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Salicylate Downregulates 11 β -HSD1 Expression in Adipose Tissue in Obese Mice and in Humans, Mediating Insulin Sensitization

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Recent trials show salicylates improve glycemic control in type 2 diabetes, but the mechanism is poorly understood. Expression of the glucocorticoid-generating enzyme 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) in adipose tissue is increased in vitro by proinflammatory cytokines and upregulated in obesity. 11 β -HSD1 inhibition enhances insulin sensitivity. We hypothesized that salicylates downregulate 11 β -HSD1 expression, contributing to their metabolic efficacy. We treated diet-induced obese (DIO) 11 β -HSD1-deficient mice and C57Bl/6 mice with sodium salicylate for 4 weeks. Glucose tolerance was assessed in vivo. Tissue transcript levels were assessed by quantitative PCR and enzyme activity by incubation with ³H-steroid. Two weeks' administration of salsalate was also investigated in a randomized double-blind placebo-controlled crossover study in 16 men, with measurement of liver 11 β -HSD1 activity in vivo and adipose tissue 11 β -HSD1 transcript levels ex vivo. In C57Bl/6 DIO mice, salicylate improved glucose tolerance and downregulated 11 β -HSD1 mRNA and activity selectively in visceral adipose. DIO 11 β -HSD1-deficient mice were resistant to these metabolic effects of salicylate. In men, salsalate reduced 11 β -HSD1 expression in subcutaneous adipose, and in vitro salicylate treatment reduced adipocyte 11 β -HSD1 expression and induced adiponectin expression only in the presence of 11 β -HSD1 substrate. Reduced intra-adipose glucocorticoid regeneration by 11 β -HSD1 is a novel mechanism that contributes to the metabolic efficacy of salicylates. *Diabetes* 61:790–796, 2012

Salicylate anti-inflammatory agents, including acetylsalicylic acid (aspirin) and salicylsalicylic acid (salsalate) (1), improve insulin sensitivity in animal models (2–5) and in healthy or obese humans (6–9) and improve glycemic control in patients with type 2 diabetes (10–12). The mechanism of insulin sensitization is uncertain and may involve blockade of inhibitor of κ B kinase- β and, hence, nuclear factor- κ B effects (13,3,14) and/or interference with phosphorylation and activity of CCAAT enhancer binding protein- β (a transcription factor involved in metabolic and inflammatory pathways) (15,16), with associated reductions in proinflammatory cytokines.

11 β -Hydroxysteroid dehydrogenase type 1 (11 β -HSD1) catalyzes the regeneration of active cortisol from inert cortisone (corticosterone from 11-dehydrocorticosterone in rodents), including in liver and adipose tissue (17). In genetically modified mice, overexpression of 11 β -HSD1 in

adipose tissue (18) or liver (19) results in increased glucocorticoid action and insulin resistance, whereas targeted disruption of *Hsd11b1* protects against insulin resistance and central obesity on high-fat feeding (20–22). Pharmacological inhibition of 11 β -HSD1 lowers intracellular glucocorticoid levels and thereby enhances insulin sensitivity in rodents and humans (23–26). Circumstantial evidence suggests that salicylates may reduce 11 β -HSD1 in adipose tissue. Proinflammatory cytokines, including tumor necrosis factor (TNF)- α , interleukin-1 β , and interleukin-6, upregulate 11 β -HSD1 in several cell types in culture, including in human preadipocytes (27–29). In human obesity, a state of systemic and intra-adipose inflammation (14), 11 β -HSD1 mRNA levels and activity are decreased in liver (30,31) but increased in subcutaneous adipose tissue (32,31,33)—reviewed in (34)—and less consistently in visceral adipose tissue (35,36), explaining elevated intra-adipose cortisol regeneration (37). These observations raise the possibility that intra-adipose 11 β -HSD1 expression is increased by the proinflammatory state in obesity. Indeed, in HIV lipodystrophy, a paradigm of extreme intra-adipose inflammation, there is increased intra-adipose and systemic 11 β -HSD1 activity (38).

We hypothesized that salicylates reduce 11 β -HSD1 expression in adipose tissue and that this contributes to their insulin-sensitizing effect. We examined the effects of salicylate on adipose tissue 11 β -HSD1 in vivo in lean and obese mice and humans.

RESEARCH DESIGN AND METHODS

Chemicals were purchased from Sigma-Aldrich (Dorset, U.K.) unless otherwise stated.

Cell culture experiments. Simpson-Golabi-Behmel syndrome (SGBS) cells are a human preadipocyte cell line characterized by a high capacity for in vitro differentiation (39). Cells were cultured in Dulbecco's modified Eagle's medium-F12, supplemented with 10% (v/v) FCS, 50 units/mL penicillin per 50 units/mL streptomycin, biotin (33 μ mol/L), and pantothenic acid (17 μ mol/L) at 37°C in 5% CO₂. Adipogenic differentiation was induced after reaching near confluence as described previously (39). At day 14, cells were incubated for 24 h in stripped serum medium prior to treatment with salicylic acid (10, 30, and 100 μ mol/L) or vehicle (0.1% ethanol) for 24 h, and RNA was extracted for analysis of 11 β -HSD1 transcript. To assess the interaction between salicylate and 11 β -HSD1 in determining adiponectin (AdiQ) transcript levels, day 14 cells were incubated for 24 h in cortisol-free, stripped serum medium prior to treatment with cortisol (0.1 μ mol/L), cortisone (0.1 μ mol/L), or vehicle (0.1% ethanol) with or without salicylic acid alone (100 μ mol/L), or vehicle (0.1% ethanol) for 24 h. RNA was extracted for analysis.

Experiments in mice. Experiments were performed under license from the U.K. Home Office. Mice were maintained under controlled conditions of light (0700–1900 h) and temperature (18–20°C) and allowed access to food and drinking water ad libitum. Adult male C57Bl/6 mice were obtained (Harlan Olac, Oxfordshire, U.K.) at age 12 weeks. C57Bl/6 Lean animals were maintained on normal chow diet (2.27% fat, 4.05% sucrose; 801151; Special Diet Services). Diet-induced obese C57Bl/6 mice (C57Bl/6 DIO) were given 10 weeks of high-fat diet (58% fat, 12% sucrose; D12331; Research Diets, New Brunswick, NJ) before

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treatment. Homozygous male 11 β -HSD1-deficient (HSD1KO) mice were bred from an in-house colony on a C57Bl/6 background (>10 generations backcross) (21) and fed a high-fat diet until weight matched with C57Bl/6 DIO mice (~38 g).

Sodium salicylate (salicylate) (120 mg/kg/day) or distilled water (vehicle) was administered from 1 week after arriving (C57Bl/6 Lean), after 10 weeks of high-fat feeding (C57Bl/6 DIO), or after achieving target weight (HSD1KO-DIO) for 4 weeks to groups of $n = 8$ via osmotic minipumps implanted subcutaneously between the scapulae.

For glucose tolerance tests, mice were fasted for 6 h before administration of glucose (2 g/kg) by intraperitoneal injection and blood sampling by tail nicks for 90 min thereafter.

Animals were killed by decapitation and tissues dissected onto dry ice and stored at -80°C until analysis.

Experiments in humans. A total of 16 otherwise healthy men, selected to represent a wide range of BMIs, from 20 to 50 kg/m², took part in a double-blind balanced randomized crossover study to compare salsalate (Disalcid; 3M Pharmaceuticals, Northridge, CA; 1 g p.o. every 8 h for 2 weeks) with placebo. There was a 2-week washout period between phases, consistent with the short half-life of salsalate (<8 h). Inclusion criteria included normal thyroid, renal, and hepatic function; alcohol intake <28 units/week; no glucocorticoid use in the previous 6 months; no medication, including nonsteroidal anti-inflammatory medication in the previous month or during the study; and no history of dyspepsia or peptic ulcer disease. Local ethical committee approval and written informed consent were obtained.

At the end of each phase, volunteers attended the clinical research facility on 2 consecutive days. On day 1, they completed a 24-h urine collection and attended at 0830 h after fasting overnight. Blood was taken for glucose, insulin, lipid profile, and plasma cortisol. Height, weight, waist, and hip circumferences; percent fat by bioimpedance (Omron BF302, Milton Keynes, U.K.); and blood pressure by mercury sphygmomanometer were recorded. An adipose biopsy was obtained from periumbilical subcutaneous adipose tissue under local anesthesia (lignocaine 1%; Braun; Sheffield, U.K.) using a 14G biopsy needle (Sterican; B. Braun Melsungen AG) attached to a sterile 60 mL syringe. The tissue was then washed with diethyl pyrocarbonate-treated water, frozen immediately on dry ice, and stored at -80°C .

On day 2, volunteers took oral dexamethasone 1 mg (Merck Sharp & Dohme, Hertfordshire, U.K.) at 2300 h the evening before, fasted overnight, and attended at 0900 h. A venous cannula was inserted and oral cortisone acetate 25 mg (Pantheon Inc., Swindon, U.K.) was administered with water. Plasma samples were collected at intervals shown in Fig. 5 in lithium heparin and stored at -20°C .

Laboratory methods

Biochemical assays. Mouse glucose and insulin were measured by hexokinase assay (Thermo Electron, Victoria, Australia) and ELISA (Crystal Chem Inc., Downers Grove, IL), respectively.

Human plasma cortisol was measured using the ImmunChem cortisol ¹²⁵I radioimmunoassay coated tube kit (MP Biomedicals, High Wycombe, U.K.). Enzyme immunoassays (Eurogenetics Tasah Corp. UK Ltd., Hampton, U.K.) were used to measure plasma insulin and C-peptide. Electrolytes and salicylate were measured by dry-slide chemistry with a Vitros 950 (Ortho Clinical Diagnostics, Raritan, NJ), and glucose was determined on a Cabas Mira Plus (Roche, Mannheim, Germany). Triglycerides, total cholesterol, and HDL cholesterol were measured using ELISA kits (TG, CHOL, and HDLC-plus, respectively; Roche).

Human urinary cortisol and its metabolites (5β -tetrahydrocortisol [THF], 5α -THF, 5β -tetrahydrocortisone [THE], cortols, cortolones, and cortisone) were measured in 24-h samples by electron impact gas chromatography/mass spectrometry as previously described (40). The balance between 11 β -HSD activities in all tissues was assessed as the ratio of (5β -THF + 5α -THF) to THE.

Quantification of mRNA. For cultured cells and mouse tissue samples, total RNA was extracted using TRIzol reagent (Invitrogen, Paisley, U.K.) and RNeasy Mini Kit (QIAGEN, West Sussex, U.K.). RNA integrity was verified on an agarose gel (1.2% w/v in 0.5 \times Tris/borate/EDTA). RNA (0.25 μg) was reverse transcribed with random primers using the QuantiTect Reverse Transcription Kit (QIAGEN). Real-time PCR was performed using the Roche LightCycler 480 (Roche Applied Science, Burgess Hill, U.K.). Primers (Invitrogen) were designed for use with intron-spanning probes within the Roche Universal Probe Library. Results from adipose tissue from lean and obese mice were corrected for abundance of 18S and/or TATA binding protein mRNA, which were not affected by treatment. Results from SGBS cells were corrected for abundance of TATA-binding protein mRNA, which was not affected by treatment. All samples were analyzed in triplicate and amplification curves plotted (y -axis fluorescence, x -axis cycle number). Triplicates were deemed acceptable if the SD of the crossing point was <0.5 cycles. A standard curve (y -axis crossing point, x -axis log concentration) for each gene was generated by serial dilution of cDNAs pooled from different samples, fitted with a straight line, and deemed acceptable if reaction efficiency was between 1.7 and 2.1.

For human adipose tissue, 100 mg tissue was homogenized and RNA extracted using the QIAGEN RNeasy Lipid Tissue Mini kit (West Sussex, U.K.) and quantified using the Ribogreen quantitation kit (Molecular Probes, Eugene, OR). RNA integrity was verified on a 1.2% agarose/Tris borate/EDTA gel. Oligo dT-primed cDNA was synthesized from 0.5 μg RNA using Promega Reverse Transcription System (Promega, Southampton, U.K.), together with controls from which reverse transcriptase was omitted. Transcript quantification was performed as above for mouse samples. Primer-probe sets for cyclophilin A and 11 β -HSD1 were purchased from Applied Biosystems (Cheshire, U.K.). Random primers were used for 18S. The results are expressed as a ratio to the mean of cyclophilin A and 18S, as internal controls that did not differ between groups.

Enzymology. 11 β -HSD1 functions as a reductase in vivo, converting inactive 11-dehydrocorticosterone to corticosterone. However, in vitro, dehydrogenase activity predominates, so velocity of 11 β -HSD1 activity in adipose was quantified through conversion of corticosterone to 11-dehydrocorticosterone. Enzyme kinetics were determined from whole-adipose tissue homogenate. Standardized amounts of protein for each tissue (mesenteric adipose 300 $\mu\text{g}/\text{mL}$; subcutaneous adipose 100 $\mu\text{g}/\text{mL}$) were incubated (37°C) in Krebs-Ringer buffer containing glucose (1% w/v), NADP (2 mmol/L), [³H]₄-corticosterone (10 nmol/L), and unlabeled corticosterone (9.99 $\mu\text{mol}/\text{L}$). Steroids were extracted after incubation (mesenteric adipose 24 h; subcutaneous adipose 22 h) with ethyl acetate, the organic phase was evaporated under nitrogen, and extracts were solubilized in the mobile phase (water to acetonitrile to methanol; 60:15:25, 1.5 mL/min). Steroids were separated by high-performance liquid chromatography using a C₁₈ Sunfire column (Waters, Hertfordshire, U.K.) at 42°C and quantified by on-line liquid scintillation counting.

Statistical analyses. Results are presented as mean \pm SEM and analyzed by Student t tests or two-way ANOVA with Bonferroni post hoc tests, as indicated in the figure legends. Incremental changes induced by salsalate (i.e., value after salsalate minus value after placebo) were correlated with indices of obesity and plasma salsalate concentrations using Pearson correlation tests.

RESULTS

Salicylate improves glucose tolerance in obese but not lean mice. Ten weeks of high-fat feeding caused diet-induced obesity in male C57Bl/6 DIO mice, with elevated fasting plasma insulin levels compared with C57Bl/6 Lean mice (Table 1). On glucose tolerance testing, C57Bl/6 DIO mice were hyperglycemic and hyperinsulinemic compared with C57Bl/6 Lean mice, indicating development of insulin resistance (Table 1 and Fig. 1).

Four weeks of treatment with salicylate (120 mg/kg/day) had little measurable effect in C57Bl/6 Lean mice, except for elevated fasting plasma insulin. In C57Bl/6 DIO mice, salicylate decreased both fasting and postprandial plasma glucose levels (Table 1 and Fig. 1). Furthermore, there was a trend to reduced plasma triglyceride levels after salicylate treatment in C57Bl/6 DIO mice ($P = 0.059$) (Table 1). **Salicylate downregulates visceral adipose 11 β -HSD1 in DIO mice.** Salicylate significantly reduced 11 β -HSD1 mRNA in omental adipose tissue in C57Bl/6 DIO mice, with a similar trend in mesenteric adipose ($P = 0.057$) (Fig. 2A). In mesenteric adipose of C57Bl/6 DIO mice, salicylate also reduced 11 β -HSD1 enzyme activity (Fig. 2B).

11 β -HSD1 deficiency blocks insulin-sensitizing effects of salicylate. HSD1KO-DIO mice were protected against fasting hyperinsulinemia compared with weight-matched C57Bl/6 DIO controls. However, HSD1KO-DIO mice were still hyperinsulinemic, both fasting and postprandial, compared with C57Bl/6 Lean mice (Table 1 and Fig. 1). In marked contrast to C57Bl/6 DIO mice, salicylate had no effects on biochemical indices in HSD1KO-DIO mice (Table 1 and Fig. 1).

11 β -HSD1 deficiency prevents salicylate-induced changes in adipose transcript levels. In C57Bl/6 DIO mice (Fig. 3), salicylate altered mRNA levels of several genes. In mesenteric adipose (Fig. 3B), salicylate increased AdiQ and decreased TNF- α , monocyte chemoattractant protein-1 (MCP-1), adipose triglyceride lipase (ATGL), and angiotensinogen (AGT) mRNA levels. This

TABLE 1

Effects of salicylate on body weight and fasting plasma biochemistry in mice, with biochemical indices, age, high-fat diet duration, and weight measurements for cohorts of mice

	C57Bl/6 Lean		C57Bl/6 DIO		HSD1KO-DIO	
	Vehicle	Salicylate	Vehicle	Salicylate	Vehicle	Salicylate
Weight at start (g)	28.8 \pm 0.6	29.2 \pm 0.5	38.4 \pm 1.1	39.7 \pm 1.7	38.0 \pm 0.8	38.3 \pm 0.5
Weight at end (g)	29.2 \pm 0.8	29.5 \pm 0.7	39.0 \pm 1.4	40.3 \pm 2.3	38.0 \pm 0.8	39.0 \pm 0.8
Age at end (weeks)	17.0 \pm 0.0	17.0 \pm 0.0	24.0 \pm 0.0	24.0 \pm 0.0	22.7 \pm 3.3	22.7 \pm 3.3
Time on HFD (weeks)	0	0	10.0 \pm 0	10.0 \pm 0	10.8 \pm 0.8	10.8 \pm 0.8
Fasting plasma						
Insulin (ng/mL)	0.95 \pm 0.11	1.45 \pm 0.20*	2.69 \pm 0.28###	2.74 \pm 0.32	2.01 \pm 0.15 ϕ ###	2.65 \pm 0.45
Glucose (mg/dL)	112.5 \pm 7.8	113.2 \pm 7.1	131.7 \pm 12.2	109.0 \pm 5.7*	119.5 \pm 4.4	119.3 \pm 7.5
Triglycerides (mmol/L)	0.59 \pm 0.04	0.63 \pm 0.04	0.83 \pm 0.04##	0.70 \pm 0.05	0.84 \pm 0.05	0.81 \pm 0.04

Data are mean \pm SEM for $n = 7-8$ per group. Comparisons were by two-way ANOVA with Bonferroni post hoc tests. HFD, high-fat diet. * $P < 0.05$ vs. vehicle of same group. ## $P < 0.01$ vs. C57Bl/6 Lean vehicle. ### $P < 0.001$ vs. C57Bl/6 Lean vehicle. $\phi P < 0.05$ vs. C57Bl/6 DIO vehicle.

pattern of salicylate-induced changes was similar in omental adipose (Fig. 3A). With the exception of increasing AdiQ mRNA levels, these effects were absent in subcutaneous adipose tissue (Fig. 3C).

Compared with salicylate-treated C57Bl/6 DIO mice, HSD1KO-DIO mice had a similar pattern of transcript changes in visceral adipose, with increased AdiQ and reduced TNF- α and ATGL mRNA levels (Fig. 3). However, in HSD1KO-DIO mice, salicylate treatment did not alter mRNA levels of any of the genes affected in C57Bl/6 DIO mice (Fig. 3).

Salsalate downregulates adipose 11 β -HSD1 in vivo in men. Characteristics of the 16 male participants are shown in Table 2. Salicylate levels in plasma averaged 134 \pm 33 mg/L during active treatment and were undetectable during placebo. Only 1 of the participants reported tinnitus during salsalate therapy, and there were no changes in indices of

insulin sensitivity or lipid profile. Nevertheless, salsalate reduced 11 β -HSD1 mRNA levels in subcutaneous adipose, an effect which was unrelated to BMI (Fig. 4) or plasma salicylate levels (data not shown). This was accompanied by reduced transcript levels for MCP-1 (33.6 \pm 5.6% suppression, $P < 0.02$), but not TNF- α or AdiQ. The effect of salsalate on 11 β -HSD1 activity appeared restricted to adipose tissue since there was no change in first pass conversion of cortisone to cortisol in liver or in the ratio of cortisol to cortisone metabolites in urine (Fig. 4).

Salicylate-induced upregulation of AdiQ is mediated by reduced 11 β -HSD1 in human adipocytes. In fully differentiated human SGBS adipocytes, 24-h incubation with salicylic acid in the absence of steroid dose dependently reduced 11 β -HSD1 mRNA levels (Fig. 5A) but had no effect on AdiQ mRNA (Fig. 5B). Both cortisol and cortisone treatment (0.1 μ mol/L for 24 h) reduced mRNA

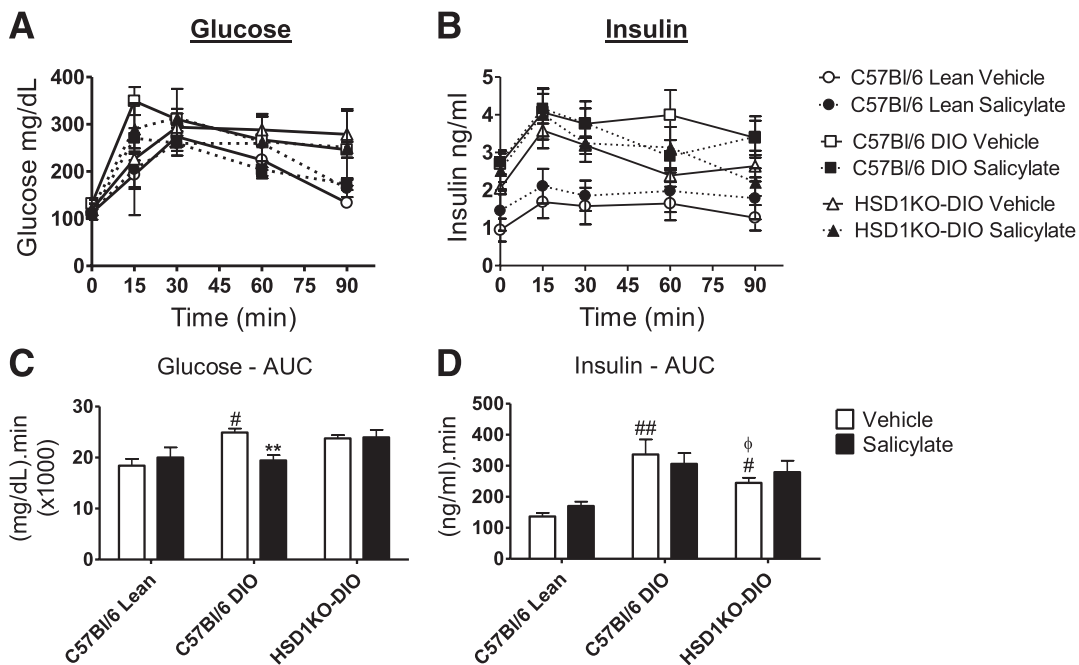


FIG. 1. Effects of salicylate on insulin sensitivity in C57Bl/6 Lean and DIO mice. Plasma glucose (A and C) and insulin (B and D) after intraperitoneal glucose injection in C57Bl/6 Lean, C57Bl/6 DIO, and HSD1KO-DIO mice. Area under the curve (AUC) for all groups shown for glucose (C) and insulin (D). Data are mean \pm SEM for $n = 7-8$ per group for individual time points. Comparisons for AUC were by two-way ANOVA with Bonferroni post hoc tests. ** $P < 0.01$ vs. vehicle in same group; # $P < 0.05$, ## $P < 0.01$ vs. Lean vehicle; $\phi P < 0.05$ vs. C57Bl/6 DIO vehicle.

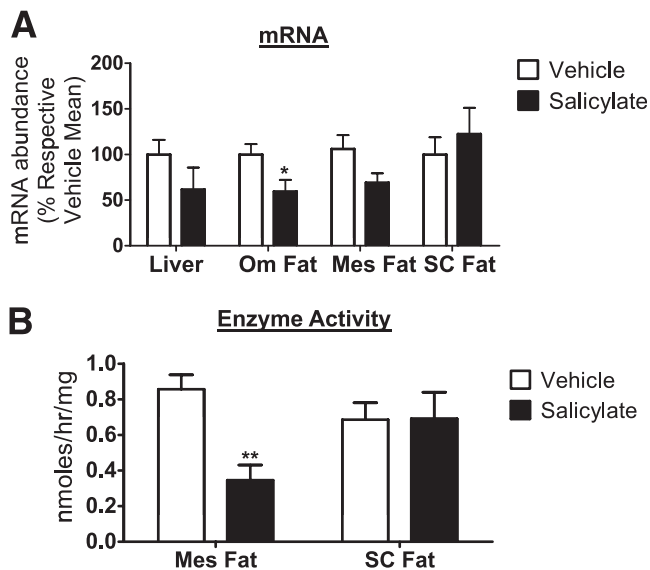


FIG. 2. Effects of salicylate on 11 β -HSD1 in DIO mice. 11 β -HSD1 mRNA expression in tissues from C57Bl/6 DIO mice (A). 11 β -HSD1 enzyme activity in mesenteric and subcutaneous adipose tissue from C57Bl/6 DIO mice (B). Om, omental; Mes, mesenteric; SC, subcutaneous. Data are mean \pm SEM for $n = 4-8$ per group. Comparisons were by Student unpaired t tests. * $P < 0.05$, ** $P < 0.01$ vs. vehicle in same tissue.

levels of AdiQ. In the presence of cortisone, coinubation with salicylic acid attenuated the suppression of AdiQ, whereas salicylate had no effect on suppression of AdiQ by cortisol (Fig. 5B).

DISCUSSION

These results identify a novel mechanism by which salicylates may enhance insulin action in diet-induced obesity, involving downregulation of adipose 11 β -HSD1 expression. This downregulation occurs selectively in adipose tissue depots in mice and in humans in vivo, is likely mediated directly within adipose, and is mimicked in human adipocytes in culture. It is crucial that mice deficient in 11 β -HSD1 display similar changes in in vivo metabolism and intra-adipose gene expression as salicylate-treated wild-type mice, and salicylate has no additional effects on these parameters in the absence of 11 β -HSD1, implicating 11 β -HSD1 as a mediator of the beneficial effects of salicylate.

The magnitude and components of the metabolic effects of salicylate remain somewhat contentious. For example, in humans, in addition to reports of improved insulin sensitivity (10,6,41,7), several reports describe worsening of insulin resistance (42-44) or attribute improved metabolism to an increase in insulin concentrations (9). Here, we confirm variable efficacy of salicylate in different settings. In lean mice, salicylate modestly increased plasma insulin concentration but had no other metabolic effects. In DIO mice, salicylate lowered fasting plasma glucose concentrations and improved glucose tolerance, as well as reducing 11 β -HSD1 in visceral adipose (omental and mesenteric) but not subcutaneous adipose tissue. These effects, together with the lack of change in plasma insulin concentrations, are consistent with insulin sensitization primarily in visceral adipose tissue due to reduced local glucocorticoid regeneration by 11 β -HSD1 (45,46), with secondary improvements in insulin sensitivity in other organs potentially mediated by altered adipokine secretion. The HSD1KO-DIO mice were <2 weeks younger than the

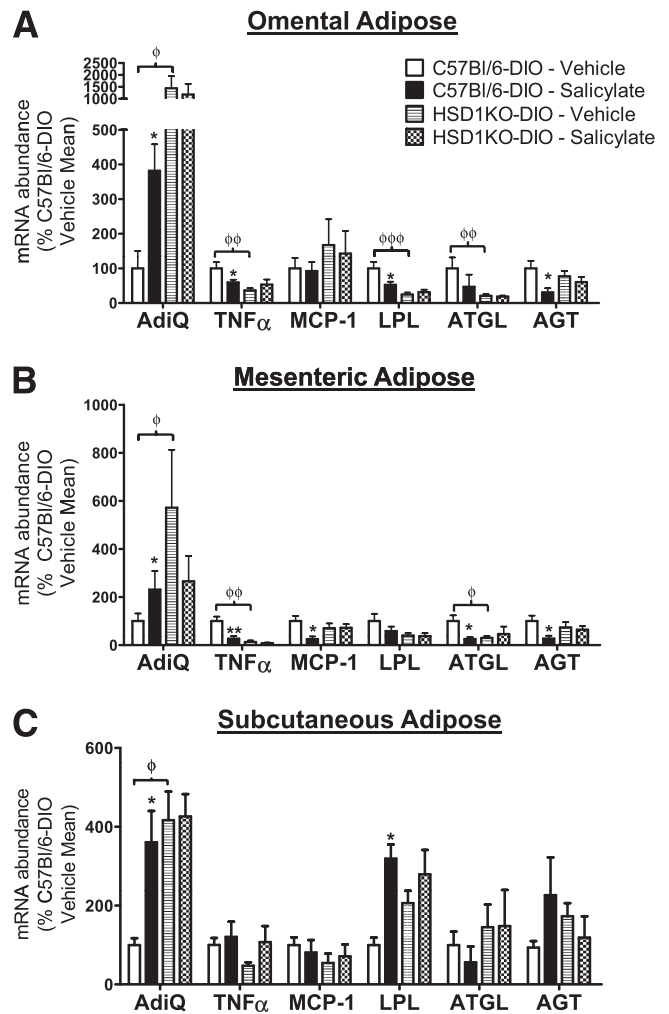


FIG. 3. Effects of salicylate on adipose mRNA levels in mice. mRNA levels were measured for AdiQ, TNF- α , MCP-1, lipoprotein lipase (LPL), adipose triglyceride lipase (ATGL), and angiotensinogen (AGT) by qPCR in omental (A), mesenteric (B), and subcutaneous (C) adipose tissue from C57Bl/6 DIO and HSD1KO-DIO mice. Data are mean \pm SEM for $n = 4-8$ per group. Comparisons were by two-way ANOVA with Bonferroni post hoc tests. * $P < 0.05$, ** $P < 0.01$ vs. vehicle of same genotype; $\phi P < 0.05$, $\phi\phi P < 0.01$, $\phi\phi\phi P < 0.001$ vs. C57Bl/6 DIO vehicle.

C57Bl/6 DIO mice, so it is highly unlikely that age-dependent effects are responsible for the discrepancies in responses to salicylate between groups. In contrast, in the human study, although we did not undertake sensitive measurements of insulin sensitivity, such as euglycemic clamps, there were no measured effects of salicylate on fasting insulin or lipid profile; this was the case even if we examined only obese participants (not shown). It may be that high doses are required to induce robust insulin sensitization in humans: the salicylate concentrations achieved in this study (mean 134 mg/L, ~ 1 mmol/L) (Table 2) are lower than in some other studies (e.g., 270 mg/L) (41). This inconsistency is in keeping with a mechanism of action mediated by 11 β -HSD1 downregulation, since recent phase 2 clinical trials of selective 11 β -HSD1 inhibitors demonstrate that metabolic effects are of relatively small magnitude and not always statistically significant (26,47). Previous studies demonstrating efficacy of salicylates in rodents use genetic models of obesity, including Zucker obese rats and *ob/ob* mice (32,2,18,3). To our knowledge, this is the first study to demonstrate the insulin-sensitizing

TABLE 2
Effects of salsalate on anthropometry and biochemistry in participating men

	Placebo	Salsalate
Age (years)	40.1 \pm 10.0	
BMI (kg/m ²)	31.9 \pm 8.0	32.0 \pm 8.0
Waist-to-hip ratio	0.93 \pm 0.23	0.94 \pm 0.23
Percent body fat	25.9 \pm 6.5	26.1 \pm 6.5
Systolic BP (mmHg)	136.8 \pm 34.2	137.9 \pm 34.5
Diastolic BP (mmHg)	79.7 \pm 19.9	81.9 \pm 20.5
Fasting plasma		
Salicylate (mg/L)	ND	134 \pm 33*
Triglycerides (mmol/L)	1.5 \pm 0.4	1.3 \pm 0.3
Total cholesterol (mmol/L)	4.7 \pm 1.2	4.4 \pm 1.1
LDL cholesterol (mmol/L)	2.7 \pm 0.7	2.6 \pm 0.6
HDL cholesterol (mmol/L)	1.3 \pm 0.3	1.3 \pm 0.3
Glucose (mmol/L)	5.2 \pm 1.3	5.1 \pm 1.3
Insulin (mU/L)	26.5 \pm 6.6	23.1 \pm 5.8

Data are mean \pm SEM for $n = 16$ per group. Comparisons were by Student paired t tests. BP, blood pressure; ND, not detected. * $P < 0.01$ vs. placebo.

effects of salicylate in a nongenetic model of obesity. Indeed, diet-induced obesity was chosen because it is a more relevant model to the obesity seen in humans. Furthermore, salicylates improve insulin sensitivity during lipid-induced insulin resistance (2,7). 11 β -HSD1 activity is increased acutely in adipose tissue by intralipid infusion (48), potentially contributing to lipid-induced insulin resistance. Blockade by salicylates of the effective increase in 11 β -HSD1 activity could therefore explain its relatively selective effect on lipid-induced insulin resistance.

Protective metabolic effects of 11 β -HSD1 deficiency in mice receiving a high-fat diet have been described previously (22) and are shown here to bear remarkable similarity to the pattern of effects of salicylates on glucose tolerance and adipose lipase expression. It might be argued that the lack of effect of salicylates in HSD1KO mice is confounded by the improved metabolic “baseline” in these animals. However, to avoid this, we administered high-fat diet for long enough to achieve similar body weight in HSD1KO mice to that observed in C57Bl/6 DIO mice and, thus, ensured these HSD1KO-DIO mice were markedly hyperinsulinemic compared with lean mice. The striking lack of efficacy of salicylate treatment in HSD1KO mice is therefore consistent with downregulation of 11 β -HSD1 playing a key role in its mechanism of action.

Downregulation of 11 β -HSD1 by salicylates is likely to reflect a direct mechanism in adipose tissue, given its replication in an adipocyte cell line. Furthermore, we have demonstrated in vitro that salicylic acid reverses cortisone-mediated suppression of the insulin-sensitizing adipokine AdiQ; since salicylic acid had no effect when the contribution of 11 β -HSD1 was negated by removal of steroid or by addition of cortisol, these data indicate that inhibition of 11 β -HSD1 by salicylates mediates the altered AdiQ expression also observed in vivo. The downregulation of 11 β -HSD1 was selective for the visceral adipose depots in mice but was evident in subcutaneous adipose in humans. Central adipose depots exhibit greater variation in lipolytic activity and more intense proinflammatory responses during high-fat feeding and are more glucocorticoid responsive (49). However, in humans, recent evidence suggests that 11 β -HSD1 regenerates more cortisol in subcutaneous than in visceral adipose tissue (50), suggesting species-specific

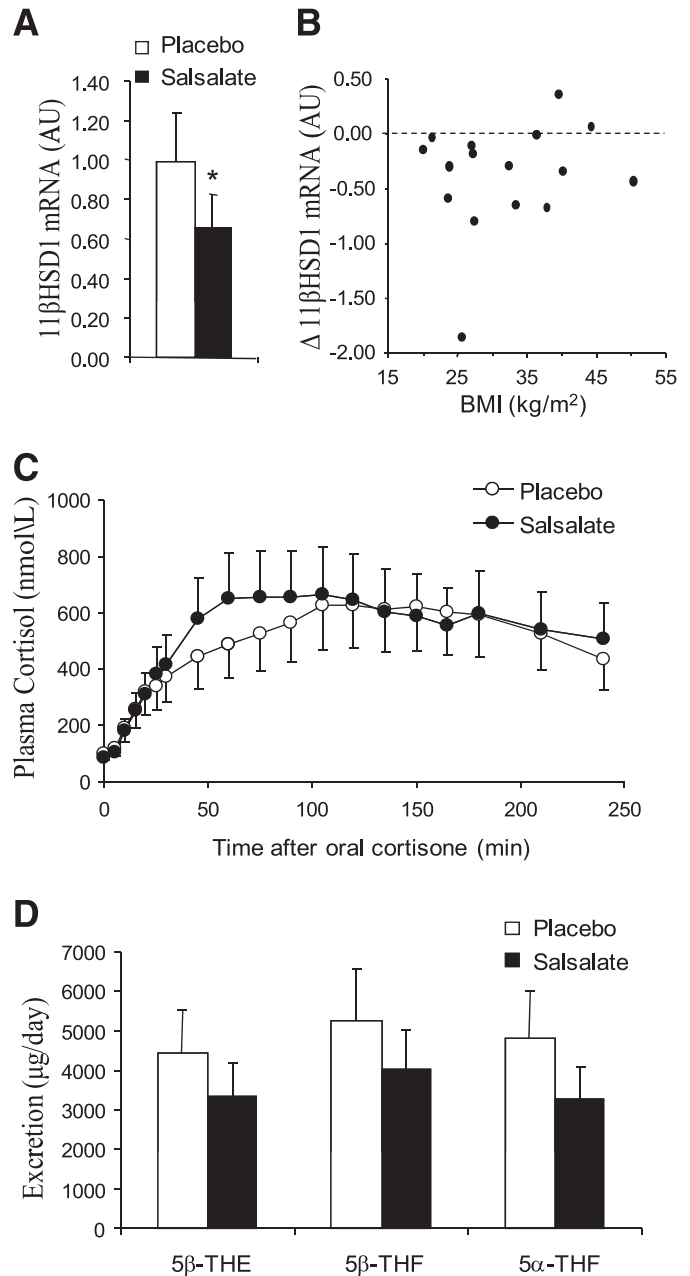


FIG. 4. Effect of salsalate on 11 β -HSD1 in humans. Subcutaneous adipose tissue 11 β -HSD1 mRNA levels (A); the correlation of change in adipose mRNA levels after salsalate with BMI (B); liver 11 β -HSD1, measured as appearance of cortisol in plasma after overnight dexamethasone suppression and administration of 25 mg cortisone by mouth at time 0 (C); and urinary cortisol metabolites (D) in 16 participants in a double-blind randomized crossover trial comparing salsalate with placebo. Data are mean \pm SEM. Comparisons were by paired Student t test (A and D), Pearson correlation (B), or two-way repeated-measures ANOVA (C). * $P < 0.02$ vs. placebo. AU, arbitrary unit.

differences. Moreover, we did not find evidence that changes in proinflammatory cytokines, such as TNF- α , mediate the effects of salicylates on 11 β -HSD1 expression; the downregulation of TNF- α by salicylates in C57Bl/6 DIO mice, but resistance to this effect in 11 β -HSD1KO mice, suggests that effects of salicylates on intra-adipose inflammation may be a consequence rather than cause of downregulation of 11 β -HSD1. This is further supported by the downregulation of TNF- α in the visceral adipose of 11 β -HSD1KO mice compared with C57Bl/6 mice. In human adipose, TNF- α mRNA

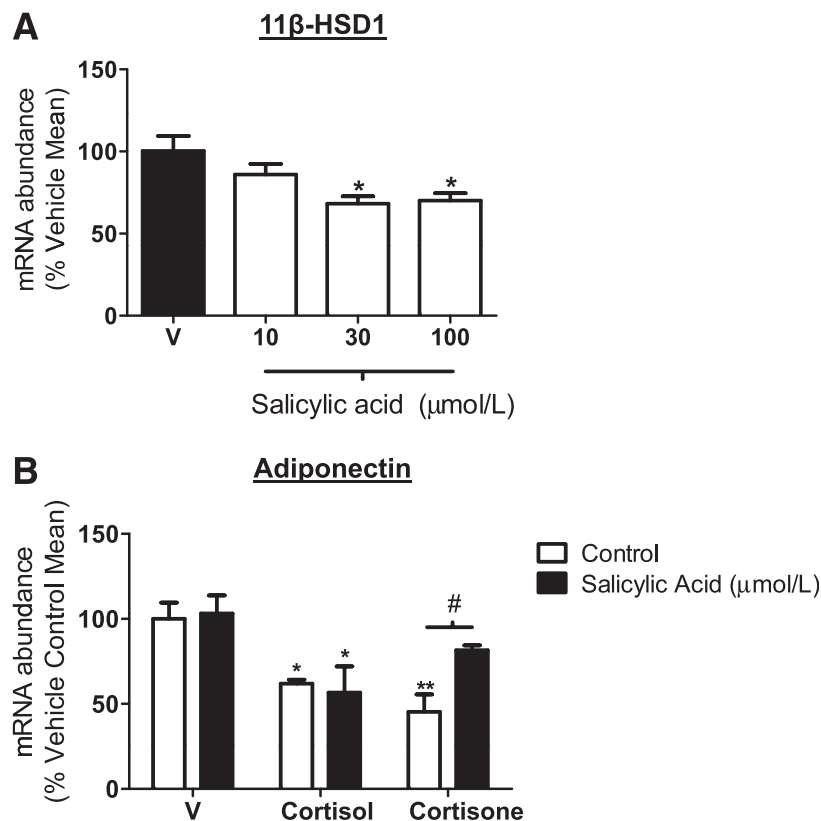


FIG. 5. Effects of salicylic acid on 11 β -HSD1 and Adiponectin mRNA levels in human cultured adipocytes. **A:** Human SGBS adipocytes were incubated for 24 h with salicylic acid (10, 30, and 100 μ mol/L) or vehicle (V) and mRNA levels measured. **B:** SGBS adipocytes were incubated for 24 h with vehicle, cortisol (0.1 μ mol/L), or cortisone (0.1 μ mol/L), with or without salicylic acid (100 μ mol/L). Data are mean \pm SEM of results from three experiments, each conducted in triplicate. Comparisons were by one-way ANOVA with Dunnett post hoc tests (A) or two-way ANOVA with Bonferroni post hoc tests (B). * P < 0.05, ** P < 0.01 vs. vehicle; # P < 0.05 vs. cortisone control.

did not change in association with altered 11 β -HSD1 mRNA. In the absence of cytokine-mediated regulation, more detailed investigation will be required to dissect the molecular mechanisms behind the salicylate-mediated regulation of 11 β -HSD1 expression.

In conclusion, these findings suggest that the anti-inflammatory agent salicylate alters glucocorticoid metabolism in mice and humans in a pattern that differs between liver and subcutaneous adipose tissue. Downregulation of intra-adipose 11 β -HSD1 may contribute to the insulin-sensitizing effect of salicylates.

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M.N. conducted the experiments, analyzed data in mice and in vitro, and wrote the manuscript. D.E.L. and C.L.E. conducted the experiments and analyzed data in mice and in vitro. D.J.W., R.H.S., and R.A. conducted the experiments and analyzed data in humans. J.R.S. and K.E.C. contributed to discussion. B.R.W. wrote the manuscript, which was

reviewed by all authors. B.R.W. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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