Long-term Fenretinide treatment prevents high-fat diet-induced obesity, insulin resistance, and hepatic steatosis

Frederic Preitner,* Nimesh Mody,* Timothy E. Graham, Odile D. Peroni, and Barbara B. Kahn

Division of Endocrinology, Diabetes, and Metabolism, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts

Submitted 3 June 2009; accepted in final form 13 October 2009

Preitner F, Mody N, Graham TE, Peroni OD, Kahn BB. Long-term Fenretinide treatment prevents high-fat diet-induced obesity, insulin resistance, and hepatic steatosis. Am J Physiol Endocrinol Metab 297: E1420–E1429, 2009. —The synthetic retinoid Fenretinide (FEN) increases insulin sensitivity in obese rodents and is in early clinical trials for treatment of insulin resistance in obese humans with hepatic steatosis (46). We aimed to determine the physiological mechanisms for the insulin-sensitizing effects of FEN. Wild-type mice were fed a high-fat diet (HFD) with or without FEN from 4–5 wk to 36–37 wk of age (preventive study) or following 22 wk of HF diet-induced obesity (12 wk intervention study). Retinol-binding protein-4 (RBP4) knockout mice were also fed the HFD with or without FEN in a preventive study. FEN had minimal effects on HFD-induced body weight gain but markedly reduced HFD-induced adiposity and hyperleptinemia in both studies. FEN-HFD mice gained epididymal fat but not subcutaneous or visceral fat mass in contrast to HFD mice without FEN. FEN did not have a measurable effect on energy expenditure, food intake, physical activity, or stool lipid content. Glucose infusion rate during hyperinsulinemic-euglycemic clamp was reduced 86% in HFD mice compared with controls and was improved 3.6-fold in FEN-HFD compared with HFD mice. FEN improved insulin action on glucose uptake and glycogen levels in muscle, insulin-stimulated suppression of hepatic glucose production, and suppression of serum FFA levels in HFD mice. Remarkably, FEN also reduced hepatic steatosis. In RBP4 knockout mice, FEN reduced the HFD-induced increase in adiposity and hyperleptinemia. In conclusion, long-term therapy with FEN partially prevents or reverses obesity, insulin resistance, and hepatic steatosis in mice on HFD. The anti-adiposity effects are independent of the RBP4 lowering effect.

Serum RBP4 levels are elevated in insulin-resistant humans and in many mouse models of obesity and insulin resistance including high-fat diet (HFD) (4, 12, 14, 20, 49), although not all studies show this effect (19, 28). In many studies, the level of elevation correlates highly with the degree of insulin resistance in both mice and humans, and serum RBP4 levels are highly predictive of metabolic syndrome risk in a large population-based study (34). Furthermore, chronic administration of RBP4 to normal mice is sufficient to cause insulin resistance, and RBP4 knockout (KO) mice have enhanced insulin sensitivity (49). The possibility that elevated RBP4 plays a causative role in type 2 diabetes is also supported by the fact that people with a single nucleotide polymorphism in the RBP4 promoter that increases RBP4 expression and serum levels have increased risk for type 2 diabetes (17, 33, 45a). Many therapeutic interventions that improve insulin sensitivity are associated with lowering of serum RBP4 levels (14, 25, 37), and efforts are underway to develop RBP4-lowering agents to treat diabetes. While several approaches that reduce serum RBP4 levels confer insulin sensitivity (49, 51), short-term treatment with one nonretinoid small molecule, an RBP4-lowering agent, failed to do so (31). The synthetic retinoid, Fenretinide [N-(4-hydroxyphenyl)retinamide (FEN)] reduces serum RBP4 levels and improves insulin sensitivity (21, 49). Currently, there is a phase II trial of Fenretinide for treatment of insulin resistance in obese humans with hepatic steatosis (46). Thus, the mechanisms by which lowering RBP4 can result in insulin sensitivity are of great interest.

Fenretinide lowers serum RBP4 levels in rodents and humans by disrupting the ternary complex of retinol-RBP4-transthryretin and thereby promoting renal clearance of RBP4 (3, 10, 49). We found that up to 16 wk of Fenretinide treatment of mice on HFD prevented elevations of serum RBP4 levels that are usually seen with HFD-induced obesity, ameliorated insulin resistance, and normalized glucose tolerance without altering food intake or HFD-induced body weight gain (49). It appeared that Fenretinide prevents insulin resistance, at least in part, by lowering serum RBP4 levels (49).

Fenretinide was originally developed as a chemotherapeutic agent (30) because of its ability to attenuate cancer cell growth and its relatively low toxicity. It inhibits cell growth through the induction of apoptosis by mechanisms that are not well defined and appear to differ in different tissues (reviewed in Refs. 9 and 15). Fenretinide is now the most widely studied retinoid in clinical trials of breast cancer chemoprevention due to its selective accumulation in breast tissue and to its favorable toxicological profile (21, 29, 38, 52). Since our initial mouse studies indicated that Fenretinide could be a promising therapeutic agent to treat or prevent insulin resistance and glucose intolerance, and since human cancer trials demonstrated few
side effects, we sought to further investigate the mechanisms by which Fenretinide improves glucose homeostasis and insulin action. We aimed to determine whether Fenretinide has more global effects on energy balance and lipid homeostasis and whether the metabolic effects of Fenretinide are mediated entirely by lowering circulating RBP4 levels or also by other mechanisms.

METHODS

Animals. FVB male mice (3–4 wk old) were obtained from Tac- tonic. Mice with disruption of the RBP4 gene (RBP4KO) on a mixed background (C57BL/6J × 129Sv) were generously provided by Drs. William Blaner, Max Gottesman, and Loredana Quadro, (Columbia University) (35). Experimental cohorts were generated from breeding pairs of RBP4KO or wild-type (WT) littermates that were first-generation offspring from mice that were heterozygous for the RBP4 gene. Studies were conducted in accordance with the principles and procedures outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the IACUC at Beth Israel Deaconess Medical Center.

Diets. Mice were housed individually in a temperature-controlled room, on a 14:10-h light-dark cycle and with ad libitum access to food and water. In prevention studies, cohorts of either FVB mice or C57BL/6J/H11003 were then placed on HFD (55% fat calories, Teklad) (49). In intervention studies, some FVB mice (fed HF for 22 wk) were then placed on HFD + FEN. During breeding, RBP4KO C57BL/6J × 129Sv mice were fed Labdiet 5053, a standard chow diet. All diets contained 15–25 IU/g vitamin A, which is more than the minimum dietary retinol required to maintain normal vitamin A status in mice (2.5 IU/g diet) (42).

Body weight and food intake. Body weights were measured weekly. Food intake was measured weekly over the period 0–3 wk and later at 9–11 wk of FEN treatment in the FVB strain prevention study and 0–9 wk of FEN treatment in the intervention study, when body weights were similar, and 17–20 wk of FEN treatment in the C57BL/6J × 129Sv strain (RBP4KO and WT) study, when body weights had begun to diverge.

Body composition. Body composition was measured in anesthetized mice by dual-energy X-ray absorptiometry (Lunar PIXIMUS Densitometer, GE Medical Systems) at 8 and 19 wk of FEN treatment in the FVB strain prevention study; 0, 3, 6, and 9 wk of FEN treatment in the intervention study, when body weights were similar, and 17–20 wk of FEN treatment in the C57BL/6J × 129Sv strain (RBP4KO and WT) study, when body weights had begun to diverge. Body composition was measured in anesthesitized mice by dual-energy X-ray absorptiometry (Lunar PIXIMUS Densitometer, GE Medical Systems) at 8 and 19 wk of FEN treatment in the FVB strain prevention study; 0, 3, 6, and 9 wk of FEN treatment in the intervention study, when body weights were similar, and 17–20 wk of FEN treatment in the C57BL/6J × 129Sv strain (RBP4KO and WT) study, when body weights had begun to diverge.

Indirect calorimetry. Metabolic rate of singly housed mice was measured at 17 wk FEN treatment in the FVB strain prevention study by indirect, open-circuit calorimetry (CLAMS; Columbus Instruments, Columbus, OH). Food was removed during the day (light period) to measure metabolic rate in the fasted state. All mice were acclimatized to monitoring cages for 48 h prior to 24-h measurements of O2 consumption.

Insulin tolerance tests. Food was removed at 8:00 AM for 5 h, and blood glucose was measured as indicated after intraperitoneal injection of human insulin (Humulin, Lilly; 1.2 mU/g in FVB mice and 1.0 mU/g in C57BL/6J × 129Sv mixed-background mice). Tests were performed after 12–16 wk FEN treatment in all studies. Hyperinsulinemic-euglycemic clamp with 2-deoxyglucose uptake. A dual tracer clamp ([3-3H]glucose infusion and 2-deoxy-D-[1,14C]glucose bolus) was performed as previously described (23), with the following changes. After 16–18 wk of HFD ≥ FEN treatment, mice received an indwelling silicone catheter in the femoral vein and were allowed to recover for 4–7 days. Following a 5-h fast, a 10 μM·kg⁻¹·min⁻¹ insulin, euglycemic clamp was conducted for 120 min in awake, free-moving mice. Rates of basal and insulin-stimulated glucose turnover and hepatic glucose production were determined by the [3-3H]glucose dilution method. Glucose uptake in individual tissues was calculated (22). The glycogen content of muscle and liver was determined as described (23).

Statistical analysis. ANOVA followed by Bonferroni or Fisher post hoc tests were performed using the Statview 4.0 software (Abacus, Baltimore, MD).

RESULTS

Long-term Fenretinide treatment protects against HFD-induced obesity: a prevention study. By 8 wk of diet treatment and thereafter, HFD mice were heavier than CHOW mice (P < 0.05; Fig. 1A). Accordingly, body fat content in HFD mice was 42% higher than CHOW after 9 wk of diet and increased further by 19 wk (Fig. 1B). As we previously showed (49), FEN-HFD treatment for 16 wk did not affect body weight compared with HFD controls. However, by 22 wk, FEN-HFD mice were lighter than HFD (P < 0.05; Fig. 1A), as a result of reduced fat mass (Fig. 1B) without a change in lean mass (data not shown). At 19 wk, body fat content was increased 45% in HFD mice but only 19% in FEN-HFD compared with CHOW mice (Fig. 1B). Fenretinide treatment strongly reduced fed hyperleptinemia after 8 wk and totally prevented 5-h-fasted hyperleptinemia at 22 wk of treatment (Fig. 1C). As expected, serum leptin levels in chow and HFD mice strongly correlated with body fat mass (see Supplemental data; supplemental materials are found in the online version of this paper). Interestingly, FEN-HFD mice had lower-than-expected serum leptin levels for their given fat mass compared with CHOW or HFD mice (Fig. 1, B and C and Supplemental data).

By 34 wk of diet treatment, Fenretinide strikingly prevented the HFD feeding-induced expansion of both visceral and subcutaneous fat masses (Fig. 1D). In contrast, epididymal fat mass was comparable in HFD and CHOW mice and it was about twofold greater in FEN-HFD. As expected, visceral and subcutaneous fat masses showed a linear relationship to body weight in all mice (Fig. 1E, left and middle). Interestingly, epididymal fat mass had a bell-shaped relationship to body weight (Fig. 1E, right). Fenretinide completely prevented the HFD-induced overaccumulation of lipid in subcutaneous adipocytes (Fig. 1F, left). Adipocyte number in subcutaneous VAT did not differ between diet groups (Fig. 1F, right).

In parallel with lower-than-expected serum leptin levels (Fig. 1C and Supplemental data), subcutaneous fat mass was lower than expected for a given body weight in FEN-HFD mice (Fig. 1E, middle). Interestingly, serum leptin levels correlated better with subcutaneous fat (Supplemental data) than with visceral or epididymal fat masses (not shown).

Leptin regulates body fat mass by decreasing food intake and increasing energy expenditure. In obese hyperleptinemic animals, leptin action is impaired, which may account, at least partly, for hyperleptinemia. Fenretinide treatment improved
HFD-induced hyperleptinemia even in mice with increased adiposity, suggesting improved leptin sensitivity. Fenretinide did not alter food intake compared with HFD alone, measured during the first 3 wk of treatment (Fig. 2A, left) or over wk 9–11 (Fig. 2A, right) when body weights were similar between HFD and FEN-HFD mice. Similar data were obtained at the onset of body weight divergence between FEN-HFD and HFD mice (see below). Thus, Fenretinide treatment neither decreased food intake nor induced food aversion. Fenretinide also did not alter stool lipid content in HFD-fed mice (Fig. 2B), suggesting normal intestinal lipid absorption. Thus, Fenretinide does not alter energy intake and may instead preserve leanness by increasing energy expenditure. Indirect calorimetry performed at 17 wk on diet (when HFD and FEN-HFD body weights were similar) did not reveal any difference in oxygen consumption during the light or dark period expressed either per mouse (Fig. 2C, left) or per gram of body weight (not shown). Resting metabolic rate and locomotor activity were also not altered (data not shown). In addition, FEN-HFD mice did not show altered substrate metabolism compared with HFD alone as measured by respiratory exchange ratio (RER; Fig. 2C, right). Both FEN-HFD and HFD mice had similar decreases in RER compared with CHOW. However, changes in body mass can develop with very small mismatches of energy intake and expenditure especially over prolonged periods of time (see DISCUSSION).

Fenretinide partially protected mice from HFD-induced insulin resistance. We previously showed (49) that Fenretinide limits HFD-induced insulin resistance. To determine in which

Fig. 1. Fenretinide partially prevents high-fat diet (HFD)-induced obesity. A: body weight curves of male FVB mice on standard CHOW (○), HFD (▲), or HFD plus 0.1% wt/wt Fenretinide (FEN-HFD, Δ) from 4–5 wk of age. B: body fat content at 9 and 19 wk on diet (CHOW, open bar; HFD, filled bar, or FEN-HFD, gray bar). C: serum leptin levels at 8 and 22 wk on diet. D: visceral (VIS), subcutaneous (SC), and epididymal (EPI) white adipose tissue (WAT) depot weights dissected at 34 wk diet. E: association of dissected WAT depot weights with body weight at 34 wk diet. F: average size of adipocytes in SC-WAT expressed as amount of lipid per adipocyte (left), number of adipocytes in SC-WAT depot per mouse (right). Results are means ± SE from 12–14 mice per dietary group. *P < 0.05 vs. CHOW, #P < 0.05 vs. HFD.
Table 1. Euglycemic-hyperinsulinemic clamp parameters

<table>
<thead>
<tr>
<th>Metric</th>
<th>CHOW</th>
<th>HFD</th>
<th>FEN-HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>32.1±1.2</td>
<td>40.3±1.1**</td>
<td>38.0±1.4*</td>
</tr>
<tr>
<td>Plasma insulin, ng/ml</td>
<td>1.5±0.2</td>
<td>6.7±1.2***</td>
<td>2.9±0.6†</td>
</tr>
<tr>
<td>Clamp</td>
<td>6.1±0.5</td>
<td>10.0±1.2**</td>
<td>7.4±0.7</td>
</tr>
<tr>
<td>Plasma glucose, mg/dl</td>
<td>157.9±8.9</td>
<td>181.2±8.9</td>
<td>166.7±10.2</td>
</tr>
<tr>
<td>Basal</td>
<td>130.2±3.2</td>
<td>136.2±4.9</td>
<td>121.4±3.8†</td>
</tr>
<tr>
<td>Clamp</td>
<td>15.3±0.5</td>
<td>17.2±0.7</td>
<td>17.9±1.8</td>
</tr>
<tr>
<td>Hepatic glucose production, mg·kg⁻¹·min⁻¹</td>
<td>2.0±0.6</td>
<td>14.4±1.1***</td>
<td>4.1±1.7†††</td>
</tr>
<tr>
<td>Basal</td>
<td>47.7±15.6</td>
<td>146.5±25.3*</td>
<td>129.8±19.3*</td>
</tr>
<tr>
<td>Liver glycogen, µg/ng</td>
<td>1.1±0.06</td>
<td>1.04±0.11</td>
<td>1.35±0.10</td>
</tr>
<tr>
<td>Serum free fatty acid, mM</td>
<td>0.47±0.1</td>
<td>0.90±0.10***</td>
<td>0.71±0.1</td>
</tr>
<tr>
<td>Basal</td>
<td>29.1±4.1</td>
<td>47.0±29.7</td>
<td>36.8±23.0</td>
</tr>
<tr>
<td>Plasma AST activity, arbitrary units</td>
<td>26.4±13.8</td>
<td>28.0±13.5</td>
<td>34.0±17.6</td>
</tr>
<tr>
<td>No. of mice</td>
<td>13</td>
<td>14</td>
<td>11</td>
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</tbody>
</table>

Male FVB mice were placed on high-fat diet (HFD) or HFD with Fenretinide (FEN+HFD) at 4–5 wk old, and a euglycemic-hyperinsulinemic (100µU·kg⁻¹·min⁻¹ insulin) clamp was performed at 16 wk on diet. AST, aspartate aminotransferase; ALT, alanine aminotransferase. Data are means ± SE. *Significantly different vs. CHOW (P < 0.05, **P < 0.001, ***P < 0.0001); †significantly different vs. HFD (†P < 0.05, ††P < 0.005, †††P < 0.0001).

Table 2. Serum parameters: prevention study

<table>
<thead>
<tr>
<th>Metric</th>
<th>CHOW</th>
<th>HFD</th>
<th>FEN+HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin, (ng/ml)</td>
<td>2.1±0.2</td>
<td>5.4±0.7*</td>
<td>5.1±1.0**†</td>
</tr>
<tr>
<td>8 wk</td>
<td>2.9±0.4</td>
<td>44.3±10.4***</td>
<td>18.6±5.3†</td>
</tr>
<tr>
<td>Glucose, (mg/dl)</td>
<td>199±4.9</td>
<td>226.8±6.8*</td>
<td>228.8±8.1***</td>
</tr>
<tr>
<td>8 wk</td>
<td>194±7.4</td>
<td>238±13.6*</td>
<td>190.5±4.6†</td>
</tr>
<tr>
<td>Resistin, (ng/ml)</td>
<td>5.6±0.2</td>
<td>13±0.5***</td>
<td>14.3±0.9***</td>
</tr>
<tr>
<td>Adiponectin, (µg/ml)</td>
<td>0.66±0.03</td>
<td>0.59±0.02</td>
<td>0.72±0.05†</td>
</tr>
<tr>
<td>Free fatty acids, (meq/l)</td>
<td>0.93±0.04</td>
<td>1.06±0.01</td>
<td>0.94±0.04</td>
</tr>
<tr>
<td>Triglycerides, (mg/dl)</td>
<td>203±12</td>
<td>263±21</td>
<td>240±23</td>
</tr>
<tr>
<td>Glycerol, (mg/dl)</td>
<td>196±18</td>
<td>330±30*</td>
<td>258±21†</td>
</tr>
<tr>
<td>Serum parameters: prevention study</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of mice</td>
<td>15</td>
<td>18</td>
<td>18</td>
</tr>
</tbody>
</table>

Serum parameters in the ad libitum-fed state in male FVB mice. Mice were placed on HFD or FEN+HFD at 4–5 wk old and bled at 13 wk on diet (except where specified). Data are means ± SE. *Significantly different vs. CHOW (P < 0.05), **P < 0.01, ***P < 0.005; †significantly different vs. HFD (P < 0.05).
infusion stimulated whole body glucose uptake 4.8-fold over in all three groups of mice (Fig. 3).

Basal (5-h-fasted) whole body glucose uptake (Rd) was similar in liver and skeletal muscle in HFD-induced obese FVB mice. Thus, overall, Fenretinide’s insulin-sensitizing actions in FVB mice started by 13 wk on A.

In the basal state, endogenous glucose production (mainly hepatic, HGP) was similar in all diet groups (Table 1). Insulin infusion suppressed HGP 87% in CHOW mice but only 16% in HFD mice, indicating marked hepatic insulin resistance (Table 1 and Fig. 3E). Remarkably, Fenretinide completely prevented this defect despite the obese phenotype of FEN-HFD mice (Table 1 and Fig. 3E). Livers were ~60% heavier in HFD mice but only 40% heavier in FEN-HFD compared with CHOW (Fig. 3F, left). Accordingly, triglyceride accumulation in liver of HFD mice was increased 15-fold compared with CHOW controls. This severe hepatic steatosis was reduced by 50% with Fenretinide treatment (Fig. 3F, right). Thus, Fenretinide reduces HFD-induced hepatic steatosis and nearly normalizes insulin action on HGP even when hepatic lipid content is increased sevenfold above levels in CHOW mice.

Some retinoids used for cancer or acne treatment cause hypertriglyceridemia (7, 13). In this study, Fenretinide did not increase circulating triglycerides, FFAs, or glycerol (Table 2). FFAs levels in FEN-HFD and HFD mice were comparable to CHOW values in the fed and 5-h-fasted states, (Tables 1 and 2). During the clamp, insulin infusion suppressed serum FFA levels 59% in CHOW mice but only 12% in HFD mice (Fig. 3G and Table 1). Strikingly, Fenretinide completely prevented this defect (Table 1 and Fig. 3G). Thus, Fenretinide does not cause hypertriglyceridemia as other retinoids do, but instead preserves normal insulin action on lipid homeostasis in obese mice.

Many retinoids, including retinoic acid, cause hepatotoxicity (32, 40). In contrast, Fenretinide treatment in HFD mice did not increase plasma levels of aspartate amino transferase and alanine aminotransferase, two hepatic enzymes that are used as indexes of hepatocyte damage (Table 1).

Fenretinide reversed obesity and insulin resistance in HFD-fed mice: an intervention study. Fenretinide’s potential therapeutic value would be increased if, in addition to its preventive actions, Fenretinide could reverse or slow down the progression of existing obesity and insulin resistance. Thus, we performed an interventional study in FVB mice with established HFD-induced obesity and insulin resistance. After 32 wk on HFD, obese mice were randomized into groups matched for body weight, adiposity, plasma insulin levels, and insulin tolerance and either continued on HFD alone or given FEN-HFD. Remarkably, Fenretinide treatment of obese mice for 12 wk stopped the progression of obesity (Fig. 4, A–C). Body weight gain tended to be slightly less in mice switched to FEN-HFD compared with HFD alone (Fig. 4A). Fenretinide treatment totally suppressed gain of fat mass (Fig. 4, B and C), whereas gain of lean mass was unchanged (Fig. 4C). Leptin levels in HFD mice prior to Fenretinide intervention were increased more than twofold compared with CHOW mice (Fig. 4D). Strik-
ingly, Fenretinide intervention in FEN-HFD mice for 12 wk normalized to CHOW values not only hyperleptinemia (Fig. 4D) but also both visceral and subcutaneous fat pad masses (Fig. 4E). In contrast, epididymal WAT mass in both HFD and FEN-HFD mice was approximately twofold greater than in CHOW mice. Fenretinide specifically suppressed HFD-induced accumulation of lipid in subcutaneous adipocytes (Fig. 4F, left), whereas adipocyte number in subcutaneous WAT was similar in all diet groups (Fig. 4F, right). Fenretinide treatment did not alter cumulative food intake over the whole intervention period (Fig. 4G) or stool lipid content (data not shown).

Serum insulin was 75% higher in HFD mice than in CHOW pretreatment and increased to threefold higher over the next 12 wk of diet. Fenretinide not only prevented that progression, but normalized HFD-induced hyperinsulinemia to CHOW values (Fig. 4H), indicating improved insulin sensitivity even though adiposity was still somewhat increased in FEN-HFD mice compared with CHOW. Accordingly, Fenretinide treatment improved insulin tolerance at 12 wk of treatment (Fig. 4I).

Thus, Fenretinide can both prevent and reverse obesity and insulin resistance in HFD mice.

Fenretinide partially prevented HFD-induced obesity in RBP4KO mice. We recently showed (49) that elevated serum RBP4 levels contribute to insulin resistance and that lowering serum RBP4 levels genetically or pharmacologically (with Fenretinide) improves insulin sensitivity independently of changes in body weight. However, since longer treatment with...
Fenretinide dramatically reduces adipose mass and serum leptin levels, we hypothesized that Fenretinide has other mechanisms for its metabolic actions in addition to lowering serum RBP4 levels. Therefore, we investigated whether Fenretinide can prevent HFD-induced obesity in RBP4KO mice and in their WT controls on the C57BL/6J × 129Sv mixed background.

Twenty-two weeks of HFD treatment in WT mice elevated serum RBP4 levels twofold, whereas Fenretinide treatment prevented this (Fig. 5A). RBP4 was expectedly absent in sera from all RBP4KO groups (Fig. 5A). WT mice on FEN-HFD diet gained less weight than on HFD without Fenretinide over the diet treatment period (Fig. 5B, left, \( P < 3 \times 10^{-8} \), repeated-measures ANOVA), and body weights were significantly lower after 21 wk of Fenretinide treatment (Fig. 5B, left, \( P < 0.05 \), ANOVA with Bonferroni post hoc test). Similarly, RBP4KO mice on FEN-HFD diet gained less weight than on HFD without Fenretinide (Fig. 5B, right, over the diet treatment period (\( P < 2 \times 10^{-7} \), repeated-measures ANOVA)). Furthermore, Fenretinide treatment in WT mice reduced fat mass gain induced by HFD (Fig. 5C) without a change in lean mass (data not shown). In RBP4KO mice, Fenretinide similarly limited the increase of fat mass elicited by HFD diet treatment (Fig. 5C). After 22 wk of HFD, fed serum leptin levels were substantially increased in both WT HFD and RBP4KO HFD mice compared with their respective CHOW controls. This effect was strongly attenuated by Fenretinide treatment in both genotypes (Fig. 5D). Fenretinide treatment did not alter cumulative food intake in either WT or RBP4KO HFD mice (measured at 17–20 wk on the diet when body weights had begun to diverge) even when expressed per gram of body weight (Fig. 5E).

**DISCUSSION**

Insulin resistance is closely associated with weight gain and obesity. We (49) and others (51) recently showed that altering RBP4 levels genetically or pharmacologically results in alterations in insulin sensitivity without changes in adiposity. Treatment with the synthetic retinoid Fenretinide inhibits the severity of insulin resistance in mice fed an HFD for 16 wk without affecting weight gain (49). Here, we now show that, with prolonged exposure to HFD (34 wk), Fenretinide prevents the severity of obesity. The antiobesity effects progress over time and do not involve a measurable alteration in either caloric intake or energy expenditure. There are a number of examples of genetically modified mice for which the obese phenotype could not be accounted for by measurable alterations in either energy intake or energy expenditure (1). Accordingly, a mismatch in energy balance of only a few percent over months can lead to a difference in adipose tissue accretion of several grams.

Fenretinide’s total inhibition of visceral and subcutaneous WAT expansion occurred in both the 34-wk prevention study and the 12-wk intervention study and is probably a major

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**Fig. 5.** Fenretinide partially prevents HFD-induced obesity in mice lacking serum RBP4. A: serum RBP4 levels in WT and retinol-binding protein-4 knockout (RBP4KO) male mice on C57BL/6J × 129Sv mixed background after 22 wk of either standard CHOW (open bar), HFD (filled bar), or HFD + Fenretinide (0.1% wt/wt; FEN-HFD, gray bar) from 4–5 wk of age. Right: representative Western blot. B: body weight progression of WT and RBP4KO mice on either CHOW (○), HFD (▲), or FEN-HFD (▲) from 4–5 wk of age. In both WT and RBP4KO genotypes, body weight progression was significantly different among all 3 treatment groups (repeated-measures ANOVA, \( P < 2 \times 10^{-7} \)). Body weights in WT FEN-HFD were significantly different from WT HFD at weeks 21 and 22 of diet treatment (\( P < 0.05 \), ANOVA with Bonferroni post hoc test). C: body fat content at 20 wk on diet. (CHOW, open bar; HFD, filled bar; FEN-HFD, gray bar). D: ad libitum-fed serum leptin levels at 22 wk on diet. E: cumulative food intake during 17–20 wk of diet. Results are means ± SE from 10–15 mice per dietary group. *\( P < 0.05 \) vs. CHOW, #\( P < 0.05 \) vs. HFD.
that Fenretinide markedly prevented hepatic insulin resistance in vivo.

suggesting that the Fenretinide effects to reduce adiposity are adipocytes per fat depot was not changed in our experiments, cancer cells. However, we found that the total number of retinoic acid signaling in adipocytes. Alternatively, it could it could inhibit adipose tissue expansion via increases in initially accumulates in adipose and mammary tissue (18, 29, 43), acid can completely inhibit adipogenesis by inhibiting CCAAT-or other members of the family of cytochrome P-450 enzymes that degrade retinoids) may lead to increases in tissue retinoic acid levels (44, 47). Retinoic acid can inhibit or promote adipogenesis depending on the stage at which pre-adipocytes are exposed (41, 48). With early exposure, retinoic acid can completely inhibit adipogenesis by inhibiting CCAAT-enhancer-binding protein-β (39). Since Fenretinide preferentially accumulates in adipose and mammary tissue (18, 29, 43), it could inhibit adipose tissue expansion via increases in retinoic acid signaling in adipocytes. Alternatively, it could induce apoptosis, as is seen with Fenretinide treatment of cancer cells. However, we found that the total number of adipocytes per fat depot was not changed in our experiments, suggesting that the Fenretinide effects to reduce adiposity are not due to inhibition of adipogenesis or apoptosis of adipocytes in vivo.

Our euglycemic-hyperinsulinemic clamp studies showed that Fenretinide markedly prevented hepatic insulin resistance and improved glucose uptake in muscle but only partly prevented whole body insulin resistance (Rd and GINF). The mechanism of Fenretinide’s effects on hepatic insulin sensitivity may involve partial reduction in hepatic steatosis and lowering RBP4, since elevated RBP4 induces PEPCK expression and impairs insulin action to suppress glucose production in cultured hepatocytes (49). Fenretinide’s near normalization of insulin action on HGP in HFD-fed mice is particularly impressive, since it occurs even with a considerable residual increase in hepatic steatosis compared with CHOW-fed mice. The effects on insulin action in muscle may be due to improved insulin signaling with Fenretinide treatment (49). We previously published (49) that insulin stimulated phosphorylation of IRS-1 threefold on Tyr612 in skeletal muscle of chow-fed FVB mice; this response was reduced by more than 50% in HFD mice, but Fenretinide completely prevented this impairment in IRS-1 phosphorylation. We did not observe differences in the expression of IRS-1 or the expression or tyrosine phosphorylation of the insulin receptor (data not shown). Thus, improved post-receptor insulin signaling in muscle may contribute to the improved insulin sensitivity parameters seen in Fenretinide-treated mice (e.g., glucose infusion rate and 2-deoxyglucose uptake into muscle during the hyperinsulinemic-euglycemic clamp).

There is an urgent need for efficient and non toxic drugs to treat obesity and insulin resistance. Unlike other retinoids, Fenretinide is relatively nontoxic and has been tested in phase III clinical trials for cancer for periods of up to 5 years (11, 38). Moreover, a recent trial found that prolonged Fenretinide treatment in overweight premenopausal women improved insulin sensitivity and decreased serum leptin levels, suggestive of a decrease in fat stores (21). In addition, in normal-weight women who showed a decrease in insulin sensitivity, Fenretinide prevented an increase in serum triglyceride levels (21). Thus, Fenretinide could be a novel and safe drug not only for treatment of type 2 diabetes but also for prevention and treatment of obesity and dyslipidemia. The antiobesity effects appear to be independent of Fenretinide’s RBP4-lowering effects.

ACKNOWLEDGMENTS

We thank Simon J. Fisher for invaluable discussions about the euglycemic clamp technique, Anna Lee for expert technical help, and Bill Blaner, Loredana Quadro, and Max Gottessman for the RBP4 knockout mice and for helpful discussions about retinoid biology.

Current address of F. Preitner: Cardiomet Mouse Metabolic Facility, University of Lausanne, Lausanne, Switzerland.

Current address of N. Mody: Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen, Scotland, UK.

GRANTS

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants R37 DK-43051, P01 DK-56116 and P30 DK-57521 and a research grant from Takeda Pharmaceutical (to B. B. Kahn), postdoctoral fellowships from the Swiss National Foundation, no. PA00A-101447 (to F. Preitner), and American Heart Association and American Diabetes Association-European Association for the Study of Diabetes (to N. Mody), and Grants K08 DK-69624 and R03 DK-080195 and the Smith Family New Investigator Award (to T. E. Graham).

DISCLOSURES

B. B. Kahn, T. E. Graham, and O. D. Peroni are inventors on a patent related to RBP4. B. B. Kahn has a research grant from Takeda Pharmaceutical Co.
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