The effect of high-dose sodium salicylate on chronically elevated plasma nonesterified fatty acid-induced insulin resistance and β-cell dysfunction in overweight and obese nondiabetic men

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Xiao C, Giacca A, Lewis GF. The effect of high-dose sodium salicylate on chronically elevated plasma nonesterified fatty acid-induced insulin resistance and β-cell dysfunction in overweight and obese nondiabetic men. Am J Physiol Endocrinol Metab 297:E1205–E1211, 2009. First published September 15, 2009; doi:10.1152/ajpendo.00313.2009.—Prolonged elevation of plasma nonesterified fatty acids (NEFA) induces insulin resistance and impairs pancreatic β-cell adaptation to insulin resistance. Studies in rodents suggest that inflammation may play a role in this “lipotoxicity.” We studied the effects of sodium salicylate, an anti-inflammatory agent, on lipid-induced alterations in β-cell function and insulin sensitivity in six overweight and obese nondiabetic men. Each subject underwent four separate studies, 4–6 wk apart, in random order: 1) SAL, 1-wk placebo followed by intravenous (iv) infusion of saline for 48 h; 2) IH, 1-wk placebo followed by iv infusion of intralipid plus heparin for 48 h to raise plasma NEFA approximately twofold; 3) IH + SS, 1-wk sodium salicylate (4.5 g/day) followed by 48-h IH infusion; and 4) SS, 1-wk oral sodium salicylate followed by 48-h saline infusion. After 48-h saline or lipid infusion, insulin secretion and sensitivity were assessed by hyperglycemic clamp and euglycemic hyperinsulinemic clamp, respectively, in sequential order. Insulin sensitivity was reduced by lipid infusion (IH = 67% of SAL) and was not improved by salicylate (IH + SS = 36% of SAL). Lipid infusion also reduced the disposition index (P < 0.05), which was not prevented by sodium salicylate. Salicylate reduced insulin clearance. These data suggest that oral sodium salicylate at this dose impairs insulin clearance but does not ameliorate lipid-induced insulin resistance and β-cell dysfunction in overweight and obese nondiabetic men.

insulin secretion; insulin sensitivity; lipid; human

TYPE 2 DIABETES IS CHARACTERIZED BY DEFECTS IN BOTH INSULIN ACTION AND INSULIN SECRETION (26). The underlying cause of β-cell dysfunction is not clear, but chronically elevated plasma nonesterified fatty acids (NEFA) may play a role, an effect referred to as “lipotoxicity” (51). It is generally accepted that plasma NEFA are essential for maintaining basal insulin secretion in the fasted state (48) and that an acute increase in plasma NEFA potentiates glucose-stimulated insulin secretion (5, 45). On the other hand, most in vitro studies and studies in rodent models have demonstrated impairing effects of prolonged elevation of plasma NEFA on glucose-stimulated insulin secretion (24, 39). In contrast, the in vivo effects of prolonged elevation of plasma NEFA on insulin secretion in humans are more controversial (2, 9, 33). Since chronic elevation of NEFA induces insulin resistance (4, 6, 10, 47, 54), it is more appropriate to assess β-cell function using an index of secretion that accounts for the whole body insulin sensitivity, the disposition index (a product of insulin sensitivity and secretion) (27, 34). In previous studies, we and others have shown in humans that chronic elevation of plasma NEFA through lipid infusion or fat ingestion induces insulin resistance that is not appropriately compensated by insulin secretion, demonstrating the detrimental effects to β-cells, particularly in obese individuals and in offspring of parents with type 2 diabetes (9–11, 13, 28, 32, 54).

The mechanisms by which chronically elevated NEFA impair β-cell function are not well understood. We have shown previously in humans and rats that the β-cell functional impairment induced by chronic elevation of NEFA is ameliorated by antioxidant therapy (41, 54). Recent studies in animal models suggest a role of inflammation in development of insulin resistance; thus pharmacological or genetic activation of the NF-κB pathways may also play a role in chronic elevation of NEFA-induced β-cell dysfunction in overweight and obese or high-fat-fed rodents (8, 55). Treatment with the high-dose anti-inflammatory agent sodium salicylate (an IKKß inhibitor) or IKKß deficiency prevented fat-induced insulin resistance in skeletal muscle of rodents (29). We have recently reported the protective effects of high-dose sodium salicylate against short-term (7-h) lipid infusion-induced hepatic insulin resistance in rats (43). In humans, salicylates have yielded increased (17), decreased (7, 21, 40), or unchanged (15, 30, 46) effects on insulin sensitivity, which might be attributed to the differences in the characteristics of the subjects and methodologies used. Acute administration of acetylsalicylic acid at low dose to healthy humans attenuated lipid-induced insulin resistance (38). Salicylates have also been shown to increase insulin secretion in vitro in rat islets (50) and human islets (56) and in healthy obese subjects (15). In rats, we have shown thatactivation of inflammatory pathways may also play a role in chronic elevation of NEFA-induced β-cell dysfunction, and this lipotoxicity is relieved by treatment with sodium salicylate (42). However, the effects of high-dose salicylate on β-cell function in the presence of prolonged elevation of NEFA have not been examined in humans. The objective of this study was to examine the potential of high-dose sodium salicylate in ameliorating chronically increased lipid-induced β-cell dysfunction in humans.

MATERIALS AND METHODS

Subjects. Six overweight or obese but otherwise healthy Caucasian men participated in the study (Table 1). To exclude the influences of family history of type 2 diabetes and impaired glucose tolerance on the response to lipotoxicity (12), no subjects with family history of type 2 diabetes or impaired oral glucose tolerance tests were included in the study. None of the participants was taking any medication or had any known systemic illness. Informed, written consent was obtained from all participants in accordance with the guidelines of the...
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Table 2. Fasting levels of plasma glucose, insulin, C-peptide, TG, and NEFA before and after 48-h infusion of saline or intralipid plus heparin

<table>
<thead>
<tr>
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<th>SAL</th>
<th>IH</th>
<th>IH + SS</th>
<th>SS</th>
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</thead>
<tbody>
<tr>
<td>Glucose, mmol/l</td>
<td>5.2±0.2</td>
<td>5.2±0.1</td>
<td>5.1±0.3</td>
<td>5.1±0.2</td>
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<tr>
<td>Preinfusion</td>
<td></td>
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<tr>
<td>Preclamp</td>
<td>5.4±0.1</td>
<td>5.6±0.2</td>
<td>5.5±0.2</td>
<td>5.1±0.1</td>
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<tr>
<td>Preinfusion</td>
<td>85.6±15.7</td>
<td>76.5±11.3</td>
<td>119.6±29.1</td>
<td>103.0±24.8</td>
</tr>
<tr>
<td>Preclamp</td>
<td>67.5±9.5</td>
<td>82.2±8.3</td>
<td>127.8±28.5</td>
<td>84.7±10.7</td>
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<tr>
<td>C-peptide, mmol/l</td>
<td>0.78±0.14</td>
<td>0.67±0.15</td>
<td>0.74±0.08</td>
<td>0.76±0.16</td>
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<tr>
<td>Preinfusion</td>
<td>0.72±0.09</td>
<td>0.86±0.11</td>
<td>0.81±0.11</td>
<td>0.68±0.09</td>
</tr>
<tr>
<td>Preclamp</td>
<td>1.74±0.42</td>
<td>1.15±0.19</td>
<td>1.53±0.43</td>
<td>1.28±0.29</td>
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<tr>
<td>Fasting TG, mmol/l</td>
<td>1.67±0.31</td>
<td>3.47±0.77</td>
<td>3.5±0.65</td>
<td>1.41±0.27</td>
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<tr>
<td>Fasting NEFA, mmol/l</td>
<td>0.45±0.06</td>
<td>0.48±0.09</td>
<td>0.47±0.10</td>
<td>0.40±0.10</td>
</tr>
<tr>
<td>Preinfusion</td>
<td>0.39±0.09</td>
<td>0.88±0.13</td>
<td>0.72±0.14</td>
<td>0.43±0.07</td>
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<tr>
<td>Preclamp</td>
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Data are means ± SE; n = 6. SAL, saline infusion; IH, intralipid plus heparin infusion; IH + SS, 1-wk oral sodium salicylate followed by intralipid plus heparin infusion; SS, 1-wk oral sodium salicylate followed by saline infusion. Insulin levels in saline or intralipid plus heparin were started 48 h before clamp and continued throughout the clamps. Preinfusion, before beginning of saline or intralipid plus heparin infusion. Preclamp, 48 h after beginning of saline or intralipid plus heparin infusion and before beginning of the clamp. *P < 0.05 vs. SAL; †P < 0.05 vs. IH; ‡P < 0.05 vs. SS; §P < 0.05 vs. preinfusion.

IH + SS was greater than in SAL and IH, respectively (SS = 135% of SAL, P < 0.05; IH + SS = 163% of IH, P < 0.05) (Fig. 1A, inset). In contrast to the differences in insulin levels, C-peptide levels (Fig. 1B) and area under the C-peptide vs. time curve for the first 10 min of the hyperglycemic clamp (Fig. 1B, inset) were similar among treatments, suggesting that the greater first-phase insulin response was due not to increased insulin secretion but mainly to decreased insulin clearance, as discussed below and shown in Fig. 2D.

During the last 30-min steady state of the 2-h hyperglycemic clamp, insulin concentration (Fig. 2A) was higher in IH compared with SAL (P < 0.05). Insulin concentration was further elevated in IH + SS (Fig. 2A). SS treatment alone was also associated with a higher insulin concentration than in SAL (P < 0.05). C-peptide concentration during this time period was not significantly different among treatments (Fig. 2B). As a result, the calculated insulin secretion rates did not differ among treatments (Fig. 2C). Higher insulin levels in the IH and SS treatment groups combined with similar insulin secretion rates suggests decreased insulin clearance. This was confirmed by the significantly lower calculated insulin clearance rates in IH, IH + SS, and SS studies compared with SAL (P < 0.05; Fig. 2D). Decreased insulin clearance was shown in our previous studies after 24-h oral fat ingestion (53) or 48-h IH infusion (10, 11).

Euglycemic hyperinsulinemic clamp. During the last 30 min of the euglycemic hyperinsulinemic clamp, to maintain plasma glucose at the clamped level (~5.6 mmol/l), lower glucose infusion rates were required in the lipid infusion treatment groups (P < 0.05, IH and IH + SS vs. SAL) (Fig. 3A). IH was associated with higher plasma insulin concentration during the euglycemic hyperinsulinemic clamp study compared with SAL (P < 0.05). IH + SS was associated with a further elevation of insulin compared with IH infusion alone (P < 0.05). Insulin in the SS treatment group was also higher than SAL (P < 0.05; Fig. 3B). Since endogenous insulin secretion is completely or almost completely suppressed at the fixed and identical insulin infusion rate used in all four studies (40 mU·m²·min⁻¹), the higher insulin levels in the lipid infusion and/or SS treatment groups were likely due to decreased insulin clearance (P < 0.05), as shown in Fig. 3C.

Sf and DI. Sf was derived from the last 30 min of the euglycemic hyperinsulinemic clamp. In accord with the reduced glucose infusion rates and/or higher insulin levels, Sf in IH, IH + SS, and SS were lower than in SAL (IH = 67% of IH infusion; 10.2 ± 3.3 on April 12, 2017 http://ajpendo.physiology.org/ Downloaded from
SAL, IH + SS = 56% of SAL, and SS = 69% of SAL, $P < 0.05$, no difference between these 3 treatment groups; Fig. 4A). Since the absolute insulin secretion rates were not significantly affected by IH infusion or oral SS, DI in IH, IH + SS, and SS were similar and all lower than in SAL ($P < 0.05$; Fig. 4B).

**DISCUSSION**

In the present study we examined the effects of oral high-dose (4.5 g/day, 1 wk) sodium salicylate treatment, with and without prolonged lipid infusion, on insulin sensitivity and pancreatic β-cell function in obese and overweight nondiabetic men. Since prolonged elevation of plasma NEFA induces insulin resistance (3), we assessed β-cell function by reporting the disposition index, a product of insulin secretion and insulin sensitivity that is well accepted as an accurate measure of insulin secretion (1, 27). Sodium salicylate markedly decreased insulin clearance but did not prevent lipid-induced insulin resistance, nor did it ameliorate the lipid-induced impairment in β-cell function that we have described consistently in previous studies in humans (10, 11, 13, 28, 32, 54). The similar patterns of insulin sensitivity, secretion, and disposition index in the lipid infusion groups (with and without pretreatment with high-dose oral sodium salicylate) do not support a role for sodium salicylate in ameliorating lipid-induced impairment in β-cell function that we have described consistently in previous studies in humans (10, 11, 13, 28, 32, 54). The similar patterns of insulin sensitivity, secretion, and disposition index in the lipid infusion groups (with and without pretreatment with high-dose oral sodium salicylate) do not support a role for sodium salicylate in ameliorating lipid-induced impairment in β-cell function that we have described consistently in previous studies in humans (10, 11, 13, 28, 32, 54).

Salicylates, including acetylated (acetylsalicylic acid, i.e., aspirin) and nonacetylated forms (e.g., sodium salicylate, salicylate, triflusal), are members of a class of nonsteroidal anti-inflammatory agents. Salicylates were shown to be beneficial in some diabetic patients, with a rise in insulin concentration and amelioration of hyperglycemia (7, 16, 18, 20, 23, 25, 36). However, the effects of salicylates in nondiabetic subjects have been more variable, with improved (7, 21, 40), or unchanged (15, 30, 46) insulin sensitivity and mixed results on insulin secretion (7, 25, 30, 40, 46). Therefore, our result of salicylate alone having a neutral effect on insulin secretion and impairing insulin sensitivity is not actually that surprising and in fact is consistent with the findings of some of these previous studies. The differences between the above studies (7, 15–23, 25, 30, 36, 40, 46) might be due to the dosage of salicylate ($1$ to $7$ g/day), duration of administration (acute, 3 days, 1 wk, 2 wk, 4 wk), the substance used [acetylsalicylic acid, sodium salicylate, salsalate (disaltsate), triflusal], and characteristics of the subjects studied (diabetic, impaired glucose-intolerant, healthy lean, young obese, and obese subjects). The ability of salicylate to improve insulin sensitivity in some studies has been attributed to its inhibition of IKKβ at high dose (8, 55), with an effective dose of 4–7 g/day in humans (17, 25). Several studies using lower dosages also reported beneficial effects, such as increased insulin secretion (15, 19, 22). It is noted that, in the latter studies, the more potent anti-inflammatory salicylate derivative triflusal was used at a lower dose (600 and 900 mg/day) (15) or acetylsalicylate was administered intravenously (19, 22). Our study used 4.5 g/day of sodium salicylate, a dose equivalent to 3.6 g/day salsalate and comparable with those used in several recent studies in type 2 diabetic patients (23) or obese nondiabetic subjects (17, 30) where salsalate, a dimer of salicylate, was used at the dose of 3–4.5 g/day for...
1–4 wk. In these latter studies, β-cell function was not directly assessed. In the study by Fleischman et al. (17), insulin sensitivity was not directly assessed by euglycemic hyperinsulinemic clamp. Most studies reporting increased insulin secretion examined the acute or first-phase insulin response, which may not be indicative of the β-cell’s maximal capability to secrete insulin and was not interpreted in relation to the associated changes in insulin sensitivity.

The one finding that is consistent across studies (e.g., Refs. 7, 23, and 30), including the present study, is that salicylates decrease insulin clearance. The reduction in insulin clearance is manifest during both basal and glucose-infused conditions, as indicated by increased insulin levels before and during both the hyper- and euglycemic clamps in our study and in studies by others (23, 30). It is not known whether sodium salicylate affects C-peptide clearance. Therefore, insulin clearance in the hyperglycemic clamp, which is calculated using a measurement of prehepatic insulin secretion derived by deconvolution of plasma C-peptide levels, might not be 100% accurate were salicylate to affect C-peptide clearance. However, insulin clearance assessed in the euglycemic hyperinsulinemic clamp was independent of C-peptide concentrations and was clearly reduced by salicylate. The effects of salicylates on insulin clearance may contribute to the controversy with regard to their reported effects on insulin secretion and action. The reduction in insulin clearance contributes to peripheral hyperinsulinemia, which can erroneously be interpreted as an increase in insulin secretion unless accompanied by an increase in plasma C-peptide concentrations or calculated insulin secretion rate, as we have done in the present study. As can be seen from our results, the increase in first-phase insulin response with salicylate treatment was not accompanied by an increase in insulin secretion and therefore likely occurred as a result of decreased insulin clearance. In addition, measurements of glucose disposal must be assessed in relation to circulating insulin concentration. If this calculation is not performed, as we have done in the present study, the increased glucose disposal associated with higher circulating insulin concentrations may be misinterpreted as improved insulin sensitivity. Not all previous studies have taken these important factors into account. Indeed, in a recent study in obese nonobieject subjects, salsalate increased glucose disposal along with increased insulin levels as a result of decreased insulin clearance. The effect of salsalate on glucose disposal was abolished after normalizing for

Fig. 3. Glucose infusion rates (A), plasma concentrations of insulin (B), and calculated insulin clearance (C) during the last 30 min of the euglycemic hyperinsulinemic clamp. Euglycemic hyperinsulinemic clamps were performed on subjects in 4 randomized visits after SAL, IH, IH + SS, or SS. *P < 0.05 vs. SAL; †P < 0.05 vs. IH.

Fig. 4. Insulin sensitivity index (SI) calculated from the last 30 min of the euglycemic hyperinsulinemic clamp (A) and disposition index (DI; B). Euglycemic hyperinsulinemic clamps were performed on subjects in 4 randomized visits after SAL, IH, IH + SS, or SS. *P < 0.05 vs SAL.
the increased insulin levels (30). In our study, the glucose infusion rates might have been even lower had the insulin levels not increased due to the impaired insulin clearance. Insulin clearance is mediated by similar and partly overlapping pathways affecting insulin sensitivity, and indeed salicylate also decreased insulin action. This may be due to a decrease in cyclooxygenase-1- and cyclooxygenase-2-mediated production of prostaglandin E₂, which increases insulin actions (35, 37, 50). Prostaglandin E₂ instead decreases insulin secretion (49), which is one of the reasons why salicylate alone might affect insulin secretion. The mechanism by which salicylates reduce insulin clearance warrants further study.

In the present study, we found that the anti-inflammatory agent sodium salicylate did not ameliorate or prevent lipid-induced impairment of insulin secretion and insulin sensitivity in humans. One recent study in healthy men reported that acetylsalicylic acid (a total of 4 g given in 48 h) attenuated lipid-induced insulin resistance (38). In that study, insulin concentrations during a clamp were not reported, and potential effects of salicylates on insulin secretion were not assessed. In a study in rats, 24-h treatment with iv salicylate prevented whole body and skeletal muscle insulin resistance induced by 5-h iv lipid infusion (29). In our recent studies in rats, iv coinfusion of sodium salicylate prevented short-term (7-h) lipid-induced hepatic and peripheral insulin resistance (43). However, we have recently observed reduced effectiveness of sodium salicylate in preventing hepatic insulin resistance induced by prolonged (48-h) lipid infusion in rats (44). However, in the same model, salicylate prevented lipid-induced β-cell dysfunction (42). We do not have an adequate explanation for the different effects of salicylates in humans vs. rodents. Although blood salicylate levels were comparable with those reported that had similar doses of various salicylate products, CRP levels were at the lower end of the normal range, indicating low inflammation. Lipid infusion did not increase circulating CRP, and salicylate at the current dose did not suppress CRP beyond basal level. We cannot rule out the possibility that amelioration of lipid-induced impairment in β-cell function may be observed with a higher oral dose and/or different route of administration. However, higher oral doses of salicylates, including sodium salicylate, are poorly tolerated because of tinnitus, thus limiting their use in practice (23).

In conclusion, the current study does not show that the anti-inflammatory agent sodium salicylate, administered orally at this dose and for this duration, ameliorates or reverses lipid-induced impairment of insulin secretion or insulin sensitivity in nondiabetic, overweight, and obese men. However, on the basis of these data, we cannot exclude a role for inflammation in mediating these lipid effects. Antagonism of interleukin-1β receptor has recently been shown to improve both insulin sensitivity and β-cell function in type 2 diabetic patients (31), and thus more specific and potent anti-inflammatory agents should be examined in future studies.

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