weight gain in the FEN group than the O group, we did not observe any significant modification of adiposity or plasma leptin in fenretinide-treated obese mice. Reported responses to fenretinide have varied among different treatment durations and among subjects with different genetic backgrounds. The differences in the delivery route or the duration of drug treatment, and animal strains could be the reasons for the difference in the effect of fenretinide on fat accumulation and the expression SREBP1, an indicator of lipogenesis. While Preitner et al. provided relatively higher dose of fenretinide by diet, we provided it by intraperitoneal injection to achieve the similar level with the direct systemic absorption of fenretinide. In addition, in the present study, obese mice were subjected for 3 weeks of relatively short-term treatment to avoid the projected effect of fenretinide as an anti-proliferative/anti-cancer drug. Since our ob/ob mice had defects in leptin production, fenretinide could not show any significant reduction in plasma leptin. Nevertheless, fenretinide increased plasma adiponectin and decreased body weight gain and FER in ob/ob mice in the present study. Fenretinide could increase metabolic rate based on the observation lower FER in FEN mice than that in O mice. In addition, despite no significant fat weight reduction, fenretinide-treated obese mice showed some improvement in the insulin sensitivity of adipocytes, based on reduced fat cell size. Similar to the results of Mody et al., we found that fenretinide decreased circulating RBP4 and TTR levels. Also, a significant reduction of RBP4 level in adipose tissue was observed in fenretinide-treated obese mice. O mice showed greater whole-body insulin resistance as assessed by the GTT, ITT, and HOMA-IR index than C mice. However, fenretinide treatment decreased whole-body insulin resistance. Thus, the reduction in insulin resistance could be observed in consistent with other studies even without a significant decrease in fat weight.

Fenretinide also partly inhibited increases in the plasma levels of FFA, TG, and cholesterol in the present study, and less hepatic fat accumulation was observed in FEN mice than in O mice (Figs. 4C, D). It is possible that lower levels of circulating lipids are transported to tissues, including the liver, following fenretinide treatment. Fat buildup in the liver is regulated by the integrated activities of cellular enzymes that catalyze lipid uptake, synthesis, oxidation, and export. To investigate the mechanism by which fenretinide affects lipid metabolism in obese mice, we examined the fenretinide-associated changes in expression of several genes related to lipid metabolism and energy production.

As described above, the plasma adiponectin level differed between vehicle-treated and fenretinide-treated mice in the present study. Adiponectin activates the AMPK pathway, and this may provide the mechanistic link between adiponectin levels and the associated fatty acid oxidation, lipid synthesis, and hepatic steatosis. Activated AMPK phosphorylates and inactivates ACCα, consequently preventing the production of malonyl-CoA, which is an allosteric inhibitor of CPT-I, the rate-limiting enzyme in fatty acid oxidation, and resulting in reduced lipid synthesis and enhanced fatty acid oxidation. In the present study, fenretinide treatment induced the plasma adiponectin level, the activation of AMPK, and the phosphorylation of ACCα, resulting in reduced TG synthesis and enhanced fatty acid oxidation.

Fenretinide treatment also increased the level of adiponectin-stimulated transcription factor PPARα, which controls the transcription of genes encoding fatty acid oxidation enzymes such as AOX and UCP2. In our study, the protein levels of peroxisomal enzyme AOX and mitochondrial enzyme UCP2 were increased with fenretinide treatment. These results sug-
gest that fenretinide may universally affect fatty acid oxidizing enzymes in obese mice. Although activated AMPK can downregulate the expression of SREBP1, a transcription factor that regulates cholesterol and lipid synthesis, we did not detect reduced expression of SREBP1 or FAS, a SREBP1-induced lipogenic enzyme. Thus, as fenretinide affects AMPK activation and PPARα induction, which increases AOX expression, it appears that the lower hepatic fat accumulation seen with fenretinide treatment originates from increased fatty acid oxidation and not from reduced lipogenesis.

Based on the results presented above, short duration of fenretinide treatment at low dose could decrease FER, fat cell size, insulin resistance, serum lipids level and liver fat accumulation. Those beneficial effects on fenretinide could be from the decrease in circulating RBP4, and the increases in plasma adiponectin. Even though we did not show the direct effect that adiponectin leads to AMPK activation and PPARα induction, the increase in adiponectin was coincided with down-stream consequences of AMPK activation and PPARα induction in fenretinide treated mice. Both AMPK activation and PPARα induction could increase fat oxidation.

We suggested fenretinide could be a safe anti-lipidemic and anti-diabetic drug for alleviating insulin resistance, dyslipidemia, and fatty liver when used at low dose. Fenretinide can regulate the degree of insulin resistance and fatty liver in obese models, at least in part, by reducing the plasma RBP4 level and increasing hepatic fatty acid oxidation.

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REFERENCES


