

Salicylates increase insulin secretion in healthy obese subjects

JOSÉ-MANUEL FERNÁNDEZ-REAL¹, ABEL LÓPEZ-BERMEJO¹, ANA-BELÉN ROPERO²,
SANDRA PIQUER³, ANGEL NADAL², JUDIT BASSOLS¹, ROSER CASAMITJANA³,
RAMÓN GOMIS³, EVA ARNAIZ⁴, IÑAKI PÉREZ⁴ AND WIFREDO RICART¹

¹Department of Diabetes, Endocrinology and Nutrition. Institut d'Investigació Biomèdica de Girona. Girona. (Spain) and CIBER Fisiopatología de la Obesidad y Nutrición. CB06/03/010.

²Bioengineering Institute. University of Elche "Miguel Hernández". Elche. Alicante. (Spain)

³Department of Endocrinology and Hormonal Laboratory. CIBER Diabetes y Enfermedades Metabólicas (CIBERDEM). Hospital Clínic. Barcelona.

⁴R+D Clinical Unit. J. Uriach and Company, Barcelona (Spain)

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Address for correspondence:

J.M.Fernández-Real, M.D., Ph.D.

Section of Diabetes, Endocrinology and Nutrition.

Institut d'Investigació Biomèdica de Girona.

Avinguda de França s/n, 17007 Girona, SPAIN.

Phone: 34-972-94 02 00; Fax: 34-972-94 02 70;

e-mail: uden.jmfernandezreal@htrueta.scs.es

Abstract

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: To study the effects of a salicylate derivative (triflusal) on insulin sensitivity and insulin secretion.

Design, Setting and Participants: This was a double-blind, randomised, cross-over 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 intravenous glucose tolerance test that was performed after each treatment period. 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 $[\text{Ca}^{2+}]_i$ level. This was followed by a significantly increase in insulin secretion. In human islets, 200 μM of HTB 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.

Registered trial NCT00162799

Sodium salicylate at high doses was first shown to be beneficial in some cases of diabetes over 100 years ago (1,2). In 1957 Reid et al. rediscovered aspirin's effect to reduce glucosuria and blood glucose in a diabetic patient (3). 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 normal 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 IKK β activity (9,10).

These actions of salicylate are not attributed to the well-known ability of low-dose aspirin to inhibit the cyclooxygenase enzymes, Cox1 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 favours displacement of NF- κ B from its complex with I κ B and its movement from the cytosol to the nucleus (9,10).

Both genetic (Zucker *fa/fa* rats) and diet-induced (high fat intake) obesity in rodents was associated with a two-fold increase in hepatic NF- κ B activity. Transgenic mice which selectively express IKK β in hepatocytes had a type 2 diabetic phenotype with hyperglycaemia, 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 ageing 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-week 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 gr. 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 hypothesised an improvement in both insulin sensitivity and

glucose metabolism in these obese subjects after treatment with salicylates. Thus, we designed a 12 week 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 (HTB) on insulin secretion in isolated islets of Langerhans from mice.

Research Design and Methods

Study design

This was a double-blind, randomised, cross-over study with three treatment periods (each of 4 weeks) corresponding to two dose levels (600 mg or 900 mg p.o. once a day) and a placebo (registered trial NCT00162799). Each treatment period was followed by a 2 weeks wash-out 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 three 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 $<40 \text{ kg/m}^2$. The cut-point for BMI was 27.5 Kg/m^2 because in our population 27.5 is equivalent to 30 Kg/m^2 in other Caucasian populations (16); 2) absence of any systemic disease, 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 (WHR) 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 prior to the different tests.

Insulin sensitivity and secretion

All subjects had fasting plasma glucose $< 7.0 \text{ mM}$. *Insulin sensitivity* was measured

using the frequently sampled intravenous glucose tolerance test (FSIVGTT). *Insulin secretion* was calculated as the insulin area during the first 10 minutes of the FSIVGTT.

In brief, the experimental protocol started between 8:00 and 8:30 AM 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 minutes, after which glucose (300 mg/ Kg body weight) was injected over 1 minute starting at time 0, and insulin (Actrapid, Novo, Denmark; 0.03 U/kg) was administered at time 20. Additional samples were obtained from a contra-lateral antecubital vein up to 180 minutes, as previously described (17,18).

Analytical methods

Blood samples were drawn from each subject after an overnight fasting period. Serum was centrifuged at 4000g for 10 minutes, 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 Analyser 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 intra-assay coefficient of variation was 5.2% at a concentration of 10 mU/l and 3.4% at 130 mU/l. The interassay coefficients of variation were 6.9 and 4.5% at 14 and 89 mU/l, respectively. These coefficients were similar to those previously reported (17). HbA1c was measured by high performance liquid chromatography by means of a fully automated glycosylated hemoglobin analyser system (Hitachi L-9100, USA).

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/L.

Plasma soluble TNFR-1 and sTNF-R2 (BioSource Europe S.A., Zoning Industriel B-6220, Fleunes, Belgium) were determined as previously described (17).

In vitro studies

Islets isolation and intracellular calcium measurement

Swiss albino OF1 male mice (8-10 weeks 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 1h at 37°C in the incubator and then loaded at room temperature with Fura-2 by incubation for at least 1h (5 μ M, Molecular

Probes, Eugene, OR, USA). 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 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 (Zeiss, Axiovert 200). Images were acquired every \sim 3 s with an extended Hamamatsu Digital Camera C4742-95 (Hamamatsu Photonics, Barcelona, Spain) using a dual filter wheel (Sutter Instrument CO, CA, USA) equipped with 340 and 380 nm, 10 nm bandpass

filters (Omega optics, Madrid, Spain). Data were acquired using ORCA software from Hamamatsu (Hamamatsu Photonics, Barcelona, Spain). 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).

Insulin secretion measurement

To measure insulin release from isolated islets, groups of five islets were incubated in 0.4ml of modified Ringer solution in the presence of stimuli for 1 hour. The stock solution of HTB was freshly prepared at 250mM in DMSO. The maximum concentration of DMSO, equivalent to 800 μ M HTB, was always present in the stimuli. After that, 0.1ml 5% BSA modified Ringer solution was added to the islets and the medium was collected to measure insulin using radioimmunoassay (Coat-a-count, DPC, Los Angeles, CA, USA). 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, 5min centrifugation was used to measure protein concentration. The results were expressed as normalized with respect to the insulin secretion (UI/mg protein) at 5mM 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 hours at 37°C in RPMI 1640 (11.1mM glucose). The human islets were hand picked and preincubated in RPMI medium at 5.5mM glucose for 2 hours. 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 1ml bicarbonate-buffered medium containing bovine serum albumin (5 mg/ml) in the presence of stimuli. The stock solution of HTB was freshly prepared at 250mM in DMSO. The maximum concentration of DMSO, equivalent to 800 μ M HTB, was always present in the stimuli. Insulin release was measured by radioimmunoassay (BioSource INS-IRMA kit, BioSource, Nivelles, Belgium). The

results were expressed as $\mu\text{UI/ml}$ islet at 5mM glucose.

Statistical methods.

Descriptive results of continuous variables are expressed as mean (SD) if 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 analysed on a log scale and tested for significance on that scale. The anti-log transformed values of the means are reported in the Table. 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 modelling 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 carry over 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).

Results

Thirty-one subjects fulfilled the inclusion criteria. Of these, 3 subjects were lost during follow-up due to voluntary patient withdrawal. We finally studied 28 subjects (9 men and 29 women), mean age 48.8 ± 10.1 years, mean BMI 33.9 ± 3.5 Kg/m², waist-to-hip ratio 1.01 ± 0.06 (men) and 0.88 ± 0.04 (women), with a mean fasting glucose 98.6 ± 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 glycated hemoglobin among women ($p=0.04$).

No significant changes were observed in plasma lipids, or serum concentration of the soluble fraction of tumour necrosis factor-2 receptor (sTNFR2), a surrogate of tumour necrosis factor- 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 (Figure 1, upper panel). Insulin secretion, however, significantly increased in a dose dependent fashion after triflusal treatment (Figure 1, lower panel). Side

effects were similar after placebo and after each dose of triflusal.

Serum C-reactive protein also significantly decreased after anti-inflammatory treatment but only with triflusal 600 mg. However, this was due to unexplained increased C-reactive protein in 1 subject (4 mg/l). When this subject was excluded from the analysis, C-reactive protein significantly also decreased after triflusal 900 mg (Figure 3, upper panel). Administration of triflusal led to significantly decreased serum uric acid levels (Figure 3, lower panel).

In vitro studies

Intracellular calcium concentration, $[Ca^{2+}]_i$, was measured in whole islets of Langerhans isolated from mice. 5mM Glucose is not enough to produce neither a change in $[Ca^{2+}]_i$ levels nor insulin secretion above baseline. However, when 800 μ M HTB was added, there was a sustained increase in $[Ca^{2+}]_i$ level, which was maintained as long as the chemical was present (Figure 3A). This effect was reversible, since $[Ca^{2+}]_i$ decreased to basal levels after washout.

Lower concentrations of HTB as 100 μM produced no effect on $[\text{Ca}^{2+}]_i$. After the treatment with HTB, islets were perfused with a stimulatory concentration of glucose, 11mM, to show that HTB was not toxic for the islets and these were still metabolically responsive.

As insulin secretion depends on movements in $[\text{Ca}^{2+}]_i$, it was expected to have an increase in insulin secretion in response to HTB. Therefore, radioimmunoassays were performed to measure insulin secretion in groups of 5 islets. Several concentrations of HTB were used. Only 800 μM HTB increased significantly the insulin secretion in the presence of 5mM glucose (Figure 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 ($p=0.01$, Figure 4).

Discussion

Aspirin therapy has been described to improve glucose tolerance and to reduce insulin requirements in diabetic subjects (3-5). However, not all studies were concordant. While early reports suggested a salutary effect of aspirin on glucose metabolism in diabetic patients (1-5), other clinical trials have 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 weeks).

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 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- κ B activation (15). Its deacetylated metabolite, 2-hydroxy-4-trifluoromethylbenzoic acid (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 ASA (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 stimulates nitric oxide release by human neutrophils (30,31). Even though Triflusal

is less potent than ASA as COX-1 inhibitor (15, 22-24), triflusal and its main metabolite HTB are more potent than aspirin in inhibiting the activation of NF- κ B (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- κ B to a higher extent than aspirin and sodium salicylate (15). This is the rationale for using triflusal in this study. We have used 2 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 (4 weeks) treatment period and the known 4-6 weeks 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 methodologies 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 an stimulatory glucose concentration (11 mM). Of note was that we confirmed these effects in human islets at lower HTB concentrations. However, it remains to be

demonstrated that other NSAID 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 Ca²⁺ (43). Salicylate increased the red cell permeability for Ca²⁺ and there was no saturation of the Ca²⁺ transfer with respect to salicylate up to 150 mM and with respect to external Ca²⁺ up to 30 mM. Importantly, the effects of salicylate on Ca²⁺ permeability were reversible upon washing the cells (43).

Finally, given the well-known uricosuric effects of salicylates, 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 randomised 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 (tri-fluo-methyl 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. As insulin sensitivity did not significantly change, the effects on inflammation-associated insulin resistance seem less plausible.

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Figure Legends

Figure 1. 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.

Figure 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/L) during treatment with triflusal 900 mg was excluded in the upper panel.

Figure 3. Panel A. Study of $[Ca^{2+}]_i$, measured in whole islets of Langerhans isolated from mice. 5mM Glucose is not enough to produce neither a change in $[Ca^{2+}]_i$ levels nor insulin secretion above baseline. When 800 μ M HTB was added, there was a sustained increase in $[Ca^{2+}]_i$ level. Panel B. Radioimmunoassays measuring insulin secretion in groups of 5 islets. Several concentrations of HTB were used. Only 800 μ M HTB increased significantly the insulin secretion in the presence of 5mM glucose.

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

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

| | Baseline | Triflusal 600 mg | Triflusal 900 mg | p |
|--|-----------------------|-------------------------|-------------------------|-------------------|
| Sex (M/F) | 9/19 | - | - | - |
| Age (years) | 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/l) | 13.7 (7.4) | 13.3 (8.1) | 13.8 (7.5) | 0.3 |
| HbA _{1c} (%)Men | 4.7 (0.47) | 4.9 (0.63) | 5.0 (0.61) | 0.8 |
| HbA _{1c} (%)Women | 4.6 (0.48) | 4.6 (0.57) | 4.4 (0.47) | 0.04 |
| HDL-cholesterol (mg/dl) Men | 51.3 (14.9) | 50.4 (13.1) | 49.8 (13.8) | 0.4 |
| HDL-cholesterol (mg/dl) Women | 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 (mg/dl) Men | 7.0 (1.5) | 6.1 (1.4) | 6.3 (1.2) | <0.0001 |
| Uric acid (mg/dl) Women | 4.6 (1.4) | 3.7 (0.7) | 3.4 (0.8) | <0.0001 |
| C-reactive protein (mg/L) ^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 [†] (10 ⁻⁴ *mU/L) ^a | 1.78 (0.74 – 2.96) | 1.63 (0.99 – 2.37) | 1.69 (0.78 – 2.63) | 0.4 |
| Insulin secretion [†] (mU/L/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 for ^aexpressed as median (interquartile range)

* p <0.01, † p <0.05,

Figure 1

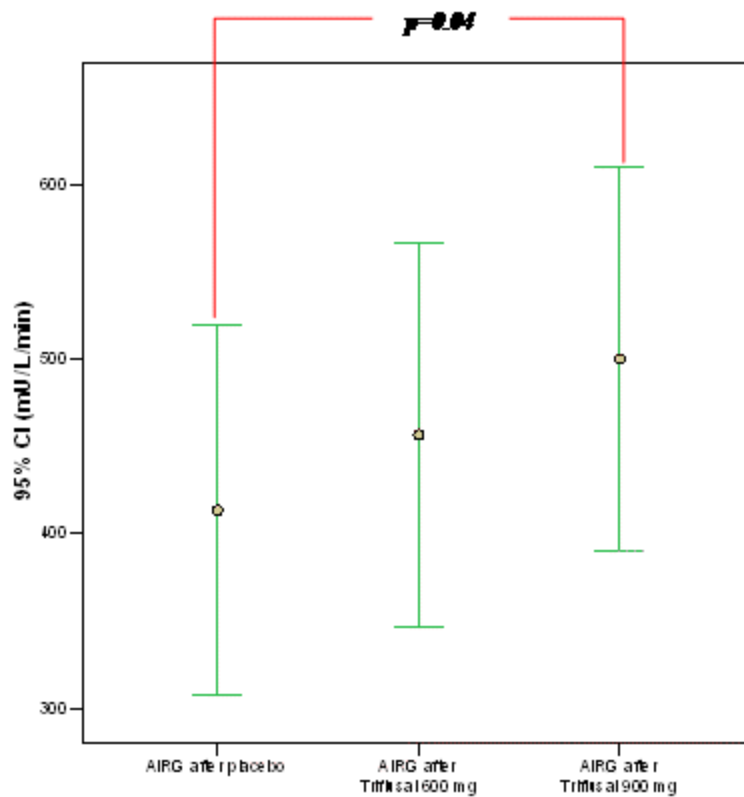
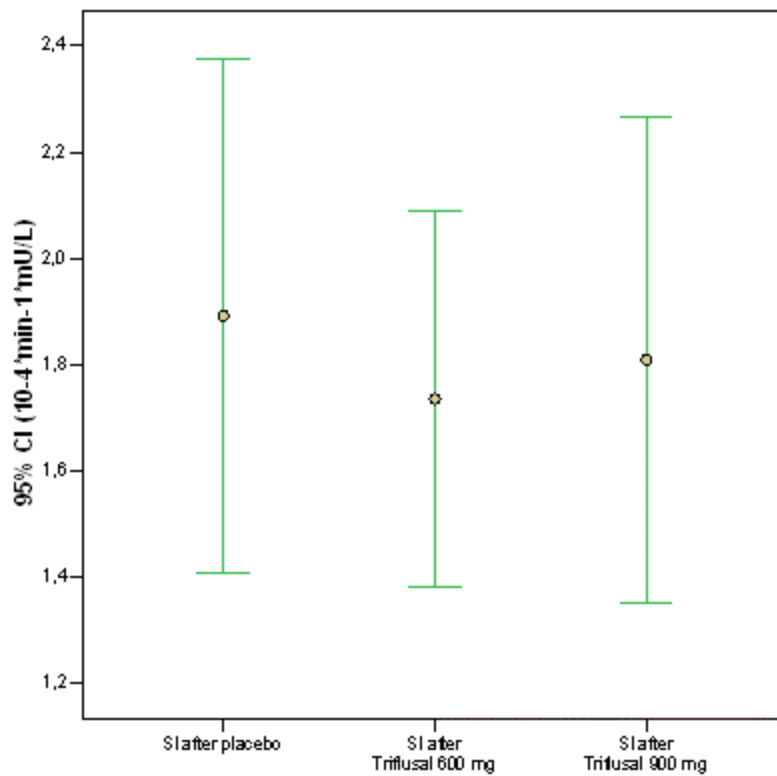


Figure 2

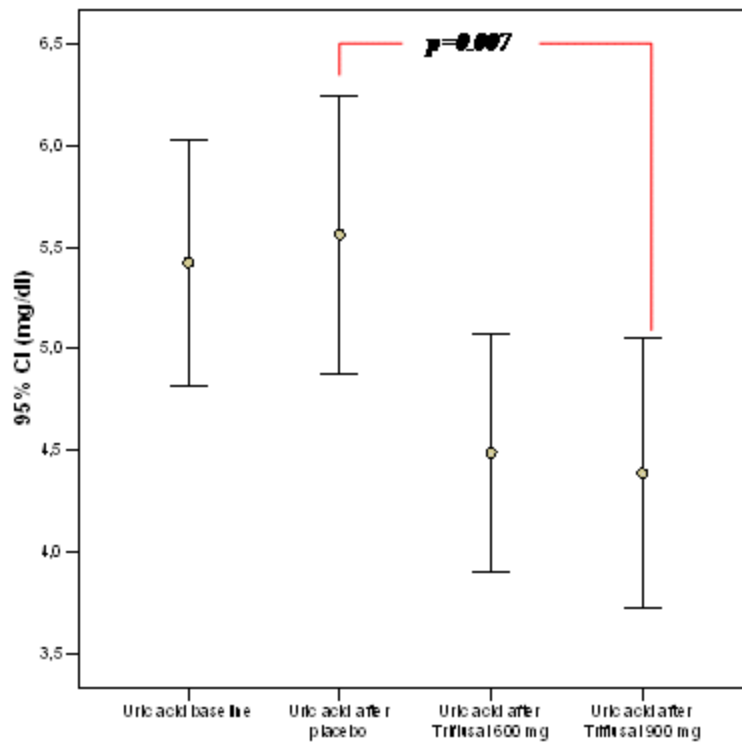
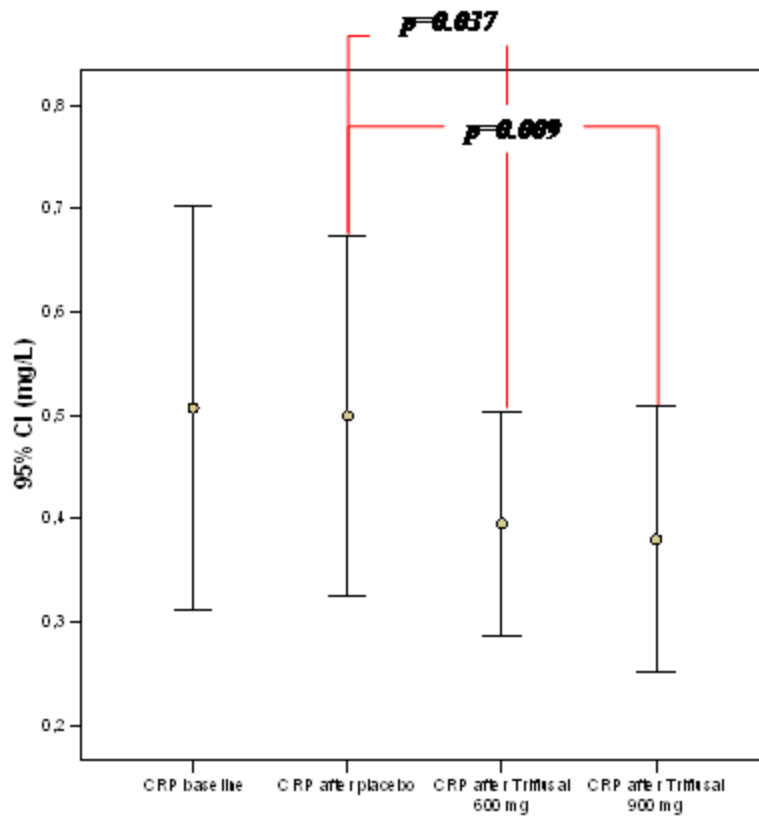
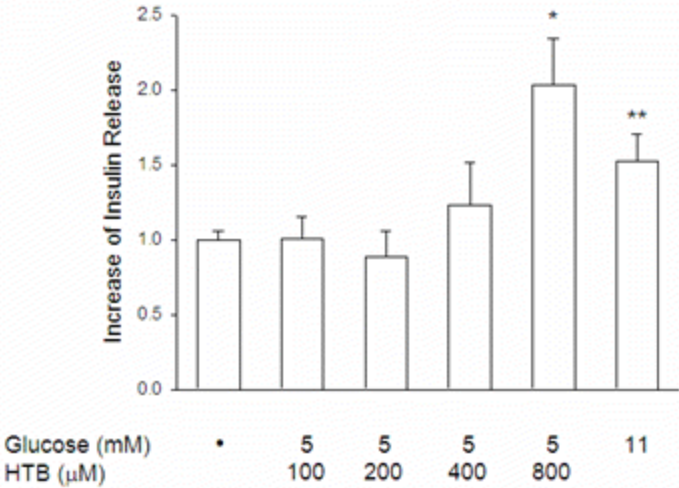
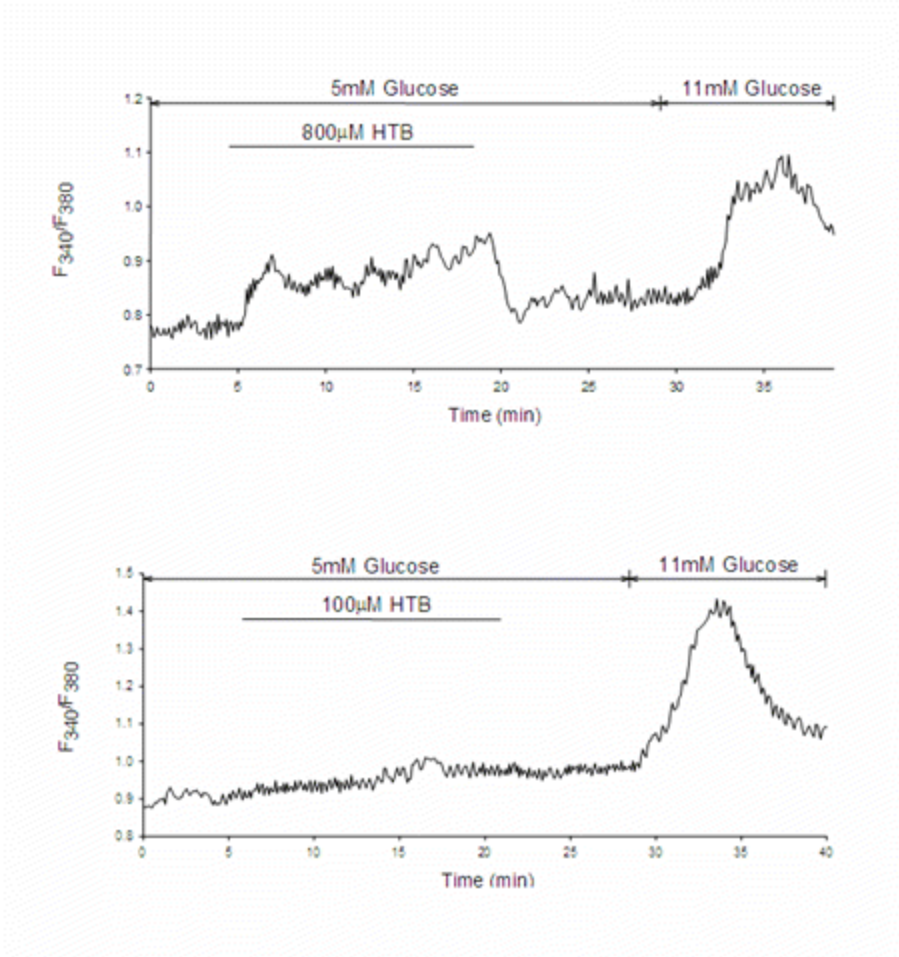


Figure 3



* p<0.01
** p<0.05

Figure 4

