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Production of insulin resistance by hyperinsulinaemia in man

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Summary. It has been proposed that hyperinsulinaemia may cause or exacerbate insulin resistance. The present studies were undertaken to test this hypothesis in man. Glucose utilization, glucose production, and overall glucose metabolism at submaximally and maximally effective plasma insulin concentrations (~80 and ~1700 mU/l), and monocyte and adipocyte insulin binding were measured in normal volunteers on two occasions: once after 40 h of hyperinsulinaemia (25–35 mU/l) produced by infusion of insulin and once after infusion of saline (75 mmol/l; plasma insulin ~10 mU/l). After 40 h of hyperinsulinaemia, glucose utilization and overall glucose metabolism at submaximally and maximally effective plasma insulin concentrations were both slightly, but significantly, reduced compared with values observed after the infusion of saline ($p < 0.05$), whereas glucose production rates were unaffected. Monocyte and adipocyte binding were also unaffected. These results indicate that hyperinsulinaemia of the magnitude observed in insulin resistant states, such as obesity, can produce insulin resistance in man. Assuming that human insulin sensitive tissues possess spare insulin receptors and that monocyte and adipocyte insulin binding accurately reflect insulin binding in insulin-sensitive tissues, the decreased maximal responses to insulin and the lack of change in insulin binding suggest that this insulin resistance occurred at a post-binding site.

Key words: Hyperinsulinaemia, insulin resistance, glucose utilization.

The relationship between plasma insulin concentration and insulin resistance in man is unclear. Insulin resistance is usually associated with hyperinsulinaemia when there is sufficient pancreatic β-cell reserve [1, 2]. It is generally thought that insulin secretion increases in these conditions to compensate for the decrease in insulin action. On the other hand, there is evidence that increased insulin concentrations can down-regulate insulin receptors [3, 4] and in animals, under certain experimental conditions, may impair intracellular responses subsequent to insulin receptor binding [5, 6]. It has been inferred from the above considerations that hyperinsulinaemia may play an important role in the production or exacerbation of insulin resistance in man [2, 3, 8].

To date, there has been no direct demonstration that hyperinsulinaemia can produce insulin resistance in vivo in man. The correlations observed between plasma insulin concentrations and insulin resistance in conditions such as obesity [7, 9, 10] may merely reflect a compensatory increase in insulin secretion rather than hyperinsulinaemia being the cause of insulin resistance. In fact, in some studies, when animal tissues had been previously exposed in vivo or in vitro to increased concentrations of insulin, subsequent tissue responses to insulin have been increased rather than decreased [11, 12]. The present studies were undertaken, therefore, to test the hypothesis that hyperinsulinaemia, similar to that observed in insulin-resistant states such as obesity, can cause insulin resistance in man and, if so, to determine whether this impairment in insulin action occurred at a receptor or a post-binding site.

Subjects and methods

Subjects

Following approval by the Institutional Ethical Committee, informed written consent was obtained from 12 non-obese male volunteers (aged 29 ± 3 years, body mass index 23 ± 1 kg/m^2) who were within 10% of their ideal body weight and had no family history of diabetes mellitus. Subjects were admitted twice to the Mayo Clinic Research Center, and were infused with either NaCl (75 mmol/l) or with crystalline pork insulin (Eli Lilly, Indianapolis, Indiana, USA; 0.4 mU · kg^{-1} · min^{-1} made up in 1% human serum albumin) for 40 h using a portable infusion pump (Travenol Laboratories, Deerfield, Illinois, USA). During the insulin infusion, glucose (20 g/100 ml) was also infused at a rate of approximately 16 μmol · kg^{-1} · min^{-1} to maintain plasma glucose concentrations within the euglycaemic range. The in-

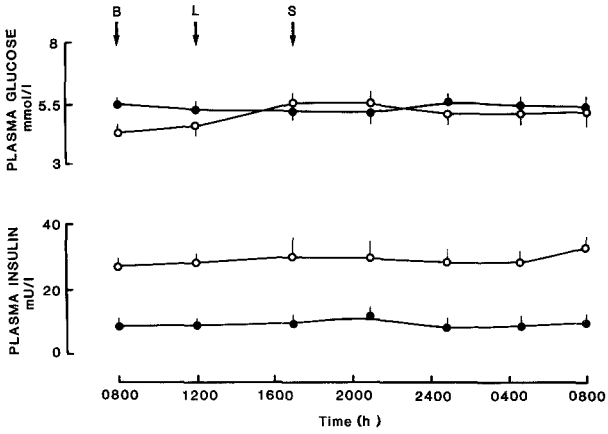

Fig. 1. Pre-prandial and fasting plasma glucose and insulin concentrations during infusion of saline (2.5 mmol/min) or insulin (0.4 mU·kg^{-1}·min^{-1}) during the 24-h period prior to the insulin dose-response study. B, L, and S indicate times at which breakfast, lunch, and supper were digested. Saline: ●—●, insulin: ○—○, mean ± SEM, $n = 7$

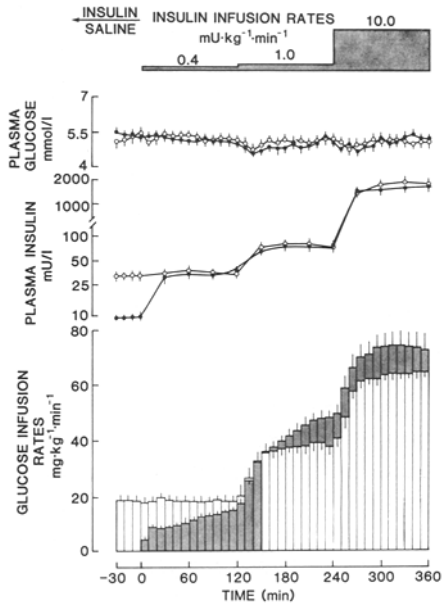

Fig. 2. Plasma glucose and insulin concentrations and glucose infusion rates necessary to maintain euglycaemia during the initial 120-min equilibration period and the subsequent dose-response study during which insulin was infused at submaximal (1.0 mU·kg^{-1}·min^{-1}) and maximal (10 mU·kg^{-1}·min^{-1}) effective rates. Saline: ●—● and shaded bars, insulin: ○—○ and open bars, mean ± SEM, $n = 7$

sulin and saline infusions were given in random order and separated by at least one week (median 7, range 7–60 days). The subjects remained ambulatory and ingested identical meals (totaling 30 Kcal/kg as 40% carbohydrate, 35% fat, 15% protein) at 0800, 1200, and 1700 h on both occasions. Following both infusions, insulin action and monocyte binding were assessed in seven subjects and adipocyte insulin binding in five additional subjects.

Assessment of insulin action on glucose production and utilization

Towards the end of the insulin and saline infusions (0700 h), the subjects were placed at bed rest and maintained supine thereafter. A primed (22 μCi/min) continuous (0.22 μCi/min) infusion of [3-^{3}H] glucose (New England Nuclear, Boston, Massachusetts, USA) was begun, and a 4-h equilibration period was allowed before the initiation of dose-response experiments.

Since several hours are required for the response to an increment in plasma insulin to reach a steady-state [13, 14] and in order to provide identical increments in plasma insulin during the dose-response studies under both experimental conditions, subjects were infused with insulin in the saline infusion experiments for 2 h at a rate (0.4 mU·kg^{-1}·min^{-1}) equal to the rate at which insulin had been infused for 40 h. Thereafter, in both experiments, insulin was infused sequentially as described previously [14] at 1.0 and 10.0 mU·kg^{-1}·min^{-1} which stimulates glucose disposal submaximally and maximally, respectively [7, 14, 15]; arterialized venous plasma glucose concentrations were clamped at approximately 5.3 mmol/l [14].

Rates of total glucose appearance (endogenously produced and exogenously infused) and glucose utilization were determined isotopically using the equations of DeBodo et al. [16]. Endogenous glucose production rate was calculated by subtracting the exogenous glucose infusion rate from the isotopically determined total glucose appearance rate. The amount of glucose infused was calculated at 10-min intervals throughout all experiments. The glucose infusion rates and the rates of glucose production and utilization over the last 40 min of each 120-min insulin infusion were used for calculation of responses to insulin [7, 14].

Monocyte receptor studies

At the end of the saline and 40-h insulin infusions, mononuclear cells were isolated from heparinized blood by a modification of the Ficoll-Hypaque technique [17] and were counted with a Coulter counter (Coulter Electronics, Hialeah, Florida, USA). The proportion of monocytes in each mononuclear cell preparation was determined by non-specific esterase staining [18]. Insulin binding was determined by the method of De Meyts [19].

Adipocyte isolation and binding

Adipocytes were isolated by a modification [20] of the collagenase digestion method of Rodbell [21]. Approximately 6 g of subcutaneous adipose tissue was obtained from the left or right lower abdominal quadrant from the centre of an area anaesthetized locally in a square-field fashion [7]. The tissue was washed in warm (37 °C) saline and 1-g portions, which had been cut into 5 × 5 mm pieces, were placed into polyethylene vessels. Each vessel contained 6 ml Krebs-Ringer-bicarbonate buffer in mmol/l: NaCl, 116; $NaHCO_3$, 29; KCL, 4.4; $MgSo_4$, 1.2; $CaCl_2$. $2H_2O$, 1.2; pH 7.6 at 37 °C after gassing with 95% O_2:5% CO_2, containing 3.5% bovine plasma albumin (Armour Pharmaceuticals, Kankakee, Illinois, USA), 5 mmol/l glucose, 3 mg collagenase (Worthing Biochemicals, Millipore Corporation, Freehold, New Jersey, USA). After shaking for 1 h at 37 °C, the digested tissue was passed first through a 1000-μm and then through a 250-μm nylon mesh. Care was taken to maintain the adipocytes at 37 °C at all times. The cells were then centrifuged at 100 *g* for 1 min in 16 × 100 mm polyethylene tubes (AS Nunc, Roskilde, Denmark), the infranatant removed, and an equal volume of buffer at 37 °C added. After resuspension by gentle inversion, the cells were recentrifuged and washed twice in the same manner into a Hepes buffer in mmol/l: Hepes-2-hydroxyethylpiperazine-N′-2-ethane sulphonic acid, 10; NaCl, 135; KCl, 4.8; $CaCl_2 \cdot H_2O$, 2.5; $MgSO_4$, 1.7; NaH_2PO_4, 1.0], pH 7.6 at 37 °C after gassing, containing 5% bovine plasma albumin, bacitracin (0.5 mg/ml; Sigma, St. Louis, Missouri, USA) and glucose (5 mmol/l). Following final resuspension of the cells, an aliquot was fixed in 1% osmium tetroxide in collidine-HCl buffer for cell counting and size analysis by a modification of the method of Hirsch and Gallian [22].

Adipocyte insulin binding was determined by a modification of the method of Pedersen et al. [20]. Isolated adipocytes (approximately 5 × 10^5/ml) were incubated in triplicate in a final volume of 0.5 ml Hepes buffer, containing 0.2 ng/ml of [^{125}I]-insulin bound in the pres-

ence of 100,000 ng/ml unlabelled insulin and was subtracted from all other values. Incubations were carried out by slow shaking at 37 °C for 1 h. Following incubation, duplicate aliquots (0.2 ml) were removed from each tube and centrifuged for 1 min at 2500 *g* through 0.2 ml silicone oil (Silwet 722, Union Carbide, Danbury, Connecticut, USA) in a microcentrifuge (Beckman, Arlington Heights, Illinois, USA). The portion of the centrifuge tube containing the cells, which float on the oil, was excised and its radioactivity was counted using a gamma counter (Nuclear, Chicago, Illinois, USA). Results were expressed as insulin bound specifically per 2×10^5 adipocytes. Incubations carried out in this manner result in a steady-state binding of 30–45 min which is maintained for at last 150 min.

Analytical techniques and statistical analysis

All glucose and insulin concentrations were determined on arterialized-venous samples. Blood for insulin [23] and glucose determinations were obtained before meals at 2100, 0100, 0500, and 0900 h over the final 24-h period before the dose-response studies. During the dose response studies, blood samples were collected at 10-min intervals for glucose and glucose specific activity and at 30-min intervals for insulin as described previously [14]. Data are given as mean ± SEM. Statistical analyses were performed using two-tailed paired Student's t-tests. A p value of < 0.05 was considered to be statistically significant.

Results

Plasma glucose and insulin concentrations

Plasma glucose and insulin concentrations over the 24-h period before the dose-response studies, during which either insulin or saline was infused, are shown in Figure 1. Plasma insulin concentrations during the insulin infusion (26 ± 2 mU/l) were significantly greater than those during the saline infusion (9 ± 1 mU/l, $p <$ 0.001). However, plasma glucose concentrations during the insulin and saline infusions were comparable (5.1 ± 0.2 and 5.4 ± 0.1 mmol/l, respectively).

Plasma glucose and insulin concentrations during the equilibration period following the saline infusions and the subsequent dose-response studies are shown in Figure 2. During the 2-h equilibration period following the saline infusion, subjects were infused with insulin at the same rate as that used during the prolonged insulin infusions ($0.4\,mU \cdot kg^{-1} \cdot min^{-1}$); the plasma insulin and glucose concentrations (34 ± 3 mU/l and 5.3 ± 0.1 mmol/l) during this period were comparable to those during the same interval of the hyperinsulinaemia experiment (36 ± 2 mU/l and 5.4 ± 0.1 mmol/l). During the dose-response studies, plasma glucose concentrations were clamped at 5.3 ± 0.1 mmol/l (coefficient of variation 4.4 ± 0.3%) during the hyperinsulinaemia experiment and at 5.2 ± 0.1 mmol/l (coefficient of variation 5.1 ± 0.3%) during the saline experiment. Plasma insulin concentrations reached comparable plateau within 30 min during the $1.0\,mU \cdot kg^{-1} min^{-1}$ (79 ± 5 versus 81 ± 8 mU/l) and during the $10\,mU \cdot kg^{-1} \cdot min^{-1}$ (1779 ± 135 versus 1630 ± 145 mU/l) insulin infusions in the hyperinsulinaemia and saline experiments, respectively.

Table 1. Effect of antecedent hyperinsulinaemia on stimulation of glucose utilization and suppression of glucose production by insulin

	Insulin infusion rates	
	($1\,mU \cdot kg^{-1} \cdot min^{-1}$)	($10\,mU \cdot kg^{-1} \cdot min^{-1}$)
Glucose utilization ($n = 7$) ($mmol \cdot kg^{-1} \cdot min^{-1}$)		
Saline	44 ± 3	68 ± 5
Hyperinsulinaemia	37 ± 3	60 ± 3
	$p < 0.01$	$p < 0.05$
Glucose production ($n = 7$) ($mmol \cdot kg^{-1} \cdot min^{-1}$)		
Saline	0.0 ± 2	0.0 ± 3
Hyperinsulinaemia	2 ± 1	2 ± 1
	$p < 0.50$	$p < 0.80$

Results expressed as mean ± SEM

Rates of glucose infusion, utilization and production during the dose-response studies

Glucose infusion rates necessary to maintain euglycaemia during infusions of insulin are equal to the sum of the increase in glucose utilization and the decrease in glucose production induced by insulin, and can thus be used as a measure for the overall effect of insulin on glucose metabolism. The steady-state glucose infusion rates necessary to maintain euglycaemia during both the submaximally ($1.0\,mU \cdot kg^{-1} \cdot min^{-1}$) and maximally ($10\,U \cdot kg^{-1} \cdot min^{-1}$) effective insulin infusions were significantly less following 40 h of antecedent hyperinsulinaemia (37 ± 3 and 60 ± 2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) than those following the saline infusion (44 ± 3 and 68 ± 5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; $p < 0.05$). Glucose utilization following antecedent hyperinsulinaemia was also significantly less at both insulin infusion rates, whereas glucose production was comparably suppressed in the saline and hyperinsulinaemia experiments (Table 1).

Table 2. Effect of antecedent hyperinsulinaemia on adipocyte insulin binding

(% Bound 2×10^5 adipocytes)	Insulin concentration (mU/l)							
	5	7.5	25	250	625	1,875	2,500	12,500
Saline ($n = 5$)	3.1 ± 4	2.8 ± 0.4	2.2 ± 0.2	1.2 ± 0.2	0.8 ± 0.2	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
Hyperinsulinaemia ($n = 5$)	4.0 ± 0.4	3.8 ± 0.5	2.9 ± 0.5	1.5 ± 0.5	0.9 ± 0.3	0.5 ± 0.2	0.3 ± 0.1	0.1 ± 0.1

Results expressed as mean ± SEM

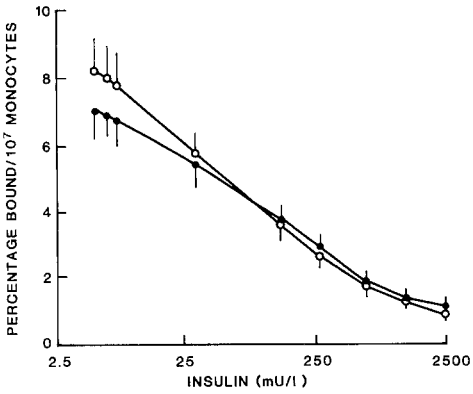

Fig. 3. Monocyte insulin binding after insulin or saline infusion. Saline: ●—●, insulin: ○—○, mean ± SEM, $n = 7$

Monocyte insulin binding

Monocyte insulin binding after 40 h or hyperinsulinaemia did not differ significantly from insulin binding after 40 h of saline (Fig. 3).

Adipocyte insulin binding

Plasma glucose concentrations over the 24-h period before the adipocyte biopsy were equivalent during the insulin (5.2 ± 0.2 mmol/l) and saline infusions (5.2 ± 0.1 mmol/l). Plasma insulin concentrations were significantly greater during the insulin infusion (35 ± 5 mU/l) than during the saline infusion (8 ± 1 mU/l; $p < 0.05$). Adipocyte insulin binding after 40 h of insulin infusion was not significantly different from insulin binding after the saline infusion at any insulin concentrations studied.

Discussion

The present studies demonstrate that sustained hyperinsulinaemia can cause insulin resistance in man. Following 40-h infusion of insulin which increased plasma insulin concentrations to levels similar to those observed in insulin-resistant conditions, such as obesity (25–35 mU/l) [1, 7], insulin-stimulated glucose utilization was slightly but significantly decreased at both submaximally and maximally effective plasma insulin concentrations. Monocyte and adipocyte insulin binding were unchanged. If one assumes that insulin sensitive tissues, such as muscle, possess spare insulin receptors and that monocyte and adipocyte insulin binding reflects insulin binding in those tissues, then the decreased maximal response to insulin and the lack of change in insulin binding both suggest that the impaired insulin action induced by the hyperinsulinaemia occurred at a post-binding site.

Previous studies in animals examining the effects of antecedent increases in insulin concentrations or the subsequent stimulation by insulin of rat adipocyte glucose metabolism have yielded contradictory results [5, 12, 24, 27]. Incubation [5], injection [12, 25] or infusion [26, 27] of insulin in rats has been reported to decrease [5, 24, 25], increase [12, 26, 27] or not to influence [5] adipocyte response to insulin. Extrapolation of these results to man is further confounded by the fact that muscle not adipose tissue is primarily responsible for insulin-stimulated glucose utilization in vivo [28]. In the rat, differences between adipocyte and muscle response to chronic hyperinsulinaemia have been reported in abstract form by Horton et al. [27], with a decrease in insulin-stimulated glucose metabolism being observed in the former but not the latter tissue. In contrast, the current studies indicate that in man, at least over the short-term, mild hyperinsulinaemia (25–35 mU/l) decreased insulin-stimulated glucose disposal implying decreased muscle glucose metabolism. Whether comparable changes occurred in human adipocyte glucose metabolism awaits further study.

The hyperinsulinaemia-induced decrease in insulin action observed in the present study, although uniformly observed and statistically significant, was relatively small (5.5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$). While it is possible that antecedent hyperinsulinaemia may not have a major regulatory influence on insulin action in man, several aspects of the current experimental design utilized could have led to an underestimation of the effect of hyperinsulinaemia, including (a) inhibition of endogenous insulin release by the exogenous insulin infusion [29] thereby minimizing differences in portal insulin concentrations during the antecedent saline and insulin infusions, (b) lack of steady-state conditions during the insulin dose-response studies following the antecedent saline infusion (i.e. a priming effect had occurred during the antecedent insulin infusion) [13, 14], (c) the degree of hyperinsulinaemia was mild, and probably most importantly, (d) the duration was brief (40 h) relative to that present in chronic hyperinsulinaemic states. Thus, although the current experiments demonstrate that hyperinsulinaemia *can* impair insulin action, due to the small magnitude of this effect, the biological significance of this phenomenon remains to be determined.

The lack of change in insulin-induced suppression of hepatic glucose production following sustained hyperinsulinaemia should be interpreted with caution. Although maximal suppression of hepatic glucose production by insulin was unaltered following 40 h of hyperinsulinaemia, the concentrations of insulin achieved during the 1.0 and 10.0 $mU \cdot kg^{-1} \cdot min^{-1}$ insulin infusion rates normally produced near-maximal suppression of hepatic glucose production [14]. Thus, a decreased response to submaximal insulin concentrations (i.e., decreased hepatic sensitivity to insulin) may have been missed. The observation that the glucose production rates during the 0.4 $mU \cdot kg^{-1} \cdot min^{-1}$ priming insulin infusions were significantly greater following hyperinsulinaemia (5.5 ± 1.1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) than following saline (3.9 ± 1.1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $p < 0.05$)

suggests that this may have been the case. Our results, therefore, do not exclude the possibility that hyperinsulinaemia also can produce hepatic resistance to insulin.

Neither monocyte nor adipocyte insulin binding were decreased after 40 h of sustained hyperinsulinaemia averaging approximately 25–35 mU/l. Other investigators [14, 30], using insulin concentrations several-fold greater than those employed in the current study, have reported that hyperinsulinaemia can decrease monocyte insulin binding. Since insulin-induced down-regulation of insulin binding appears to be both time- and concentration-dependent [31], we cannot exclude the possibility that hyperinsulinaemia at the concentrations we employed may have eventually produced a decrease in binding. The recent report [8] that adipocyte binding is decreased in patients with Type 1 (insulin-dependent) diabetes who are chronically hyperinsulinaemic due to exogenous insulin therapy supports this possibility. Nevertheless, the current studies indicate that modest sustained basal hyperinsulinaemia, under the present experimental conditions, does not cause down-regulation of monocyte and erythrocyte insulin receptor binding.

Assuming that human muscles possess spare insulin receptors, as has been demonstrated in animals [32, 33] the decreased maximal response to insulin following antecedent hyperinsulinaemia suggests a post-binding defect in insulin action. This conclusion is supported by the lack of change in either monocyte or adipocyte binding following hyperinsulinaemia (although the latter was only measured in five of the 12 subjects and, therefore, may not reflect the binding in the other seven). Our conclusion is also consistent with the studies of Marshall and Olefsky [5] and Mandarino et al. [34] in which pre-incubation of rat adipocytes with insulin [5] or 6-h infusions of insulin in normal human volunteers [34] produced a post-receptor defect in the stimulation of adipocyte glucose transport by insulin.

The mechanism responsible for the hyperinsulinaemic impairment of insulin action is unknown. In the present studies, as well as in previous animal studies [12, 24, 26], additional calories were provided as glucose during the induction of hyperinsulinaemia to prevent hypoglycaemia. Thus, it is not possible to determine whether the observed decrease in insulin action was due to a direct effect of insulin at an early post-binding step or an indirect effect of insulin resulting from a biological action of insulin (i.e. stimulation of glucose uptake and metabolism). Hyperinsulinaemia may also impair insulin action by altering the plasma concentrations of flux of the numerous other substrates and hormones. Alterations in plasma catecholamine concentrations may be an important example of the latter.

The present studies, in conjunction with previous reports [7, 9, 35], emphasize the complexity of the interactions between hyperinsulinaemia, insulin receptor binding, post-binding events, and the resultant biological response to insulin. In view of these complexities, one may not necessarily be able to deduce the sequence of events leading to insulin resistance when hyperinsulinaemia, decreased insulin receptor binding, and a post-receptor defect in insulin action are all present simultaneously.

In conclusion, the present studies demonstrate that hyperinsulinaemia produced by infusion of insulin can cause insulin resistance in man and that this decrease in insulin action probably occurs at a post-receptor site. Although the changes in the current study are small, the implication is that hyperinsulinaemia, whether due to increased secretion, decreased clearance (e.g., hepatic or renal disease), or exogenous insulin administration (Type 1 diabetes), may also impair insulin action and thus be involved in the pathogenesis of insulin resistance in a variety of disease states.

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