A new antidiabetic compound attenuates inflammation and insulin resistance in Zucker diabetic fatty rats

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Lu M, Patsouris D, Li P, Flores-Riveros J, Frincke JM, Watkins S, Schenk S, Olefsky JM. A new antidiabetic compound attenuates inflammation and insulin resistance in Zucker diabetic fatty rats. Am J Physiol Endocrinol Metab 298: E1036–E1048, 2010. First published February 16, 2010; doi:10.1152/ajpendo.00668.2009.—Tissue macrophage inflammatory pathways contribute to obesity-associated insulin resistance. Here, we have examined the efficacy and mechanisms of action of a novel anti-inflammatory compound (HE3286) in vitro and in vivo. In primary murine macrophages, HE3286 attenuates LPS- and TNFα-stimulated inflammation. In Zucker diabetic fatty rats, inflammatory cytokine/chemokine expression was downregulated in liver and adipose tissue by HE3286 treatment, as was macrophage infiltration into adipose tissue. In line with reduced inflammation, HE3286 treatment normalized fasting and fed glucose levels, improved glucose tolerance, and enhanced skeletal muscle and liver insulin sensitivity, as assessed by hyperinsulinemic euglycemic clamp studies. In phase 2 clinical trials, HE3286 treatment led to an enhancement in insulin sensitivity in humans. Gluconeogenic capacity was also reduced by HE3286 treatment, as evidenced by a reduced glycemic response during pyruvate tolerance tests and decreased basal hepatic glucose production (HGP) rates. Since serum levels of gluconeogenic substrates were decreased by HE3286, it indicates that the reduction of both intrinsic gluconeogenic capacity and substrate availability contributes to the decrease in HGP. Lipidomic analysis revealed that HE3286 treatment reduced liver cholesterol and triglyceride content, leading to a feedback elevation of LDL receptor and HMG-CoA reductase expression. Accordingly, HE3286 treatment markedly decreased total serum cholesterol. In conclusion, HE3286 is a novel anti-inflammatory compound, which displays both glucose-lowering and cholesterol-lowering effects.

HE3286

OBESE AND INSULIN RESISTANCE are dominant features in most patients with type 2 diabetes mellitus, and recent data have established the concept that chronic, low-grade tissue inflammation is an important etiological cause of decreased insulin sensitivity (34, 44). Recent studies have shown that rodent and human adipose tissues are infiltrated with macrophages and that this phenomenon is exacerbated in obesity (57, 58). Thus, the number of proinflammatory M1-like macrophages increases by severalfold in obesity, and compelling evidence indicates that these M1-like adipose tissue macrophages (ATMs) are an important component of the inflammatory/insulin resistance in obesity (26).

Strong genetic evidence supporting these concepts exists. When major proinflammatory pathways within macrophages, the chemotactic ability of macrophages, or secretion or action of TNFs and monocyte chemotactic protein-1 (MCP-1) are blocked, high-fat-diet/obesity induced insulin resistance is largely prevented (2, 8, 16, 46, 52). These studies show that inhibition of macrophage-mediated inflammatory responses results in beneficial effects on glucose metabolism and insulin resistance and suggests that anti-inflammatory therapy might be useful for the treatment of insulin resistance and diabetes.

Dehydroepiandrosterone (DHEA) is a natural steroid that serves as a precursor of male and female sex hormones (21). Produced from the adrenal gland, DHEA, together with its sulfated form (DHEA-S), is the most abundant steroid hormone in humans (21). DHEA has been postulated to have a variety of biological actions, including anti-inflammatory and anti-diabetic effects (36, 49). Despite these well-documented activities in animal models, the value of DHEA replacement in humans is controversial. Most human studies failed to show improved glucose tolerance or insulin action (5, 17, 31, 53). Moreover, the potential therapeutic use of DHEA is limited by its side effects due to its conversion to sex hormones (3).

In the current study, we have examined the efficacy and mechanism of HE3286 (17α-ethyl-5-androstene-3β, 7β, 17β-triol), an analog of a human DHEA metabolite, in cultured cell systems and in insulin resistant Zucker diabetic fatty (ZDF) rats. HE3286 does not bind to or transactivate sex hormone or PPAR receptors and exhibits anti-inflammatory activities (4, 35). Here, we show that HE3286 inhibits macrophage inflammatory pathways and decreases macrophage chemotaxis in vitro and in vivo. HE3286 effectively suppresses systemic inflammation and normalizes hyperglycemia by lowering hepatic glucose production and increasing whole body insulin sensitivity. In addition, HE3286 modulates lipid metabolism in the liver to lower circulating cholesterol levels. Preliminary clinical studies suggested that HE3286 treatment improved insulin sensitivity in humans. In summary, we conclude that HE3286 may serve as a drug candidate for treatment of obesity-related metabolic disorders.

MATERIALS AND METHODS

Materials and cell culture. LPS and recombinant TNFs were obtained from Sigma. Anti-sterol regulatory element-binding protein-2 (SREBP-2) and anti-Mac2 were purchased from Abcam (Cambridge, MA). All other primary antibodies were purchased from Cell Signaling Technology (Danvers, MA). HE3286 and rosiglitazone were obtained from Hollis Eden Pharmaceuticals (San Diego, CA).

Murine primary macrophages were elicited by intraperitoneal injection of thioglycollate (3 ml/mouse) in C57BL/6J mice. Macrophages were obtained from intraperitoneal lavage and washed twice. Cells were cultured in RPMI supplemented with 10% fetal bovine

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serum (FBS) for 3 days and then starved in RPMI supplemented with 0.5% FBS for overnight before the treatment.

RAW 264.7 cells and 3T3-L1 cells were cultured as described previously (33, 60).

Animals. The study was staggered into six cohorts conducted on different days. Forty-two male ZDF rats and six Zucker fatty rats at 7 wk of age were received from Charles River laboratories (Wilmington, MA). The rats were housed individually on a 12:12-h light-dark cycle with the lights on at 0600 and were fed ad libitum except during the experiments. After 1 wk of acclimation, the ZDF rats began daily oral treatment for 32–35 days with vehicle (n = 18), 100 mg·kg⁻¹·day⁻¹ HE3286 (n = 15), or 10 mg·kg⁻¹·day⁻¹ rosiglitazone (n = 9).

Rat procedures conformed to the Guide for Care and Use of Laboratory Animals of the National Institutes of Health (NIH) and were approved by the Animal Subjects Committee of the University of California, San Diego, California.

Metabolic studies. Glucose, insulin, and pyruvate tolerance tests were performed on 6-h-fasted rats. For glucose tolerance test (ITT), animals were orally gavaged with glucose (1 g/kg), whereas for insulin tolerance test (ITT), 0.35 U/kg insulin (Novolin R; Novo-Nordisk) was injected intraperitoneally.

Rat hyperinsulinemic euclrigemic clamp studies were performed as described previously (43) with modifications. Briefly, dual jugular venous cannulae and one carotid arterial cannula were implanted in rats. After 4–5 days of recovery, the hyperinsulinemic euclrigemic clamp experiments were begun with a priming injection (7.5 μCi/0.2 ml) and constant infusion (0.25 μCi/min) of D-[3-³H]glucose (Du Pont-NEN, Boston, MA). After 60 min of tracer equilibration and basal sampling at t = −10 and 0 min, glucose (50% dextrose, variable infusion; Abbott) and tracer (0.25 μCi/min) plus insulin (20 mU·kg⁻¹·min⁻¹) were infused into the jugular vein. The achievement of steady-state conditions (100 mg/dl ≥ 5 mg/dl) was confirmed at the end of the clamp by measuring blood glucose every 10 min and ensuring that steady state for glucose infusion and plasma glucose levels was maintained for a minimum of 30 min. Blood samples were taken at t = −60 (start of experiment), −10, 0 (basal), 110, and 120 min (end of experiment) to determine glucose-specific activity and insulin clearance.

Individual pairwise comparisons were performed using Student’s t-test. Analysis was performed using Excel (Microsoft, Redmond, WA) or Prism (GraphPad Software, San Diego, CA).

RESULTS

HE3286 inhibits LPS effects. Recent findings indicate an important contribution of macrophage inflammatory pathways in causing the obesity-related decrease in insulin sensitivity (44). HE3286 has potential anti-inflammatory actions, and therefore, we tested its effects in primary murine intraperitoneal macrophages. Treatment with the Toll-like receptor 4 ligand LPS broadly activates proinflammatory signaling cascades, including phosphorylation of IKK and MAPKs such as JNK, p38, and extracellular signal-regulated kinases (ERK) (Fig. 1A). As a result, various inflammatory genes were upregulated (Fig. 1B), as determined by a quantitative nuclease protection assay array. Pretreatment with HE3286 partially, but significantly, blocked the activation of IKK, JNK, p38, and ERK (Fig. 1A). Although IkB phosphorylation and degradation were not influenced by HE3286 treatment, NF-κB phosphorylation was attenuated. Consistent with this, we performed chromatin immunoprecipitation studies and found that drug treatment led to decreased NF-κB occupancy of the κB site in the IL-6 promoter (Supplemental Fig. S1; Supplemental Material for this article can be found at the AJP-Endocrinology and Metabolism web site). Concordantly, LPS-induced transcription of Il1b, Il6, Il12, Tnfa, Nos2, Cxcl10, and Cxcl11 was significantly reduced (Fig. 1B). These results suggest that HE3286 can ameliorate intracellular inflammatory responses elicited by Toll-like receptor 4 signaling. When macrophages were treated with HE3286 alone, no activation of phosho-JNK was detected (Supplemental Fig. S2), demonstrating that the inhibition of LPS-induced inflammation by HE3286 is...
unlikely due to macrophage tolerance caused by preconditioning.

**HE3286 inhibits TNFα signaling.** TNFα stimulates inflammation and activates NF-κB, Akt, and MAPKs by binding to TNF receptor 1 and 2. Of note, LPS-activated macrophages secrete TNFα, which then stimulates macrophages in an autocrine or paracrine fashion (24, 42). When TNFα was immunodepleted in the culture media, the effect of LPS to stimulate inflammatory signaling was reduced significantly (Supplemental Fig. S3). At the same time, when TNFα was neutralized, no additional inhibition of phospho-JNK and phospho-NF-κB by HE3286 treatment was observed. Since LPS-induced TNFα secretion was not altered by HE3286 (Supplemental Fig. S4), we speculated that the action of TNFα might be inhibited by HE3286. To test this, murine macrophages were pretreated with DMSO or HE3286, followed by TNFα stimulation. We found that HE3286 significantly decreased phosphorylation of IKK, NF-κB, and p38 but not JNK (Fig. 2A). Although changes in phosphorylation or degradation of IκB with HE3286 treatment were not observed, the recovery of IκB appeared to be enhanced (compare DMSO and HE3286-treated cells at 30 and 60 min; Fig. 2A). HE3286 attenuated TNFα-stimulated expression of *Il1b*, *Cxcl10*, *Vcam1*, *Mmp9*, and *Ccl2* (Fig. 2B). Therefore, it appears that HE3286 causes inhibition of the
macrophage inflammatory program primarily by inhibiting TNFα action.

Effects on macrophage chemotaxis. Increased macrophage infiltration and accumulation in adipose tissue occurs in obesity (57, 58), and therefore, we determined whether HE3286 could regulate macrophage chemotaxis. We used conditioned media (CM) from 3T3-L1 adipocytes to induce chemotaxis of RAW 264.7 monocyte/macrophages. Figure 3A shows that CM from TNFα-treated adipocytes markedly stimulated macrophage migration, which was reduced by ~30% ($P < 0.05$) when adipocytes were pretreated with HE3286. In addition, adipocyte secretion of inflammatory cytokines, such as MCP-1/CCL2 and chemokine (C-C motif) ligand 5 (CCL5/regulated upon activation, normal T cell expressed and secreted), was augmented in TNFα-treated adipocyte CM and was significantly impaired by HE3286-pretreatment of the adipocytes (Fig. 3, B and C).

In vivo studies in ZDF rats. Given the anti-inflammatory effects of HE3286 and the known role of systemic inflammation in insulin resistance, we assessed whether HE3286 could improve glucose metabolism in vivo. We treated ZDF rats with placebo vehicle, HE3286 (100 mg·kg$^{-1}$·day$^{-1}$), or rosiglitazone (10 mg·kg$^{-1}$·day$^{-1}$) for 4 wk. The ZDF rat, a model of obesity, insulin resistance, and diabetes, is known to develop hyperinsulinemia at 8–9 wk of age and hyperglycemia after 9–10 wk of age (11, 47). The treatment was initiated at 8 wk of age so that the progression of diabetes in the animals could be evaluated. We found that HE3286 completely normalized fasting and fed glucose levels throughout the study. Thus, 1 wk of treatment with HE3286 was sufficient to normalize fasting and fed glucose levels as well as plasma insulin levels (Fig. 4, A and B). Rosiglitazone, a known insulin sensitizer, was used as a positive control and had the expected effects to ameliorate hyperglycemia and hyperinsulinemia (Fig. 4, A and B). However, rosiglitazone treatment also caused increased weight gain, whereas body weight of the vehicle- and HE3286-treated rats was comparable (Supplemental Table S1).

Oral GTTs and ITTs. To further investigate the effect of HE3286 on insulin secretion and glucose utilization, oral GTTs (OGTT) were performed. At 10 wk of age (day 14 during treatment), vehicle-treated ZDF rats began to exhibit fasting hyperglycemia, whereas HE3286-treated animals had reduced glycemia both in the fasting state and after glucose administration (Fig. 4C). Glucose-induced insulin levels were also
HE3286 improves insulin sensitivity

To determine whether HE3286 affects insulin-induced glucose clearance, ITTs were performed on a subset of the treated rats on day 21. Upon insulin injection, both HE3286- and rosiglitazone-treated rats showed enhanced glucose clearance (Fig. 4D), consistent with improved insulin sensitivity.

Studies of gluconeogenesis. Since it has been suggested that fasting glucose level and the area under the curve for the first 30 min during a GTT largely represent insulin effects on the liver (1), we speculated that HE3286 could regulate hepatic glucose production (HGP). We reasoned that HE3286 treatment reduces gluconeogenic substrate levels. In ZDF rats, glycerol is a key gluconeogenic substrate for increased HGP, whereas other substrates such as lactate and pyruvate also contribute (30). To address this issue, plasma levels of glycerol, lactate, and pyruvate were measured on day 14. In agreement with our hypothesis, both fasting and fed glycerol levels were markedly reduced by HE3286 treatment (Fig. 4E). Likewise, lactate and pyruvate levels were also decreased (Supplemental Figs. S6 and S7). As published previously, rosiglitazone also effectively reduced the level of gluconeogenic substrates (Fig. 4E and Supplemental Figs. S5 and S6).

In addition, we measured FFA levels since FFAs can contribute indirectly to gluconeogenesis by interfering with insulin suppression on HGP and by providing an energy source for ATP generation (18). As seen in Fig. 4F, fed FFA levels were decreased in the HE3286-treated group.

Glucose clamp studies. Euglycemic hyperinsulinemic clamp studies provide a quantitative measurement of in vivo insulin sensitivity. As summarized in Fig. 5, after 4 wk of treatment, both HE3286 and rosiglitazone treatment led to an increase in the glucose infusion rate and insulin-stimulated glucose disposal rate (IS-GDR), with the effects of rosiglitazone being more robust (Fig. 5, A and B). Since 70–80% of IS-GDR is attributable to skeletal muscle, this implies that HE3286 treatment improves skeletal muscle insulin action. In support of this, Akt phosphorylation was enhanced in insulin-stimulated skeletal muscle and liver (Fig. 5E and Supplemental Fig. S9). Importantly, basal rates of HGP were markedly and equally reduced by HE3286 and rosiglitazone treatment (Fig. 5C), and since basal HGP is the major contributor to basal hyperglycemia, these results are fully consistent with the marked reduction in basal glucose levels observed in these animals. In addition, we also found that the ability of insulin to suppress HGP was enhanced in the treated rats (Fig. 5D and Supplemental Fig. S8). Taken together, these in vivo results support the conclusion that HE3286 treatment leads to robust effects on hepatic glucose metabolism, which result in a marked reduction in gluconeogenic flux, and near normalization of hyperglycemia and enhanced hepatic insulin sensitivity.

Glucose clamp studies in humans. In preliminary clinical studies, HE3286 is effective at improving insulin sensitivity in insulin-resistant, obese patients. In these studies, 10 obese, insulin-resistant subjects with impaired glucose tolerance (according to standard American Diabetes Association OGTT criteria) were treated with HE3286 (5 mg BID, n = 5, or 10 mg BID, n = 5; total, n = 10) for 4 wk (Fig. 6A). Hyperinsulinemic-euglycemic glucose clamp studies were performed on the day prior to treatment and on day 28 of treatment. The results are seen in Fig. 6B, which showed a significant 34% increase (P = 0.0304) in the glucose infusion rate (M value) in these patients. Thus, these results in insulin-resistant subjects are fully consistent with the in vitro results and the data in ZDF rats.
control and treated rats. As seen in Fig. 7A, there was a marked reduction in ATM content, as measured by staining for the macrophage-specific marker Mac-2 in the treated rats. This directly demonstrates decreased ATM accumulation, and, consistent with this, we also found a decrease in F4/80 mRNA expression in both groups of treated animals (Fig. 7B). Moreover, we observed a striking decrease in a variety of inflammatory markers, including TNFα and MCP-1, in adipose tissue from the HE3286- and rosiglitazone-treated rats. Both M1 macrophage markers (NOS2, CXCL1, and IL-1) and M2 markers (arginase-1 and IL-10) were comparably reduced by HE3286 treatment (Fig. 7B), and the ratio of M1/M2 markers remained the same (Supplemental Fig. S10). In contrast to these broad changes in adipose tissue inflammatory markers, expression of genes such as glucose transporter 4, adiponectin, adipose triglyceride lipase, and hormone-sensitive lipase was not altered by HE3286 treatment (Fig. 7B). In contrast, rosiglitazone treatment led to an increase in glucose transporter 4 and adipose triglyceride lipase expression as well as very large increases in the lipogenic program, as measured by fatty acid synthase and acetyl-CoA carboxylase expression. Consistent with the decrease in inflammatory gene expression profile in the adipose tissue, circulating TNFα and IL-1β levels were also markedly reduced in the treated rats (Fig. 7C). We measured adipocyte size and found no significant difference after HE3286 treatment, in contrast to the effects of rosiglitazone,
which decreased adipocyte size (Supplemental Fig. S11). On the basis of these findings, it seems likely that HE3286 exerts potent anti-inflammatory effects in vivo.

To determine whether HE3286 treatment also produces anti-inflammatory effects in the liver, we measured the hepatic expression of inflammatory cytokines such as TNFα, IL-1β, IL-6, CXCL1, and MCP-1. As in adipose tissue, HE3286 led to suppression of inflammatory programs (Fig. 8D). Rosiglitazone treatment led to qualitatively similar, but quantitatively less marked, effects (Fig. 8D).

In vivo lipidomic studies. Dyslipidemia usually coexists with insulin resistance/diabetes, and increased tissue lipid accumulation has been demonstrated in several animal models of obesity and diabetes, including ZDF rats (7). Therefore, we assessed lipid profiles in three key insulin-responsive tissues: liver, fat, and skeletal muscle. Figure 8A shows that HE3286 treatment led to a marked decrease in intracellular triacylglycerol content in livers, as did rosiglitazone. HE3286 significantly reduced hepatic cholesteryl ester (CE) levels by 73%, and as a result, total cholesterol content in the liver was...
HE3286 (n = 10)

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<td>BMI (kg/m²)</td>
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<td>FPG (mg/dL)</td>
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<td>FPI (µU/mL)</td>
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Data are expressed as mean ± SEM.

BMI, body mass index; FPG, fasting plasma glucose; FPI, fasting plasma insulin

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To further understand the effect of HE3286 and rosiglitazone on lipid metabolism, we examined tissue fatty acid composition. Figure 9A demonstrates that HE3286 decreased the levels of palmitate (16:0), palmitoleate (16:1n7), and linolenate (18:3n3) in the liver, whereas arachidonic acid (20:4n6) and docosahexaenoic acid (22:6n3) were increased. Interestingly, treatment with rosiglitazone increased only the level of eicosapentaenoic acid (20:5n3) but not docosahexaenoic acid (Fig. 9A).

Figure 9B summarizes the fatty acid levels in epididymal white adipose tissue. It has been suggested that palmitoleate (16:1n7) is a lipokine that can promote insulin sensitivity (6), and we found an elevation of palmitoleate levels in both HE3286- and rosiglitazone-treated animals (Fig. 9B). Since 16:1n7 is an indicator of de novo lipogenesis (6, 56), these results imply enhanced adipocyte lipogenesis in the treated rats.

We also measured the effects of HE3286 and rosiglitazone treatment on serum fatty acid profiles, as shown in Fig. 9C. Since fatty acids released by adipose tissue are the dominant source of circulating fatty acids, one would expect serum FFA patterns to largely mirror adipose tissue. Thus, we observed an increase in palmitoleate levels in FFAs in HE3286- and rosiglitazone-treated animals (Fig. 9C). Reflecting the corresponding changes in adipose tissue, de novo fatty acid synthesis in muscle is negligible, and therefore, the fatty composition of muscle is determined largely by circulating FFAs and VLDL (6). Indeed, we found an increase in palmitoleate levels in several lipid classes in skeletal muscle (Fig. 9D).

DISCUSSION

Numerous studies have shown that chronic, low-grade tissue inflammation is a key component of obesity-induced insulin resistance (2, 44, 57, 58). Several reports have found that anti-inflammatory salicylate treatment improves insulin sensitivity (19), and the discovery that proinflammatory macrophages accumulate in adipose tissue has further indicated that anti-inflammatory therapies can have beneficial effects in insulin resistance/diabetes (57, 58). Here, we show that a novel anti-inflammatory compound, HE3286, can inhibit IKKβ/ NF-κB and JNK signaling, reduce cytokine release, and decrease ATM infiltration. As a result, glucose tolerance and insulin sensitivity are substantially improved in ZDF rats treated with HE3286. These findings support the concept that inflammation is an important cause of insulin resistance and that anti-inflammatory therapy can be an effective measure to treat this class of metabolic disorders.

Despite positive findings in rodent models, indicating that DHEA treatment improves glucose tolerance and insulin sensitivity, DHEA supplementation in humans has yielded inconclusive results (5, 10, 17, 53). One possibility to explain these discrepancies is that a metabolite(s) of DHEA, rather than DHEA itself, may be necessary for its full action in human physiology. DHEA undergoes extensive conversion and derivation to multiple products by phase 1 reactions involving the cytochrome P450 system (22, 28, 29), and studies have shown that these phase 1 products can be more potent than...
parental DHEA (22, 23, 27–29). Phase 1 reactions frequently decline in elderly subjects (45), and since such subjects have been the major participants in human DHEA treatment studies, it is possible that biologically active metabolites of DHEA were not produced in adequate amounts in previous human studies. It is also possible that qualitative changes in DHEA metabolism between rodents and humans may account for these differences (12). HE3286 is a synthetic analog of a DHEA metabolite, it is chemically designed to resist microsomal oxidation, and it is metabolically stable (35). HE3286 is readily bioavailable and does not bind to or activate sex hormone receptors or PPAR family receptors (54). The hydroxyl group at the carbon 7 position of HE3286 prevents its conversion to testosterone, preventing generation of unwanted metabolic byproducts of DHEA. Consistent with our findings in ZDF rats, preliminary results from ongoing clinical trials in type 2 diabetic subjects have shown that HE3286 exerts beneficial effects on glucose metabolism and improves insulin sensitivity in humans.

Our in vivo studies showed that basal hyperglycemia was largely normalized by HE3286 treatment and that this was due to correction of the basal rate of HGP. Since the elevated HGP in ZDF rats is due largely to increased gluconeogenesis (51), these results indicate that treatment with this agent markedly inhibits hepatic gluconeogenesis, leading to the reduction in HGP and normalization of basal glycemia. HE3286 treatment significantly decreased plasma levels of a wide range of gluconeogenic substrates, including glycerol, pyruvate, and lactate. Compared with FFAs, glycerol released by adipose tissue is considered a more accurate reflection of lipolysis since FFA levels can be influenced by both the lipolytic and FFA clearance pathways. Given that plasma glycerol has been shown to be a key driving force for HGP in ZDF rats (50), the marked reduction in adipose tissue lipolysis with a decrease in circu-
HE3286 regulates lipid metabolism in vivo. A and B: lipid classes in the liver (A) and serum (B) were quantified by lipidomic analysis. Data are expressed as means ± SE. C: protein extracted from the livers of treated rats was subjected to SDS-PAGE. Sterol regulatory element-binding protein-2 (SREBP-2) precursor (P) and mature form (M) were detected by immunoblotting. Culture cells infected with adenovirus expressing SREBP-2 NH2 terminus (Ad-2N) were used as a positive control for blotting. D: total RNA extracted from the liver of treated ZDF rats was subjected to quantitative PCR analysis. Data shown are the fold induction of gene expression normalized with housekeeping genes and expressed as means ± SE. Statistical significance vs. vehicle-treated rats is indicated by *P < 0.05, †P < 0.01, or ‡P < 0.001. TAG, triacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; CE, cholesteryl ester; FC, free cholesterol; TC, total cholesterol.
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Fig. 9. HE3286 modulates fatty acid composition in vivo. A and B: mole percentages (mmol%) of palmitate (16:0), palmitoleate (16:1n7), linoleic acid (18:2n6), α-linolenic acid (18:3n3), arachidonic acid (20:4n6), eicosapentaenoic acid (20:5n3), and docosahexaenoic acid (22:6n3) in the liver (A) and white adipose tissue (B) were measured. C and D: nmol% of palmitoleate in lipid classes (16:1n7 compared with total fatty acids in indicated lipid classes) were calculated in serum (C) and muscle (D). Data are expressed as means ± SE. Statistical significance vs. vehicle-treated rats is indicated by *P < 0.05, †P < 0.01, or ‡P < 0.001. CL, cardiolipin; DAG, diacylglycerol; FFA, free fatty acid; LYPC, lysophosphatidylcholine; PS, phosphatidylserine.

Eating glycerol levels is likely to be an important factor in reducing HGP.

Hyperinsulinemic euglycemic clamp studies demonstrated further that HE3286 treatment leads to systemic insulin sensitivity with improved insulin action in muscle and liver (Figs. 4 and 5). Thus, drug treatment caused an increase in the insulin-stimulated GDR and an improved ability of insulin to suppress HGP and to lower FFA levels, indicating a broad systemic increase in insulin sensitivity. These results are qualitatively comparable with the effects of rosiglitazone treatment. However, quantitative differences were apparent in that the effects of rosiglitazone on muscle insulin sensitivity (IS-GDR) were greater, whereas the hepatic effects were comparable between the two drugs (Fig. 5).

The mechanisms by which HE3286 improves systemic insulin sensitivity are of high interest and are likely multifaceted. For example, HE3286 treatment attenuates JNK/AP1 and IKK/NF-κB signaling in macrophages, and it has been documented that genetic deletion of these pathways in myeloid cells is sufficient to alleviate systemic insulin resistance (2, 46). Inflammatory cytokines such as TNFα, IL-6, and IL-1β may be key mediators linking local tissue inflammation and increased ATM content to systemic insulin resistance (34, 44). HE3286 treatment inhibited macrophage TNFα mRNA expression and also led to a decrease in circulating TNFα and IL-1 levels in treated rats. Clearly, the latter could be largely a result of the reduced number of ATMs. These findings support the conclusion that an important aspect of HE3286-induced increased insulin sensitivity is related to its anti-inflammatory effects.

Another component of HE3286 in vivo action relates to inhibition of hepatic lipogenesis. Although it is still unclear whether hepatic lipogenesis and steatosis can directly cause insulin resistance (30, 40, 59), ample evidence exists showing that amelioration of hepatic lipid accumulation is associated with an improvement in insulin sensitivity (9, 32). Therefore, the drug-induced reduction of hepatic triglyceride and cholesterol content may participate in the effects on hepatic insulin sensitivity. Lastly, palmitoleate, a product of de novo lipogenesis, has recently been identified as a lipokine secreted by adipose tissue, which can lead to peripheral insulin sensitiza-
tion (6). We observed an increase in palmitoleate levels in the serum FFA fraction and in adipose tissue after HE3286 treatment. Close inspection shows that 16:1n7 does not increase in all serum lipid classes, and the changes in fatty acid composition in the specific lipid classes have potential physiological implications. Serum and muscle lipid profiles largely reflect events occurring in the liver and adipose tissue. For example, fasting serum phosphatidylcholine (PC), triglyceride (TG), and CE arise mainly from the liver, whereas serum FFAs come from adipose tissue (15, 20, 48). Therefore, 16:1n7 in serum PC, TG, or CE reflects enhanced hepatic lipogenesis, whereas increased 16:1n7 in FFAs reflects adipose tissue lipogenesis (6, 20, 39, 55). Drug treatment caused a significant increase in 16:1n7 FFA but not in the other serum lipid classes, suggesting a specific increase in de novo lipogenesis in fat. The reduction in hepatic TG and 16:1n7 levels by HE3286 treatment suggests decreased hepatic lipogenesis. The changes in 16:1n7 in blood are consistent with the findings in liver and adipose tissue, and together these results suggest that HE3286 treatment led to decreased lipogenesis in the liver and increased lipogenesis in the fat.

Cardiovascular disease is a major complication of type 2 diabetes and is closely associated with dyslipidemia and inflammation (41). In the current study, we observed novel effects of HE3286 on intracellular lipid metabolism. Thus, drug treatment led to a decrease in intracellular cholesterol concentration that could account for the feedback elevation of hepatic LDLR and HMG-CoA reductase expression. Elevation of LDLR-mediated cleavage would in turn lead to the reduction in circulating cholesterol levels in the treated rats. Many previous reports have confirmed that reduced intrahepatic cholesterol content will activate the key cholesterogenic transcription factor SREBP-2 via proteolytic cleavage and nuclear translocation (14). Subsequently, key enzymes involved in cholesterol biosynthesis (HMG-CoA reductase) and cholesterol uptake (LDLR) are upregulated. Our findings show that this sequence of events is triggered by HE3286 treatment, and its progression to the overt diabetic state.

References


