

Salicylates Increase Insulin Secretion in Healthy Obese Subjects

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Context: Conflicting results on the effects of salicylates on glucose tolerance in subjects with normal glucose tolerance or type 2 diabetes have been reported.

Objective: The objective of the study was to investigate the effects of a salicylate derivative (triflusal) on insulin sensitivity and insulin secretion.

Design, Setting, and Participants: This was a double-blind, randomized, crossover study with three treatment periods corresponding to two dose levels of triflusal and placebo in healthy obese subjects.

Main Outcome Measures: Insulin sensitivity and insulin secretion, evaluated through frequently sampled iv glucose tolerance test that was performed after each treatment period, were measured. Insulin secretion was also evaluated *in vitro* in mice and human islets of Langerhans.

Results: The administration of triflusal led to decreased fasting serum glucose concentration in the study subjects. Insulin sensitivity did not significantly change after each treatment period. Insulin secretion, however, significantly increased in a dose-dependent fashion after each triflusal treatment period. The administration of 800 μM of the main triflusal metabolite to whole mice islets of Langerhans led to a sustained increase in intracellular calcium concentration level. This was followed by a significantly increase in insulin secretion. In human islets, 200 μM of 2-hydroxy-4-trifluoromethylbenzoic acid was sufficient to increase insulin release.

Conclusions: The administration of a salicylate compound led to lowering of serum glucose concentration. We suggest that this effect was mediated through increased insulin secretion induced by salicylate directly on the β -cell. (*J Clin Endocrinol Metab* 93: 2523–2530, 2008)

Sodium salicylate at high doses was first shown to be beneficial in some cases of diabetes more than 100 yr ago (1, 2). In 1957 Reid *et al.* (3) rediscovered aspirin's effect to reduce glucosuria and blood glucose in a diabetic patient. Glucose tolerance was improved by aspirin therapy in diabetic subjects (3–5), and aspirin was shown to reduce insulin requirements (4). Conflicting results on the effects of aspirin in subjects with nor-

mal glucose tolerance (6, 7) and diabetic subjects (8) were subsequently reported.

Recently interest in the compound has been revived by a study demonstrating that it can reverse insulin resistance (9, 10). Rodent and human data demonstrated the ability of high-dose salicylate to improve insulin resistance in obesity by inhibiting inhibitory- κB kinase (IKK)- β activity (9, 10).

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Abbreviations: BMI, Body mass index; $[\text{Ca}^{2+}]_i$, intracellular calcium concentration; COX, cyclooxygenase; DMSO, dimethylsulfoxide; HbA1c, hemoglobin A1c; HTB, 2-hydroxy-4-trifluoromethylbenzoic acid; IKK, inhibitory- κB kinase; NF- κB , nuclear factor- κB ; s, soluble fraction; TNFR, TNF receptor.

These actions of salicylate are not attributed to the well-known ability of low-dose aspirin to inhibit the cyclooxygenase enzymes, cyclooxygenase (COX)-1 and COX2. Rather, it has been shown to be due to blocking nuclear factor- κ B (NF- κ B) action (11) and most specifically by inhibiting the regulatory kinase IKK β that normally favors displacement of NF- κ B from its complex with inhibitory- κ B and its movement from the cytosol to the nucleus (9, 10).

Both genetic (Zucker *falfa* rats) and diet-induced (high fat intake) obesity in rodents was associated with a 2-fold increase in hepatic NF- κ B activity. Transgenic mice which selectively express IKK β in hepatocytes had a type 2 diabetic phenotype with hyperglycemia, hepatic and systemic (muscle) insulin resistance, and increased circulating proinflammatory cytokines (12). Glucose tolerance was improved by salicylate treatment. These studies showed that activation of the NF- κ B pathway in the liver alone is sufficient to cause hepatic and peripheral insulin resistance and a syndrome resembling type 2 diabetes (12).

Mice lacking IKK β in hepatocytes retained insulin sensitivity in the liver in response to high-fat diet, obesity, and aging but continued to develop peripheral insulin resistance (12). In contrast, mice without myeloid cell IKK β retained both hepatic and peripheral insulin sensitivity (13).

In humans, a 2-wk trial of high-dose aspirin treatment was accompanied by significant decreases in hepatic glucose production (22%), fasting plasma glucose (24%), and triglycerides (48%) and a 19% increase in peripheral glucose disposal. The aspirin-induced increase in insulin-stimulated glucose uptake was associated with an approximately 47% increase in plasma insulin concentrations, which was interpreted as decreased insulin clearance. Aspirin therapy also resulted in significant reductions in postprandial hyperglycemia (9).

In a recent study, the acute administration of 4 g of acetylsalicylic acid to 10 healthy men attenuated lipid-induced insulin resistance (14).

We found no studies dealing with the administration of salicylates in nondiabetic obese subjects. Obesity *per se* is well known to be associated with insulin resistance. We hypothesized an improvement in both insulin sensitivity and glucose metabolism in these obese subjects after treatment with salicylates. Thus, we designed a 12-wk double-blind trial to evaluate the effects of the administration of 600 and 900 mg of triflusal [a 4-trifluoromethyl derivative of salicylate (15)] on insulin sensitivity and insulin secretion in these subjects. After knowing the results of this study, we also decided to test the effects of the triflusal metabolite [2-hydroxy-4-trifluoromethylbenzoic acid (HTB)] on insulin secretion in isolated islets of Langerhans from mice and humans.

Subjects and Methods

Study design

This was a double-blind, randomized, crossover study with three treatment periods (each of 4 wk) corresponding to two dose levels (600 or 900 mg orally once a day) and a placebo (registered trial NCT00162799). Each treatment period was followed by a 2-wk

washout period. Insulin sensitivity, insulin secretion, and metabolic and inflammatory variables (see below) was evaluated after each treatment period. The study was powered (80%) to detect significant changes in insulin sensitivity. Subjects were visited before and after each treatment period and asked for side effects. Compliance was monitored in each visit.

Inclusion and exclusion criteria

All subjects reported that their body weight had been stable for at least 3 months before the study. A food frequency questionnaire was obtained from all subjects. None of the subjects was taking any medication or had any evidence of metabolic disease other than obesity. Inclusion criteria were: 1) body mass index (BMI; weight in kilograms divided by the square of height in meters) between 27.5 and less than 40 kg/m². The cut point for BMI was 27.5 kg/m² because in our population 27.5 is equivalent to 30 kg/m² in other Caucasian populations (16); 2) absence of any systemic disease; and 3) absence of clinical symptoms and signs of infection in the previous month by structured questionnaire to the patient.

Informed consent was obtained from all subjects. Local ethics committee approved the study.

Measurements

BMI was calculated as weight (in kilograms) divided by height (in meters) squared. The subjects' waist was measured with a soft tape midway between the lowest rib and the iliac crest. The hip circumference was measured at the widest part of the gluteus region. The waist to hip ratio was then calculated. Blood pressure was measured in the supine position on the right arm after a 10-min rest; a standard sphygmomanometer of appropriate cuff size was used, and the first and fifth phases were recorded. Values used in the analysis are the average of three readings taken at 5-min intervals. Patients were requested to withhold alcohol and caffeine during at least 12 h before the different tests.

Insulin sensitivity and secretion

All subjects had fasting plasma glucose less than 7.0 mM. Insulin sensitivity was measured using the frequently sampled iv glucose tolerance test. Insulin secretion was calculated as the insulin area during the first 10 min of the frequently sampled iv glucose tolerance test.

In brief, the experimental protocol started between 0800 and 0830 h after an overnight fast. A butterfly needle was inserted into an antecubital vein, and patency was maintained with a slow saline drip. Basal blood samples were drawn at -30 , -10 , and -5 min, after which glucose (300 mg/kg body weight) was injected over 1 min starting at time 0, and insulin (Actrapid; Novo, Copenhagen, Denmark; 0.03 U/kg) was administered at time 20 min. Additional samples were obtained from a contralateral antecubital vein up to 180 min, as previously described (17, 18).

Analytical methods

Blood samples were drawn from each subject after an overnight fasting period. Serum was centrifuged at $4000 \times g$ for 10 min, immediately divided into aliquots, and frozen at -80 C until analysis.

Serum glucose concentrations were measured in duplicate by the glucose oxidase method with the use of a Beckman Glucose Analyzer II (Beckman Instruments, Brea, CA). The coefficient of variation was 1.9%. Serum insulin was measured in duplicate by monoclonal immunoradiometric assay (Medgenix Diagnostics, Fleunes, Belgium). The intraassay coefficient of variation was 5.2% at a concentration of 10 mU/liter and 3.4% at 130 mU/liter. The interassay coefficients of variation were 6.9 and 4.5% at 14 and 89 mU/liter, respectively. These coefficients were similar to those previously reported (17). Hemoglobin A1c

(HbA1c) was measured by high performance liquid chromatography by means of a fully automated glycolated hemoglobin analyzer system (L-9100; Hitachi, Indianapolis, IN).

Total serum cholesterol was measured through the reaction of cholesterol esterase/cholesterol oxidase/peroxidase. Total serum triglycerides were measured through the reaction of glycerol-phosphate-oxidase and peroxidase.

Serum C-reactive protein (ultrasensitive assay; Beckman, Fullerton, CA) was determined by routine laboratory test, with intra- and interassay coefficients of variation lower than 4%. The lower limit of detection is 0.02 mg/liter.

Plasma soluble fraction (s) of TNF receptor (TNFR)-1 and sTNFR2 (Zoning Industriel B-6220; BioSource Europe S.A., Fleunes, Belgium) were determined as previously described (17).

In vitro studies

Islets isolation and intracellular calcium measurement

Swiss albino OF1 male mice (8–10 wk old) were killed by cervical dislocation in accordance with national guidelines provided by our animal house. An internal animal care and use committee reviewed and approved the method used. Pancreatic islets of Langerhans were isolated with collagenase digestion as previously described (19), left to recover for 1 h at 37°C in the incubator, and then loaded at room temperature with fura-2 by incubation for at least 1 h (5 μ M; Molecular Probes, Eugene, OR). Loaded islets were kept in a medium containing 115 mM NaCl, 10 mM NaHCO₃, 5 mM KCl, 1.1 mM MgCl₂, 1.2 mM NaH₂PO₄, 2.5 mM CaCl₂, and 25 mM HEPES plus 1% albumin and 5 mM D-glucose, continuously gassed with a mixture of 95% O₂ and 5% CO₂ (pH 7.35). Islets were perfused at a rate of 1 ml/min with a modified Ringer solution containing 120 mM NaCl, 5 mM KCl, 25 mM NaHCO₃, 1.1 mM MgCl₂, and 2.5 mM CaCl₂ (pH 7.35 when gassed with 95% O₂ and 5% CO₂). Stimuli were added containing always the same concentration of dimethylsulfoxide (DMSO), according to the concentration of HTB used. Calcium records in the whole islet of Langerhans were obtained by imaging intracellular calcium under an inverted epifluorescence microscope (Axiovert 200; Zeiss, Jena, Germany). Images were acquired approximately every 3 sec with an extended digital camera C4742–95 (Hamamatsu Photonics, Barcelona, Spain) using a dual-filter wheel (Sutter Instruments Co., Navato, CA) equipped with 340 and 380 nm, 10 nm bandpass filters (Omega Optics, Madrid, Spain). Data were acquired using ORCA software (Hamamatsu Photonics). Fluorescence changes are expressed as the ratio of fluorescence at 340 nm and 380 nm (F_{340}/F_{380}). Results were plotted using commercially available software (Sigmaplot, Jandel Scientific, San Rafael, CA).

Insulin secretion measurement

To measure insulin release from isolated islets, groups of five islets were incubated in 0.4 ml of modified Ringer solution in the presence of stimuli for 1 h. The stock solution of HTB was freshly prepared at 250 μ M in DMSO. The maximum concentration of DMSO, equivalent to 800 μ M HTB, was always present in the stimuli. After that, 0.1 ml 5% BSA modified Ringer solution was added to the islets and the medium was collected to measure insulin using RIA (Coat-a-Count; Diagnostic Products Corp., Los Angeles, CA). The islets were transferred to lysis buffer (75% ethanol, 0.4% HCl), vortexed thoroughly, and incubated overnight in cold. Then the supernatant of a 2500-rpm, 5-min centrifugation was used to measure protein concentration. The results were expressed as normalized with respect to the insulin secretion (international units per milligram protein) at 5 mM glucose.

Insulin secretion in human islets

Human islets were isolated following a modification of Ricordi's automatic digestion technique (20, 21). The isolated islets were cultured for 12 h at 37°C in RPMI 1640 (11.1 mM glucose). The human islets were hand picked and preincubated in RPMI 1640 medium at 5.5 mM glucose for 2 h. To assess insulin secretion from isolated human islets, batches of

eight islets were incubated in a shaking water bath for 90 min at 37°C in 1 ml bicarbonate-buffered medium containing BSA (5 mg/ml) in the presence of stimuli. The stock solution of HTB was freshly prepared at 250 μ M in DMSO. The maximum concentration of DMSO, equivalent to 800 μ M HTB, was always present in the stimuli. Insulin release was measured by RIA (INS-IRMA kit; BioSource, Nivelles, Belgium). The results were expressed as microinternational units per milliliter islet at 5 mM glucose.

Statistical methods

Descriptive results of continuous variables are expressed as mean (SD) whether normally distributed, or as median and interquartile range, and as proportions for qualitative variables. Before statistical analysis, normal distribution and homogeneity of the variances were evaluated using Levene's test, and then variables were given a log transformation if necessary. These parameters (insulin sensitivity, insulin secretion, soluble TNFR-1 and TNFR-2, triglycerides) were analyzed on a log scale and tested for significance on that scale. The antilog-transformed values of the means are reported in Table 1. To compare the effects of the treatments, we used a classic crossover design in which the patients served as their own controls. In this repeated-measures design, each patient received one of the three treatments (placebo, Triflusal 600 mg, and Triflusal 900 mg). For the crossover analysis, overall mean (response) was modeling by a mixed-effects model in which period, treatment, and carryover terms were considered fixed effects and subject and error terms were considered random effects. There was not observed a carryover effect in any of the parameters. Adjustment was made for multiple testing (Bonferroni) by the requirement that the overall test results be significant before pairwise comparisons were considered. Patients were included in analyses on the basis of per protocol population. All reported *P* values are two sided. All statistical analyses were conducted with the use of SAS software (version 8.0; SAS Institute, Cary, NC).

Results

Thirty-one subjects fulfilled the inclusion criteria. Of these, three subjects were lost during follow-up due to voluntary patient withdrawal. We finally studied 28 subjects (nine men and 29 women), mean age 48.8 \pm 10.1 yr, mean BMI 33.9 \pm 3.5 kg/m², waist to hip ratio 1.01 \pm 0.06 (men) and 0.88 \pm 0.04 (women), with a mean fasting glucose 98.6 \pm 12.8 mg/dl and mean HbA1c 4.6 \pm 0.49%. Four subjects had impaired fasting glucose. Inclusion or exclusion of these subjects did not change significantly the results. We observed no significant variations in anthropometrical parameters (BMI, waist, or waist to hip ratio) or blood pressure among the study subjects (Table 1). The administration of triflusal led to decreased fasting serum glucose concentration in the study subjects (Table 1). This was accompanied by decreased glycolated hemoglobin among women (*P* = 0.04).

No significant changes were observed in plasma lipids, or serum concentration of the sTNFR2, a surrogate of TNF- α action (Table 1). Fasting insulin also remained unchanged during the trial. Contrary to our expectations, insulin sensitivity did not significantly change during the trial (Fig. 1, upper panel). Insulin secretion, however, significantly increased in a dose-dependent fashion after triflusal treatment (Fig. 1, lower panel). Side effects were similar after placebo and after each dose of triflusal.

Serum C-reactive protein also significantly decreased after antiinflammatory treatment but only with triflusal 600 mg. However, this was due to unexplained increased C-reactive protein in one subject (4 mg/liter). When this subject was excluded

TABLE 1. Study of the effects of salicylate (triflusal) on metabolic parameters

	Baseline	Triflusal, 600 mg	Triflusal, 900 mg	P
Sex (male/female)	9/19			
Age (yr)	48.8 (10.1)			
BMI (kg/m ²)	33.9 (3.5)	34.2 (3.4)	34.0 (3.4)	0.8
Waist circumference, men	106.1 (11)	105.3 (11.4)	106.3 (10)	0.2
Waist circumference, women	100.3 (5.3)	101.3 (7.7)	99.5 (5.8)	0.3
Fasting glucose (mg/dl)	98.6 (12.8)	95.8 (11.1)	94.2 (9.7)	0.03
Fasting insulin (mU/liter)	13.7 (7.4)	13.3 (8.1)	13.8 (7.5)	0.3
HbA1c, men (%)	4.7 (0.47)	4.9 (0.63)	5.0 (0.61)	0.8
HbA1c, women (%)	4.6 (0.48)	4.6 (0.57)	4.4 (0.47)	0.04
HDL cholesterol, men (mg/dl)	51.3 (14.9)	50.4 (13.1)	49.8 (13.8)	0.4
HDL cholesterol, women (mg/dl)	59.2 (11.7)	58.8 (12)	61.1 (11.2)	0.2
Triglycerides (mg/dl) ^a	77 (55–156)	80 (67.5–113)	80.5 (63.7–101.7)	0.3
Uric acid, men (mg/dl)	7.0 (1.5)	6.1 (1.4)	6.3 (1.2)	<0.0001
Uric acid, women (mg/dl)	4.6 (1.4)	3.7 (0.7)	3.4 (0.8)	<0.0001
C-reactive protein (mg/liter) ^a	0.37 (0.23–0.59)	0.31 (0.19–0.50)	0.29 (0.16–0.51)	0.07
sTNFR2 (ng/ml)	4.3 (3.7–7.0)	4.4 (3.3–7.0)	4.7 (3.7–7.6)	0.7
Insulin sensitivity ¹ (10 ⁻⁴ ·min ⁻¹ , mU/liter) ^a	1.78 (0.74–2.96)	1.63 (0.99–2.37)	1.69 (0.78–2.63)	0.4
Insulin secretion ¹ (mU/liter·min) ^a	345.2 (205.4–541.1)	403.2 (236–564.9)	460.6 (275–644.2)	0.04

Data are means (sd), except as cited. HDL, High-density lipoprotein. P values in *bold* are significant.

^a Expressed as median (interquartile range).

from the analysis, C-reactive protein significantly also decreased after triflusal 900 mg (Fig. 2, *upper panel*). Administration of triflusal led to significantly decreased serum uric acid levels (Fig. 2, *lower panel*).

In vitro studies

Intracellular calcium concentration [Ca²⁺]_i was measured in whole islets of Langerhans isolated from mice. Five millimoles glucose are not enough to produce either a change in [Ca²⁺]_i levels or insulin secretion above baseline. However, when 800 μM HTB were added, there was a sustained increase in [Ca²⁺]_i level, which was maintained as long as the chemical was present (Fig. 3A). This effect was reversible because [Ca²⁺]_i decreased to basal levels after washout. Lower concentrations of HTB (as low as 100 μM) produced no effect on [Ca²⁺]_i. After the treatment with HTB, islets were perfused with a stimulatory concentration of glucose, 11 mM, to show that HTB was not toxic for the islets, and these were still metabolically responsive.

Because insulin secretion depends on movements in [Ca²⁺]_i, it was expected to have an increase in insulin secretion in response to HTB. Therefore, RIAs were performed to measure insulin secretion in groups of five islets. Several concentrations of HTB were used. Only 800 μM HTB significantly increased the insulin secretion in the presence of 5 mM glucose (Fig. 3B).

Insulin secretion in human islets

Finally, we also studied the effects of the triflusal metabolite, HTB, on insulin secretion in human islets. We observed significantly higher insulin release after 200 μM of HTB (*P* = 0.01, Fig. 4).

Discussion

Aspirin therapy has been described to improve glucose tolerance and reduce insulin requirements in diabetic subjects (3–5). However, not all studies were concordant. Whereas early reports suggested a salutary effect of aspirin on glucose metabolism in diabetic patients (1–5), other clinical trials demonstrated a detrimental effect of aspirin therapy on insulin sensitivity (6–8). Important differences between these studies included lower aspirin dosages (<3 g/d) and therapeutic duration (a few days) in the more recent studies than in the earlier studies (6–9 g/d for 1–3 wk).

To our knowledge, this is the first study of salicylates in nondiabetic insulin-resistant obese subjects. Triflusal is a 4-trifluoromethyl derivative of salicylate (2-acetoxy-4-trifluoromethylbenzoic acid). Triflusal is not a novel salicylate. It was launched in 1981 in Spain as an antiplatelet agent and is now currently marketed in 25 countries worldwide. Triflusal is an irreversible inhibitor of COX-1 activity in different territories, including platelets, with a potency lower than that of acetylsalicylic acid (15, 22–24). Triflusal is also an inhibitor of COX-2 activity and is capable of inhibiting COX-2 expression due to its inhibitory effect on NF-κB activation (15). Its deacetylated metabolite, HTB, has also inhibitory effects on COX-1 activity (23, 24). Unlike triflusal, HTB is a reversible inhibitor of COX-1 activity (23). The main metabolite of salicylic acid is not active as antiplatelet agent, and its inhibitory effect on COX-1 activity is negligible (23). By virtue of COX-1 inhibition, triflusal also inhibits thromboxane biosynthesis (23, 24–28). Furthermore, triflusal leads to increased cAMP levels in platelets by inhibition of cAMP-phosphodiesterase activity (29), is associated with protection of prostacyclin biosynthesis due to its negligible inhibitory effect on vascular cyclooxygenase at therapeutic doses (23, 26–28), and stimu-

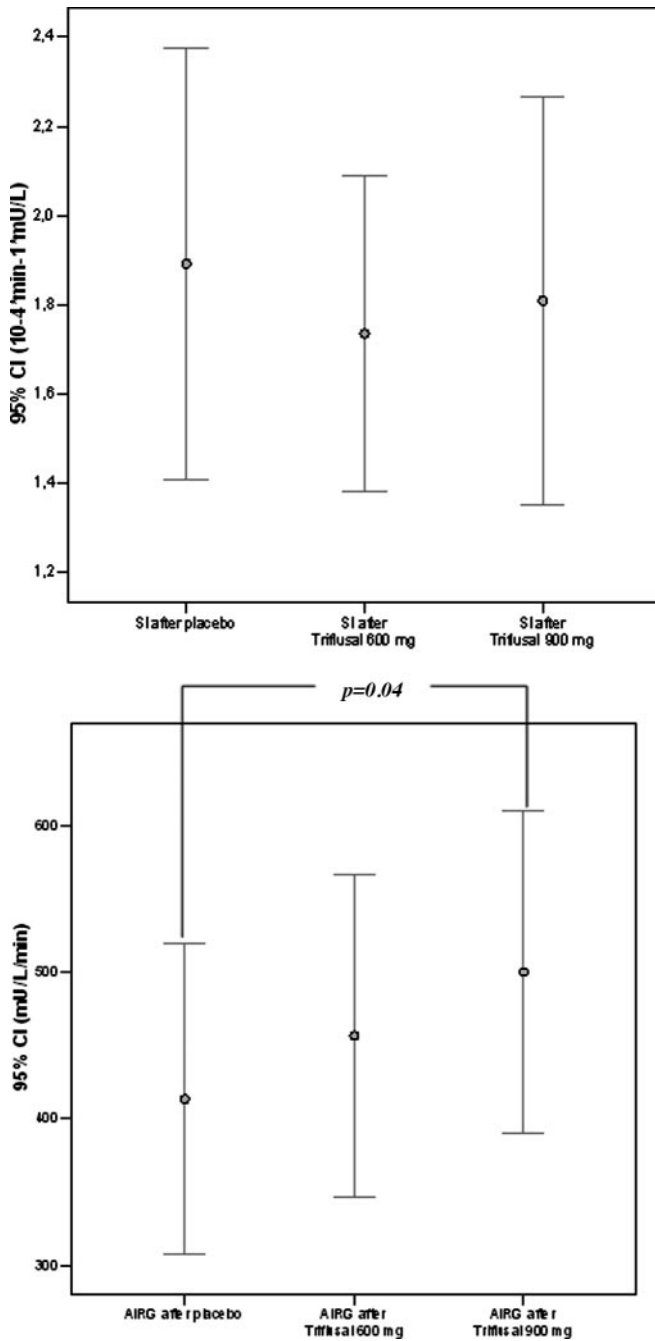


FIG. 1. The 95% confidence interval (CI) for the mean of insulin sensitivity index (upper panel) and insulin secretion (lower panel) after treatment with placebo, triflusal 600 mg, and triflusal 900 mg.

lates nitric oxide release by human neutrophils (30, 31). Even though triflusal is less potent than salicylic acid as COX-1 inhibitor (15, 22–24), triflusal and its main metabolite HTB are more potent than aspirin in inhibiting the activation of NF-κB (32), and inhibition of this transduction factor may be related with an improvement of insulin resistance. When triflusal and HTB were compared with aspirin and sodium salicylate, the former blocked the activation of NF-κB to a higher extent than aspirin and sodium salicylate (15). This is the rationale for using triflusal in this study. We used two

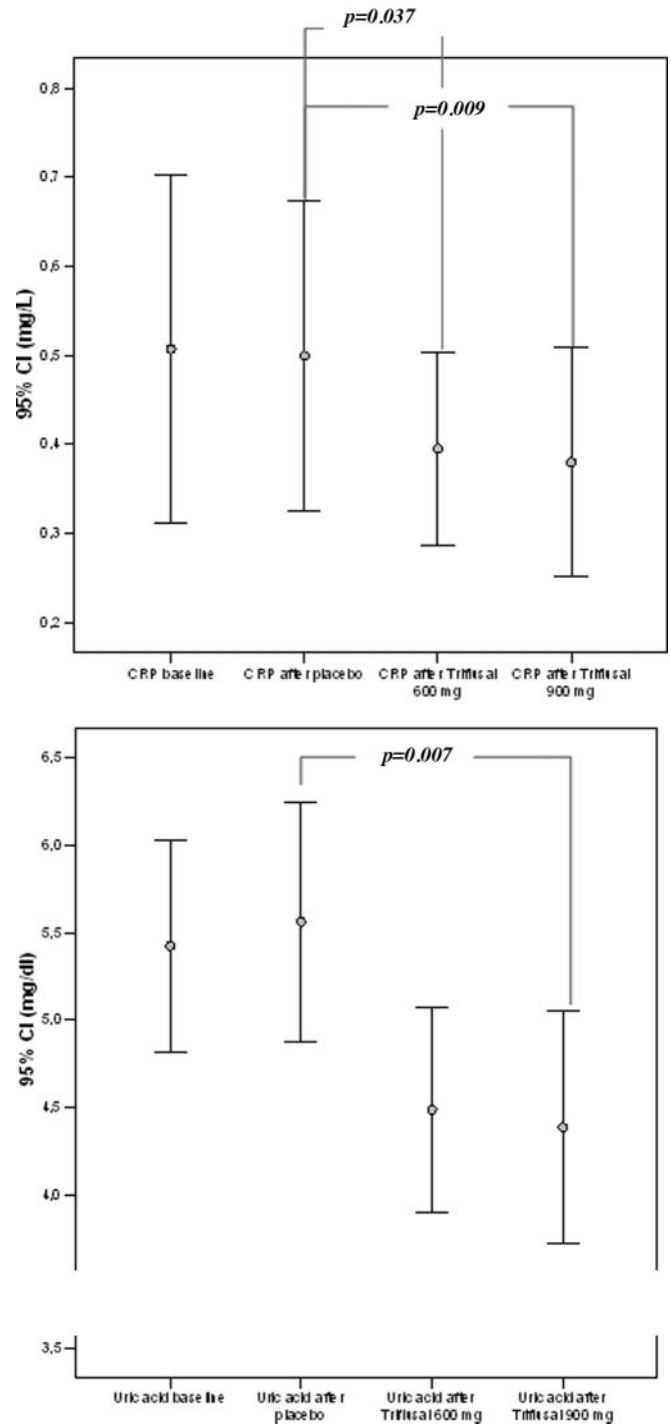


FIG. 2. Baseline 95% confidence interval (CI) for the mean of C-reactive protein (CRP, upper panel) and serum uric acid (lower panel) at baseline and after treatment with placebo, triflusal 600 mg, and triflusal 900 mg. One subject with unexplained increased C-reactive protein (4 mg/liter) during treatment with triflusal 900 mg was excluded in the upper panel.

different doses (600 and 900 mg) with demonstrated anti-inflammatory activity (15).

We observed significant changes in fasting serum glucose after both treatment periods. In women, we also found a borderline decrease in glycated hemoglobin ($P = 0.04$). This was surprising, given the relative short treatment period (4 wk) and the known

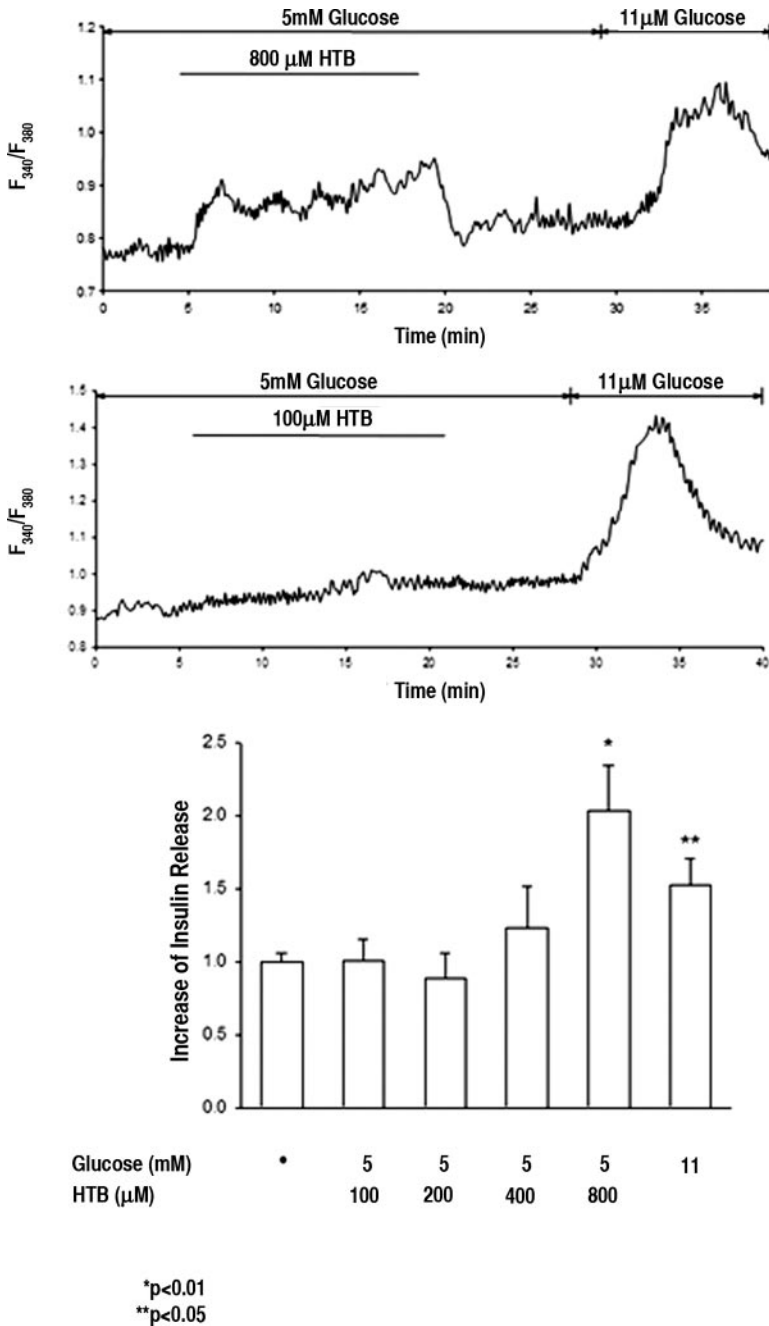


FIG. 3. Upper panels, Study of $[Ca^{2+}]_i$ measured in whole islets of Langerhans isolated from mice. Five millimoles glucose are not enough to produce either a change in $[Ca^{2+}]_i$ levels or insulin secretion above baseline. When 800 μM HTB were added, there was a sustained increase in $[Ca^{2+}]_i$ level. Lower panel, RIAs measuring insulin secretion in groups of five islets. Several concentrations of HTB were used. Only 800 μM HTB increased significantly the insulin secretion in the presence of 5 mM glucose.

4- to 6-wk period needed to find changes in glycated hemoglobin. However, we cannot exclude that this effect was due to chance.

In contrast to the effects described in type 2 diabetic patients (9), we observed no significant effects on insulin sensitivity. Interestingly, the authors of this study reported an aspirin-induced increase in plasma insulin concentrations, which was interpreted as decreased insulin clearance. No study of insulin secretion was performed (9). However, we should recognize that discrepancy between results might stem from the use of different methodol-

ogies and differing patient types. In addition, the effects of salicylates on insulin clearance in the liver may be important. The apparent lack of effect on insulin sensitivity found in this study may relate to increased insulin clearance.

We found that triflusal significantly influenced the inflammatory status, as showed by decreasing C-reactive protein. Sodium salicylate and aspirin have been shown to block activation of NF- κ B by IL-1 β in rat islets and to thereby prevent the loss of islet function normally induced by this cytokine (33–34). In fact, hyperglycemia has been shown to increase IL-1 β mRNA islet expression and secretion (35). However, we found salicylate effects on islet function that were independent of the inflammatory status.

Triflusal could affect glucose metabolism by direct effects on insulin secretion. Sodium salicylate has been demonstrated to improve defective insulin secretion in patients with type 2 diabetes (36–38). Sodium salicylate at higher supraphysiological concentrations has been shown to affect basal insulin secretion (39, 40). However, these studies were neglected in recent reports (9, 10). We found a consistent increase in insulin secretion in obese subjects, which was corroborated in *in vitro* studies.

Insulin secretion from whole islets of Langerhans follows intracellular calcium signals (41, 42). The *in vitro* experiments performed in the present work demonstrated that HTB, at a concentration reached in plasma with the doses used in this study (15), was able to increase $[Ca^{2+}]_i$ and initiate insulin release. Remarkably, the effects of HTB on $[Ca^{2+}]_i$ signals and insulin release are within the range obtained by a stimulatory glucose concentration (11 mM). Of note, we confirmed these effects in human islets at lower HTB concentrations. However, it remains to be demonstrated that other nonsteroidal antiinflammatory drugs that inhibit COX but not IKK/NF- κ B have similar effects on islet

function *in vitro*.

Older studies suggested the possibility that salicylate may act as an ionophore for Ca^{2+} (43). Salicylate increased the red cell permeability for Ca^{2+} , and there was no saturation of the Ca^{2+} transfer with respect to salicylate up to 150 mM and with respect to external Ca^{2+} up to 30 mM. Importantly, the effects of salicylate on Ca^{2+} permeability were reversible on washing the cells (43).

Finally, given the well-known uricosuric effects of salicy-

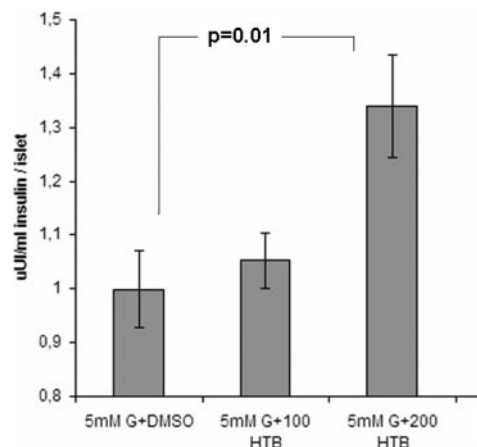


FIG. 4. Insulin secretion in human islets after DMSO (control) and 100 μ M and 200 μ M of HTB.

lates, triflusal also led to decreased serum uric acid concentration (44, 45).

Strengths of this research are the study of a homogenous sample of healthy obese subjects randomized in a double-blind fashion, with different, sequential, and random drug doses. We also used of a robust tool to measure insulin sensitivity and insulin secretion (minimal model), although less robust than clamp studies. The *in vitro* findings were in line with increased insulin secretion as observed *in vivo*.

The number of subjects studied was, however, relatively small, but it was powered 80% to find significant changes in insulin sensitivity. Liver *vs.* peripheral insulin sensitivity cannot be differentiated in the minimal model.

In summary, the administration of a salicylate compound (trifluoromethyl salicylate) led to lowering of serum glucose concentration. We suggest that this effect was mediated through increased insulin secretion induced by salicylate directly on the β -cell. Because insulin sensitivity did not significantly change, the effects on inflammation-associated insulin resistance seem less plausible.

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