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EFFECTS OF ACETYLSALICYLIC ACID ON PLASMA GLUCOSE, FREE FATTY ACID, BETAHYDROXYBUTYRATE, GLUCAGON AND C-PEPTIDE RESPONSES TO SALBUTAMOL IN INSULIN-DEPENDENT DIABETIC SUBJECTS

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The selective β_2-adrenergic agonist salbutamol is now widely used to relieve airway obstruction in asthmatic patients [32] and to prevent premature labour [23,24]. Although stimulation of β-adrenergic receptors leads to activation of the adenylcyclase system thereby enhancing the formation of cyclic AMP [30], a limited number of reports have dealt with the metabolic effects of salbutamol either in normal people [16,27,38] or in non-diabetic [25,40] and diabetic [10,35] pregnant women. All the papers hitherto published agree that this drug increases plasma glucose, lactate and insulin as well as the rate of lipolysis. Apart from the studies in diabetic pregnant women, however, there is no extensive information about the effect of this β-adrenergic agonist on insulin and glucagon secretion or on carbohydrate and lipid metabolism in insulin-dependent diabetic subjects.

The aim of present study was twofold: (a) to focus on the actions of salbutamol on plasma glucose, free fatty acid, β-hydroxybutyrate, C-peptide and glucagon circulating levels in insulin-dependent diabetics; and (b) to investigate the possible effects of acetylsalicylic acid (ASA) on these salbutamol-induced metabolic and hormonal changes. ASA has been reported to reduce the basal concentration of both free fatty acids and β-hydroxybutyrate in insulin-dependent diabetics [12].

Key-words: Acetylsalicylic acid; Betahydroxybutyrate; C-peptide; Free fatty acids; Glucagon; Glucose; Insulin-dependent diabetes; Salbutamol.

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SUBJECTS AND METHODS

Six insulin-dependent diabetic subjects (3 males and 3 females) gave their informed consent to participate in this study after a clear explanation of its experimental nature and the potential hazards involved. Ages ranged from 25 to 57 years and all subjects were within 10% of their ideal body weight as indicated by the Metropolitan Life Insurance Company tables [34]. All subjects were hospitalized in the metabolic ward of our Institution several days before in order to improve the degree of diabetic control. However, all subjects remained in good metabolic control throughout the duration of the study and no changes in their daily insulin dosage occurred. Insulin dosage ranged from 30 to 60 U of a mixture of *neutral* plus semilente insulin administered s.c. once or twice daily depending upon the kind of insulin therapy. The subjects took no drugs other than insulin during the entire study period.

All studies were done in the morning between 08^{00} and 10^{00}, after an overnight fast (12 h) and required 120 min to complete. Insulin administration was omitted on the morning of the test day so that the last insulin injection was given 24 or 14 h before depending on the type of insulin therapy. With the subject in the supine position, a No. 19 scalp vein needle was inserted into a large antecubital vein of each arm, one being used for infusion, the other for blood sampling. Patency was maintained by a slow saline (0.9%) drip. A 30-min equilibration period was allowed before collection of the two baseline samples. Salbutamol (Ventolin, Glaxo Laboratories, Italy) was infused at the constant rate of 5 μg/min (total dose 0.5 mg) with the aid of a peristaltic pump (Italfarmaco, Milan, Italy). In the salbutamol-ASA infusion study, lysine-acetylsalicylate (Flectadol, Maggioni, Italy) was infused i.v. at the constant rate of 54 mg/min. Since 0.9 g of lysine-acetylsalicylate correspond to 0.5 g of ASA, we actually infused 30 mg/min of ASA. The order in which the studies were completed was randomized, and the interval between the two studies for any one individual was 4-6 days. All blood samples were obtained retrograde from the contralateral scalp vein needle after discarding the initial 2 ml of blood.

Blood specimes were collected in prechilled tubes each containing 1.2 mg of EDTA and 500 U of Trasylol per ml of blood, kept in an ice-bath during the study period and immediately centrifuged at 4 °C upon completion of the experiments. Plasma was stored deep-frozen until assayed. Plasma glucose was estimated by the glucose-oxidase method [19]. Plasma free fatty acids were measured by the method of DOLE and MEINERTZ [7] as modified by DUNCOMBE[8]. Plasma β-hydroxybutyrate was assayed by standard enzymatic analysis [43]. Plasma glucagon was measured by radioimmunoassay with the method of FALOONA and UNGER [9] and C-peptide by the double-antibody-RIA kit of Byk-Mallinckrodt [22]. All samples from one subject were included in the same assay in order to reduce interassay variation.

Statistical analysis af the data was performed by a paired Student's *t*-test. Integration of the areas under the curves was performed with an Olivetti Program 101 desk-top computer.

RESULTS

Substrates

Glucose (fig. 1) - Basal plasma glucose concentration during the salbutamol study was 168±10 mg/dl, a value which was not statistically different from the corresponding concentration of 174±10 mg/dl in the salbutamol-ASA study.

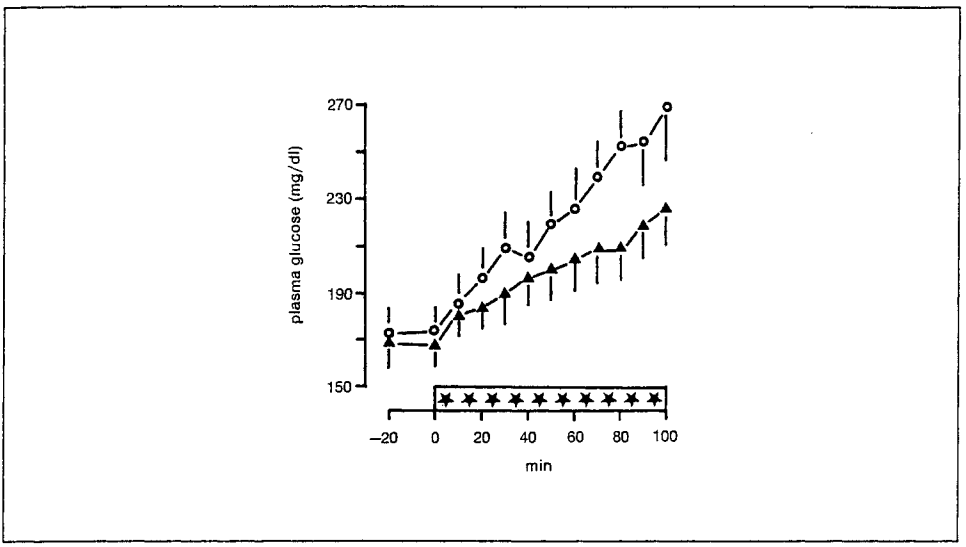

Fig. 1 - Plasma glucose concentrations in response to salbutamol (▲——▲) and salbutamol-ASA (○——○) infusion in 6 insulin-dependent diabetics. Means ± SEM.

Salbutamol infusion resulted in a progressive and significant increase in plasma glucose concentration that reached its maximum at the end of the infusion (Δ plasma glucose increase: 58±9 mg/dl). This progressive rise in plasma glucose concentration, however, was significantly less marked than that observed in the salbutamol-ASA infusion study. In fact, in the latter, plasma glucose attained a concentration of 270±24 mg/dl at the end of the infusion. (Δ plasma glucose increase: 95±14 mg/dl; $p<0.01$).

Free fatty acids (figs 2 and 5). In the salbutamol study, the basal plasma free fatty acid concentration was 380±69 μmol/l, which was not statistically different from the basal plasma free fatty acid concentration in the salbutamol-ASA study (350±58 μmol/l). After the start of salbutamol infusion, plasma free fatty acids rose promptly in all subjects and reached their maximum at 50 min (Δ plasma free fatty acid increase: 850±320 μmol/l). Compared to that obtained in the salbutamol-ASA infusion study (Δplasma free fatty acid increase: 410±217 μmol/l, 50 min), this peak response was twice as high. When the integrated area above basal concentration was calculated for the entire 100-min experimental period, the integrated rise in plasma free fatty acid concentration for the salbutamol study (44,503±11,765 μmol/l-min) was significantly higher than that observed during the salbutamol-ASA infusion study (21,672±10,091 μmol/l-min; $p<0.05$). In both series of experiments, plasma free fatty acid concentration tended to decrease after the peak response, despite continued salbutamol infusion.

β-*hydroxybutyrate* (figs 3 and 5) - Basal plasma β-hydroxybutyrate concentration was not statistically different in the salbutamol study (155±30 μmol/l) compared with the basal concentration in the salbutamol-ASA study (160±19 μmol/l). During the experimental period, a gradual rise in plasma β-hydroxybutyrate concentration occurred in both experiments. The peak response (50 min) of β-hydro-

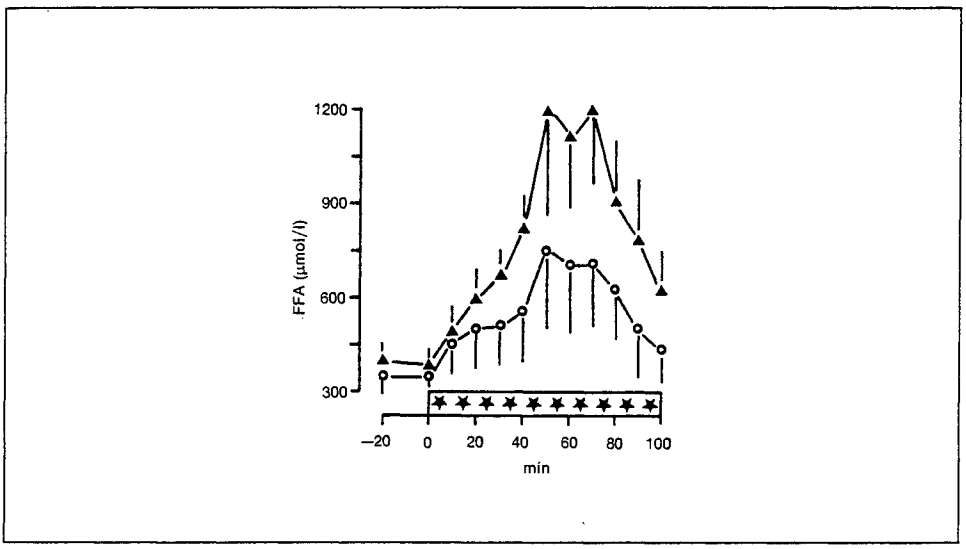

Fig. 2 - Plasma free fatty acid concentrations in response to salbutamol (▲——▲) and salbutamol-ASA (o——o) infusion in 6 insulin-dependent diabetics. Means ± SEM.

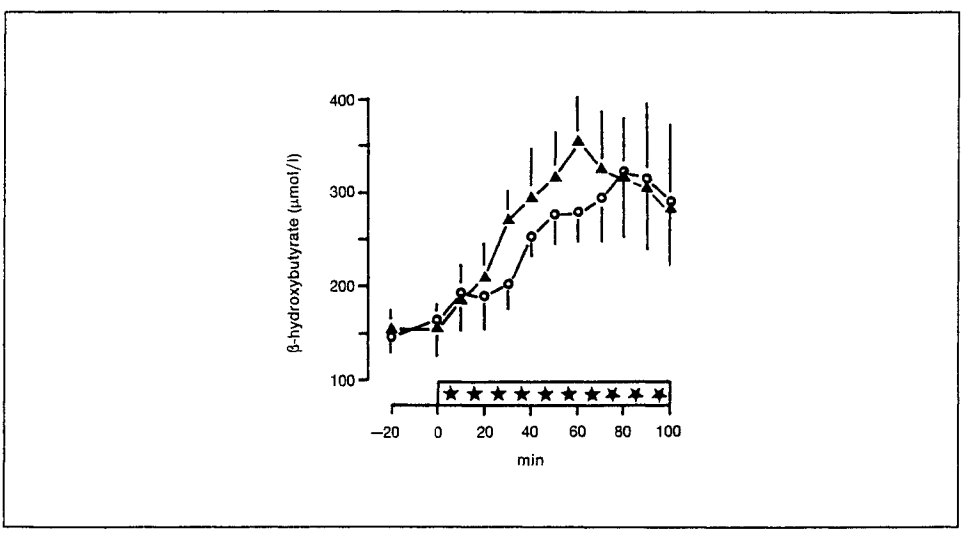

Fig. 3 - Plasma β-hydroxybutyrate concentration in response to salbutamol (▲——▲) and salbutamol-ASA (o——o) infusion in 6 insulin-dependent diabetics. Means ± SEM.

xybutyrate to salbutamol (Δ plasma β-hydroxybutyrate increase: 160±34 μmol/l) was significantly greater than that occurring at the same time during the salbutamol-ASA infusion study (Δ plasma β-hydroxybutyrate increase: 122±26 μmol/l., $p<0.05$). However, the integrated rise in plasma β-hydroxybutyrate concentration in the salbutamol study (12,356±2,958 μmol/l-min^{-1}) was not different from that obtained in the salbutamol-ASA infusion study (9,500±3,460 μmol/l-min^{-1}). Like

free fatty acids, also β-hydroxybutyrate tended to decrease after the peak response in both experiments.

Hormones

Plasma glucagon concentration (fig. 4) - Basal plasma glucagon concentration in the salbutamol study (120±19 pg/ml) was not statistically different from that of the salbutamol-ASA infusion study (129±17 pg/ml). During salbutamol infusion, a small and transient increase in plasma glucagon concentration was observed, with its maximum at 30 min (136±18 pg/ml) and return to basal values at 50 min. This occurred despite continued salbutamol infusion. By contrast, this transient increase in circulating plasma glucagon level was not seen in the salbutamol-ASA infusion study; rather, plasma glucagon concentration tended to decline slightly during the infusion. The integrated rise above basal glucagon concentration (0-40 min) was 462±130 pg/ml-min^{-1} in the salbutamol study and −313±230 pg/ml-min^{-1} in the salbutamol-ASA infusion study. This difference is statistically significant ($p<0.05$).

Plasma C-peptide concentration (fig. 4) - Basal plasma C-peptide concentration in the salbutamol study (1.3±0.2 ng/ml) was not different from the corresponding value prior to the salbutamol-ASA infusion study (1.5±0.3 ng/ml). As shown in fig. 4, only minor and not significant changes in plasma C-peptide concentration occurred in both studies.

Table 1 reports the results obtained in saline control studies. As is clearly seen, no significant changes in the plasma concentrations of the parameters investigated were observed during the infusion of saline in the 6 insulin-dependent diabetics.

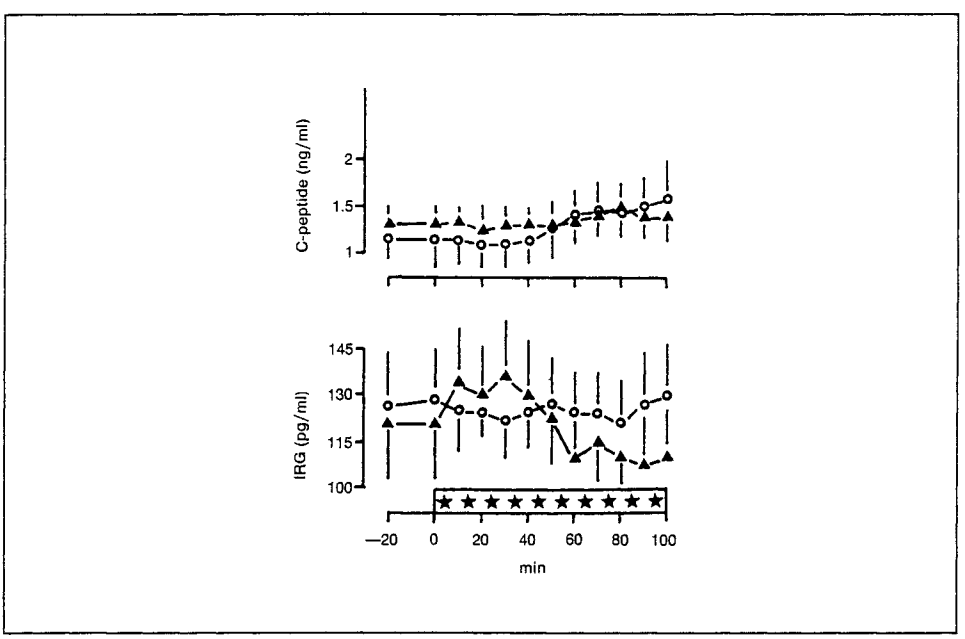

Fig. 4 - Plasma glucagon and C-peptide concentrations in response to salbutamol (▲——▲) and salbutamol-ASA (o——o) infusion in 6 insulin-dependent diabetics. Means ± SEM.

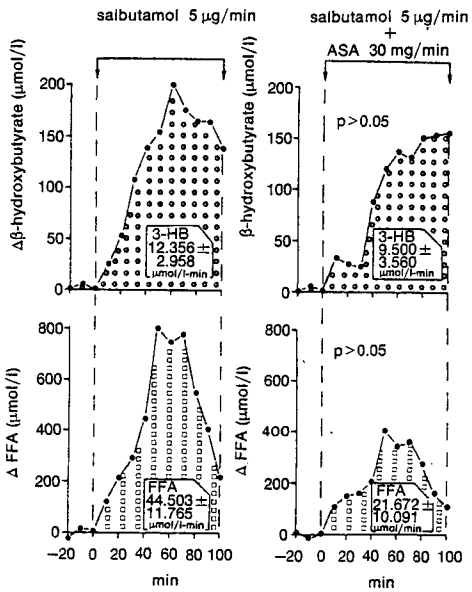

Fig. 5 - Integrated plasma β-hydroxybutyrate (top) and free fatty acid (bottom) concentrations during salbutamol (left) and salbutamol-ASA (right) infusion.

	time (min)					
	0	20	40	60	80	100
glucose (mg/dl)	180 ± 20	174 ± 19	185 ± 25	190 ± 22	187 ± 22	191 ± 24
FFA (µmol/l)	450 ± 55	500 ± 60	490 ± 55	510 ± 68	560 ± 78	540 ± 75
β-hydroxybutyrate (µmol/l)	160 ± 25	155 ± 20	163 ± 27	170 ± 30	165 ± 30	175 ± 30
IRG (pg/ml)	130 ± 20	135 ± 22	130 ± 20	127 ± 20	133 ± 22	128 ± 22
C-peptide (ng/ml)	1.2 ± 0.2	1.2 ± 0.2	1.1 ± 0.2	1.3 ± 0.3	1.2 ± 0.3	1.1 ± 0.2

Tab. 1 - Plasma concentrations of the various metabolic and hormonal parameters investigated during the saline control study in 6 insulin-dependent diabetic subjects.

DISCUSSION

The results of the present study clearly demonstrate that the synthetic non-catecholamine β-adrenoceptor stimulating drug salbutamol causes sustained increases of plasma glucose, free fatty acids and β-hydroxybutyrate, and a transient rise in plasma glucagon when infused in insulin-dependent diabetics. A concurrent infusion of ASA reduces the increase in free fatty acids, blunts the weak glucagon response but exaggerates the rise in plasma glucose following salbutamol administration.

The increase in plasma glucose concentration following salbutamol administration in the present study may be regarded as the sum of many simultaneous events: stimulation of liver glycogenolysis [2], increased gluconeogenesis from lactate [20], reduced tissue utilization of glucose [1], lack of circulating insulin and finally high levels of free fatty acids [29]. It is interesting that in normal subjects this salbutamol-induced increase of plasma glucose is abolished by pretreatment with β_1-receptor blockers propranolol [27] or practolol [16].

The marked rise in plasma free fatty acid and β-hydroxybutyrate concentration during salbutamol administration in our series of diabetics is in agreement with the results of previous studies either in normal people [16, 27] or in pregnant diabetic women [36]. Since the lipolytic effect of salbutamol is not altered by practolol [16], it seems likely that lipolysis in man is mediated by β_2-receptors. An important corollary to this study is that salbutamol, like norepinephrine, seems to exert a ketogenic effect independent of its lipolytic effect. In fact, although in the salbutamol-ASA infusion study the availabiliy of free fatty acids to support hepatic ketogenesis was reduced by half, no significant difference was observed between the two studies as far as the plasma β-hydroxybutyrate response to salbutamol is concerned. Since plasma C-peptide concentration did not show any significant change in both studies and since the change of plasma glucagon, which has been shown to possess ketogenic activity independent of its lipolytic effects [31], was very small and transient, it seems likely that the ketogenic effect of salbutamol is direct and not mediated by changes in insulin or glucagon concentrations. The fact that plasma free fatty acids as well as β-hydroxybutyrate concentration tended to decrease after the peak response despite continued salbutamol infusion may be accounted for, at least in part, by an adaptive change of hormone sensitivity at the level of the target tissue. These changes include hormone refractoriness or changes in the qualitative pattern of effects [39]. In this context it should be remembered that *in vitro* experiments with human adipose tissue showed that the lipolytic effects of the naturally occurring catecholamines are impaired in untreated juvenile diabetics [3, 4].

In the salbutamol-ASA infusion study, the increase in plasma glucose was significantly higher than that observed in the salbutamol study. This was quite unexpected, since previous studies in our laboratory had indicated that ASA tended to decrease rather than increase plasma glucose concentration both in normal subjects [15] and in maturity-onset diabetics [11]. However, in those studies, ASA augmented both basal and stimulated circulating insulin levels, which lead to the suggestion that its blood sugar lowering effect was the result of increased insulin secretion. Moreover, ASA caused a slight but not significant enhancement of plasma glucose response following arginine stimulation in insulin-dependent diabetic subjects [12].

Rapid depletion of glycogen stores in liver slices of rats treated with salicylates was found by LUTWAK-MANN [26]; this was confirmed by SPROULL [34] and extended by WINTERS and MORRILL [44]. As pointed out by HECHT and GOLDNER [18], these data suggested a theory of salicylate action, viz. 'early depletion of liver glycogen, ensuing hyperglycemia, followed by inhibition of hepatic gluconeogenesis [45] and subsequent hypoglycemia'. It thus seems likely that the potentiating effect of ASA on salbutamol-induced increase in plasma glucose may at least in part be regarded as the result of the potentiation of salbutamol-induced glycogenolysis.

Previous studies in normal, as well as in mild-untreated diabetic subjects [6], have indicated that a large dose of salicylate (5 g) decreased the basal concentration

of plasma free fatty acids. This effect was mainly due to a direct action on the adipose tissue, since parallel *in vitro* studies showed that the addition of salicylate to epididymal fat pads diminished the output of free fatty acids. GIUGLIANO et al. [12] reported that a 3-day treatment with ASA (50 mg/kg/*die*) reduced the fasting concentration of plasma free fatty acids and β-hydroxybutyrate in insulin-dependent diabetics. In the present study, an infusion of ASA reduced the increase in free fatty acid concentration following salbutamol administration by half. The mechanism underlying the effect of ASA on adipose tissue is not clarified by our study. Since a general feature of lipolysis regulation by PGE seems to be inhibition of lipolysis induced by stress hormones (catecholamines, ACTH and glucagon) [5, 36, 37], it seems unlikely that ASA may act via the inhibition of endogenous prostaglandin synthesis [17].

Unlike lipolysis, the blunting of the glucagon response to salbutamol by ASA may be linked to its ability to inhibit endogenous prostaglandin synthesis in many tissues, including pancreatic islets [21]. In fact, it is well documented that PGs stimulate glucagon release *in vitro* [29] and augment circulating glucagon levels in normal humans [13]. In addition, furosemide, which stimulates endogenous PGs synthesis [42], increases the glucagon response to arginine in man [14].

In conclusion, these studies have demonstrated that salbutamol exerts potent hyperglycemic, lipolytic and ketogenic effects in insulin-dependent diabetics. An infusion of ASA partially reverses this lipolytic effect but potentiates the hyperglycemic action. We suggest that this β-adrenergic agent be used very cautiously in insulin-dependent diabetics, as well as in asthmatics. Two cases of salbutamol-induced diabetic ketoacidosis have recently been reported [41].

SUMMARY

The selective β_2-adrenergic agonist salbutamol increases plasma glucose concentration and the rate of lipolysis when infused in pregnant diabetic women. The aim of the present study was twofold: (a) to focus on the actions of salbutamol on lipid and carbohydrate metabolism in insulin-dependent diabetics; and (b) to investigate possible interferences of acetylsalicylic acid (ASA) with the metabolic responses to i.v. salbutamol. The results obtained during salbutamol infusion (5 μg/min) in 6 insulin-dependent diabetic subjects demonstrated that this drug caused sustained increases in plasma glucose, free fatty acid and β-hydroxybutyrate concentrations, as well as a small and transient rise of plasma glucagon. No change in plasma C-peptide concentration occurred during salbutamol. A concurrent infusion of lysine acetylsalicylate reduced the increase in free fatty acids by half, blunted the weak glucagon response but enhanced the rise in plasma glucose following salbutamol administration. The present data show that salbutamol exerts a potent hyperglycemic, lipolytic and ketogenic effect in insulin-dependent diabetics. We suggest that this β-adrenergic agent should be used cautiously in human diabetes mellitus.

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