Insulin Resistance in Alloxan-Diabetic Dogs: Evidence for Reversal Following Insulin Therapy

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Summary. Hepatic glucose production and peripheral glucose utilization were measured basally and during infusion of insulin (25 and $40 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) in normal dogs and in insulin-deficient diabetic dogs, before and after a 10–14 day period of insulin treatment. Basal hepatic glucose production was significantly raised in the diabetic dogs ($21.4 \pm 2.5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $p < 0.005$) compared with normal dogs ($11.9 \pm 2.5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and fell by 20% in diabetic dogs following insulin treatment ($17.4 \pm 3.0 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). However, in all groups, hepatic glucose production suppressed equally well during the low dose insulin infusions, suggesting that the raised hepatic glucose production of diabetes is due to insulin deficiency and not hepatic insulin resistance. In addition, a marked defect of glucose utilization was found in the diabetic dogs ($25 \pm 5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $p < 0.001$) compared with normal dogs ($99 \pm 15 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) during matched hyperinsulinaemia and hyperglycaemia. This defect of glucose utilization, as defined by euglycaemic insulin dose-response curves employing insulin infusion rates between 40 – $600 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, demonstrated a marked reduction of glucose disposal in diabetic dogs. The severity of the insulin resistance closely paralleled the degree of hyperglycaemia. In contrast, following 10–14 days of insulin treatment, an improvement of glucose disposal was seen in all diabetic dogs. It is concluded that insulin deficiency leads to (a) increased hepatic glucose production, and (b) the development of marked peripheral insulin resistance, which is reversed by insulin treatment.

Key words: Alloxan, diabetic dogs, insulin resistance, insulin therapy.

Insulin deficiency is clearly the major aetiological factor in the glucose intolerance of Type 1 (insulin-dependent) diabetes in man. However, it is unclear whether the abnormalities of glucose kinetics can be ascribed to insulin deficiency alone or whether other factors, such as insulin resistance, are important. Studies in man have given conflicting results. DeFronzo et al. have described the presence of insulin resistance in established Type 1 diabetes [1], whereas Ginsberg [2] did not find this abnormality. Furthermore, the finding of insulin resistance in Type 1 diabetes does not resolve the question of whether the resistance is primary or secondary to the insulin deficiency. The latter was suggested by Reaven et al. [3] from experiments in alloxan-diabetic dogs.

In this study we sought to explore further the relationship between insulin deficiency and insulin resistance in the alloxan-diabetic dogs by (a) differentiating between hepatic and peripheral insulin resistance; (b) defining the severity of the insulin resistance; and (c) studying the effect of insulin treatment on the insulin resistance. To this end, hepatic glucose production and

glucose utilization were measured basally and during low-dose insulin infusions using $[3\text{-}^3\text{H}]\text{-glucose}$. The insulin resistance was also defined by constructing euglycaemic insulin dose-response curves both before and after a period of insulin treatment [4, 5].

Materials and Methods

Studies were conducted on 13 mongrel dogs, weighing 14–28 kg, before and after induction of diabetes, and following a period of insulin therapy. Animals had forelimb arterio-venous fistulae fashioned at least 14 days before the study to facilitate blood sampling. The IV glucose tolerance test (glucose 0.5 g/kg) or the oral glucose tolerance test (glucose 1.0 g/kg) was used to define glucose tolerance, and animals were considered diabetic if fasting blood glucose was $> 8.0 \text{ mmol/l}$. Diabetes was induced in 24-h fasted animals by IV injection of alloxan monohydrate (50 mg/kg) (Koch-Light Laboratories, London, UK). All dogs had normal standard liver function tests (aspartate transaminase, bilirubin) and kidney function tests (electrolytes, urea and creatinine), before and after alloxan administration. Two animals with life-threatening severe hyperglycaemia were intermittently given small doses of crystalline pork insulin (Actrapid, Novo Industries,

Table 1. Basal body weight and biochemical data, and glucose kinetic data in normal, untreated diabetic and treated diabetic dogs

Metabolic parameter	Study period	Diabetic groups		Control group
		Pre-treatment (n = 7)	Post-treatment (n = 5)	(n = 10)
Body weight (kg)		20.2 ± 1.5	21.4 ± 1.6	21.5 ± 1.6
Fasting plasma glucose (mmol/l)		16.3 ± 2.8 ^f	12.9 ± 2.5 ^f	4.4 ± 0.1
Fasting insulin (mU/l)		4.3 ± 0.6	4.9 ± 0.5	6.1 ± 0.9
Steady-state plasma glucose (mmol/l)	Basal ^a	14.5 ± 1.3 ^e	14.5 ± 2.7 ^e	5.1 ± 0.2
	Low dose ^b	17.2 ± 2.0 ^e	13.6 ± 2.1 ^e	5.3 ± 0.4
	High dose ^c	14.9 ± 2.3 ^d	12.5 ± 2.1 ^d	6.0 ± 0.5
Steady-state plasma insulin (mU/l)	Basal	5.0 ± 0.7	5.1 ± 0.7	7.1 ± 0.6
	Low dose	19.5 ± 9.4	17.7 ± 3.0	21.3 ± 2.6
	High dose	38.0 ± 3.3	48.5 ± 4.8	33.4 ± 2.1
Steady-state plasma glucagon (µg/l)	Basal	71 ± 9	79 ± 12	97 ± 13
	Low dose	74 ± 7	65 ± 13	81 ± 10
	High dose	45 ± 6	55 ± 12	62 ± 5
Hepatic glucose production (µmol · kg ⁻¹ · min ⁻¹)	Basal	21.4 ± 2.5 ^e	17.4 ± 3.0	11.9 ± 1.3
	Low dose	4.1 ± 1.6	1.9 ± 1.4	1.1 ± 0.9
	High dose	6.9 ± 2.8	3.2 ± 2.0	1.1 ± 0.9
Glucose utilization (µmol · kg ⁻¹ · min ⁻¹)	Basal	17.5 ± 3.5	9.6 ± 0.8	11.9 ± 1.3
	Low dose	16.0 ± 3.7	14.7 ± 2.6	15.7 ± 2.1
	High dose	24.9 ± 4.8	14.6 ± 2.9	14.9 ± 4.1
Glucose utilization during hyperglycaemia (µmol · kg ⁻¹ · min ⁻¹)	High dose	24.9 ± 4.8 ^d	–	99.1 ± 15.0

Results expressed as mean ± SEM; post-treatment studies were performed after 10–14 days of insulin therapy.

^a Basal: [3-³H] glucose infusion; ^b low dose: insulin 25 mU · kg⁻¹ · h⁻¹/glucose 2 mg · kg⁻¹ · min⁻¹/[3-³H] glucose infusion; ^c high dose: insulin 40 mU · kg⁻¹ · h⁻¹/glucose 3 mg · kg⁻¹ · min⁻¹/[3-³H] glucose infusion; ^d *p* < 0.01 compared with control group; ^e *p* < 0.005 compared with control group; ^f *p* < 0.001 compared with control group

Copenhagen, Denmark) over the initial 2 weeks, to ensure survival, but marked hyperglycaemia was present at all times. Urinary ketones were not detected in the diabetic dogs at the time of study.

Diabetic dogs were treated with a combination of highly purified neutral and lente insulins (Actrapid and Lente monocomponent insulins, Novo Industries, Copenhagen, Denmark) for 14 days. Blood glucose levels were maintained throughout between 3.5–13.0 mmol/l. Insulin treatment was withheld for 24 h before any experimental procedure.

Experimental Procedures

The glucose kinetic studies were performed on 15-h fasted, anaesthetized dogs. Anaesthesia was induced with Thiopentone Sodium 25 mg/kg (Abbott Laboratories, Melbourne, Australia) and maintained by a 2:1 mixture of N₂O:O₂ with halothane 1%–2% (ICI, Melbourne, Australia). Solutions were infused, using two Gilson Minipuls II 4-channel pumps (Gilson, Villiers le Bel, France) via a 20-cm Intracath placed in a saphenous vein. Arterialised blood samples for glucose, [3-³H]-glucose specific activity, insulin (IRI) and glucagon (IRG) were collected at appropriate intervals from an indwelling butterfly needle in the forelimb arterio-venous fistula. Control and diabetic dogs were tested with two different experimental protocols: (a) hepatic glucose production and hepatic sensitivity to insulin were determined using [3-³H]-glucose [6, 7, 8]; (b) peripheral insulin sensitivity was measured using the euglycaemic clamp technique [9]. In a separate group of control dogs, peripheral glucose utilization was also measured during controlled hyperglycaemia [9].

On day 1, basal hepatic glucose production was measured at steady state, using a primed, constant infusion (10 µCi/h) of [3-³H]-glucose. A [3-³H]-glucose bolus dose of 15–30 µCi (depending on fasting blood glucose level) was given at time zero, in order to ensure the

attainment of steady state by 90–120 min [7, 8]. Steady-state glucose turnover was also measured during insulin infusions at two dose levels (25 and 40 mU · kg⁻¹ · h⁻¹). Glucose (2 and 3 mg · kg⁻¹ · min⁻¹ respectively) was infused simultaneously with the insulin in order to prevent hypoglycaemia [7, 8]. For both insulin doses, a period of 90 min was allowed to reach a new equilibrium, at which time blood samples were collected over the next 30 min [7, 8]. The diabetic animals had fasting hyperglycaemia, and for the hepatic sensitivity studies with [3-³H]-glucose, blood glucose levels were maintained at their fasting hyperglycaemic concentrations. For these studies, urinary glucose loss was determined basally and during the two insulin infusions. At the conclusion of each study, three 5-min samples of fluid delivered by the pumps were collected into weighed tubes. From these were measured the rates of infusion of [3-³H]-glucose, unlabelled glucose and insulin [7, 8]. To overcome the problem of insulin adsorbing to the IV tubing, insulin was run for 1 h before each study [10].

In the day 2 study, peripheral insulin action was measured at higher insulin infusion rates, employing the euglycaemic clamp procedure [9], as previously described [5]. Blood samples were collected at steady state over the final 30-min period of each insulin clamp. The coefficient of variation of blood glucose at plateau was 3.9 ± 0.4%, and steady state was reached by 90–120 min [5]. Insulin infusion rates were between 40 and 600 mU · kg⁻¹ · h⁻¹, which allowed the construction of multiple-point dose-response curves. In the diabetic and the insulin-treated dogs, blood glucose was normalised before the commencement of each study by infusion of insulin alone. This required from 1 to 3 h and the clamp procedure was commenced 1 h after normoglycaemia had been attained. At the end of each experiment, samples of the infusate delivered by the pump were again collected into weighed tubes to enable measurement of infusion rates of insulin and glucose.

The final study was carried out in six normal dogs, using the hyperglycaemic clamp procedure [9]. Blood glucose was raised to 13.4 ± 1.7 mmol/l during a simultaneous insulin infusion (40 mU ·

Fig. 1. Hepatic glucose production (upper panel) and glucose utilization (lower panel) of untreated diabetic $\square$, treated diabetic ▨ and control $\square$ dogs, basally and during low-dose ($25 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) and high-dose ($40 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) insulin infusions. Glucose utilization obtained in control dogs, during the hyperglycaemic high-dose insulin infusion, is shown by the solid bar $\blacksquare$.

$\text{kg}^{-1} \cdot \text{h}^{-1}$). To prevent endogenous insulin secretion in response to the hyperglycaemia, animals were infused with cyclic somatostatin at $5.0 \mu\text{g}/\text{min}$. This latter solution contained human serum albumin 0.2% and 20,000 KIU aprotinin (Bayer Pharmaceuticals, Botany, Australia) per 200 ml solution to prevent adsorption of somatostatin to the IV tubing and its proteolytic breakdown.

Analytical Procedures

Blood for hormone estimations was centrifuged and the plasma stored at -20°C until the time of assay. Blood for IRG estimation was placed in chilled heparinised tubes containing 5000 KIU aprotinin. Plasma samples for measurement of $[3\text{-}^3\text{H}]$ -glucose specific activity were deproteinated in $\text{Ba}(\text{OH})_2\text{-ZnSO}_4$ and the supernatant divided into three aliquots for estimation of total and $[3\text{-}^3\text{H}]$ -glucose as described previously [7, 8]. Total glucose was measured with a Union Carbide Centrifichem C400 analyser (Union Carbide Corporation, New York, USA), using a hexokinase method. Plasma IRI and IRG were estimated by radioimmunoassay using dextran-charcoal for separation of bound and free fractions [11, 12]. The IRG assay used the pancreatic glucagon specific (C-terminal reacting) RCS5 antiserum (donated by Professor S. R. Bloom).

Calculations

The rates of appearance of glucose (Ra) at steady state, both basally and during the insulin/glucose infusions, were calculated using the formula $\text{Ra} = \text{F}/\text{SA}$, where F is the rate of infusion of $[3\text{-}^3\text{H}]$ glucose and SA is the specific activity of the plasma $[3\text{-}^3\text{H}]$ glucose at steady state [13]. Steady state was defined as a variation of $<10\%$ in the counts at plateau. The mean $\pm$ SEM coefficient of variation of the counts at plateau was $5.6 \pm 0.7\%$ for all studies. On three occasions in one period from different studies, the tracer counts were not at steady state. To calculate rate of appearance from these periods, the non-steady-state equation of Steele et al. was used [6], employing 0.65 as the pool fraction. During the insulin/glucose infusion, subtraction of the amount of unlabelled glucose infused from the calculated total rate of appearance (Ra), gave the net endogenous hepatic glucose production. At steady state, plasma glucose is constant and therefore $\text{Ra} = \text{rate of disappearance of glucose}$. The rate of utilization of glucose is the rate of disappearance of glucose minus urinary glucose loss. Insulin dose-response curves were constructed by plotting metabolic clearance rate of glucose (rate of glucose utilization at pla-

teau $\div$ glucose concentration [14]) against steady-state plasma insulin concentrations, as detailed previously [5]. Statistical analyses were made using paired and unpaired Student's t-tests and linear regression analyses. All results are expressed as mean $\pm$ SEM.

Results

Body weight and fasting IRI levels were similar in all groups, but fasting glucose levels were significantly higher in both diabetic and insulin-treated groups ($p < 0.002$; Table 1). As expected, oral glucose tolerance was markedly abnormal in the diabetic dogs (fasting glucose: 14.1 ± 2.5 ; glucose 60 min after glucose load: 25.3 ± 2.7 ; 120 min: $19.0 \pm 2.0 \text{ mmol/l}$), which was accompanied by marked insulinopenia (fasting IRI: 4.3 ± 0.5 ; IRI 60 min after glucose load: 6.0 ± 1.8 ; 120 min: $6.2 \pm 1.0 \text{ mU/l}$). Insulin levels in the normal dogs were 6.1 ± 0.9 , 66.5 ± 19.7 and $24.5 \pm 3.7 \text{ mU/l}$ at 0, 60 and 120 min respectively after the glucose load. The insulin treatment was ceased 24 h before each study, accounting for the similar fasting glucose levels in the two diabetic groups on the day of study (Table 1). Mean plasma levels of glucose, IRI and IRG seen in the glucose turnover studies, basally and during the two insulin infusion periods, are shown in Table 1. Mean plasma glucose levels in the diabetic and the treated diabetic groups were similar basally and during each insulin infusion period. However, glucose levels were significantly greater than in normal dogs ($p < 0.005$). Plateau insulin concentrations rose similarly in a step-wise fashion for each study period for the three groups (Table 1). With the higher infusion rate, IRI levels were unexpectedly higher in two of the diabetic treated animals, leading to a mean IRI concentration higher than in the normal and untreated diabetic groups. At steady state, the coefficients of variation of mean plateau plasma glucose and insulin concentrations were $5.8 \pm 0.9\%$ and $9.4 \pm 1.9\%$, respectively. Basal IRG levels were similar in all groups and, as expected, fell during the insulin infusions in all dogs (Table 1).

Hepatic Glucose Production

Basal hepatic glucose production was significantly elevated in the diabetic groups compared with controls (21.4 ± 2.5 versus $11.9 \pm 1.3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $p < 0.005$). Basal hepatic glucose production fell by 20% following insulin treatment ($17.4 \pm 3.0 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). During the low-dose insulin infusion, hepatic glucose production fell significantly to equally low levels in the normal, diabetic and treated diabetic groups ($p < 0.001$; Fig. 1). These hepatic glucose production data were similar when expressed as a percentage suppression from basal (93.2 ± 5.4 , 81.8 ± 7.3 and $88.4 \pm 5.5\%$, respectively). With the higher insulin infusion rate no additional decrease in hepatic glucose production occurred in the three groups, whether expressed in abso-

Fig. 2. Individual insulin dose-response curves of glucose clearance in control dogs (Panel A) and diabetic dogs (Panel B). The shaded area in panel B represents the normal range of insulin dose-response curves

lute terms or as a percentage of suppression from basal (Fig. 1). The basal hepatic glucose production correlated with fasting blood glucose ($r=0.62$) and was inversely related to fasting IRI ($r=-0.59$) in the combined diabetic and control groups ($p<0.01$). There was no correlation between the percentage suppression of hepatic glucose production and the fasting hyperglycaemia, basal IRG or basal IRI levels observed during the low dose insulin infusion period.

Glucose Utilization

In the presence of comparable insulin levels, basal glucose utilization rates were not statistically significantly different in the three experimental groups (11.9 ± 1 versus 17.5 ± 3.6 versus $9.6 \pm 0.8 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Although there was a relatively wide scatter of individual glucose utilization rates in the three groups, the glucose utilization rate of diabetic animals never fell below the range obtained for normal dogs. A 25%–50% rise of glu-

cose utilization above basal levels was observed for the three groups during the low or the higher dose insulin infusions, but this rise was not statistically significant (Fig. 1). Basal glucose utilization did not correlate with fasting insulin in the control and diabetic groups.

Comparison of glucose utilization data between normoglycaemic control dogs and the hyperglycaemic diabetic groups is difficult. It was therefore important to study the effect of a similar degree of hyperglycaemia on the glucose utilization in control animals. To this end, six separate control dogs were selected for study at the higher dose insulin infusion, during steady-state hyperglycaemia (Table 1). The levels of hyperglycaemia ($13.4 \pm 1.7 \text{ mmol/l}$) and hyperinsulinaemia ($48.6 \pm 3.3 \text{ mU/l}$) achieved in these animals were comparable to those obtained in the untreated and treated diabetic groups (Table 1). Glucose utilization was markedly higher ($99.1 \pm 15 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $p<0.01$) than in either diabetic group (Fig. 1).

Dose-Response Curve Characteristics

Plateau plasma glucose concentrations during the euglycaemic clamps were similar (control group 4.5 ± 0.2 ; untreated diabetic group 5.9 ± 0.3 ; treated diabetic group $5.1 \pm 0.2 \text{ mmol/l}$), with a coefficient of variation for steady-state plasma glucose concentrations during clamps of $3.9 \pm 0.4\%$. The coefficient of variation in the plateau insulin concentration was $7.5 \pm 1.0\%$. Individual dose-response curves (metabolic clearance rate of glucose versus IRI) for the eight control animals are shown in Figure 2. Metabolic clearance rate of glucose increased steeply between insulin concentrations of 40–150 mU/l and several of the animals had reached their maximal rate of glucose clearance at insulin concentrations between 150–400 mU/l (Fig. 2).

The dose-response curves for the six diabetic animals were strikingly different from those of the control group (Fig. 2). Only minimal increases in glucose clearance occurred in four of the six animals, even when insulin levels were raised above 500 mU/l. This indicates a gross defect in the responsiveness of peripheral tissues to insulin in these four diabetic dogs, which were also noted to be the more diabetic members of the group with the highest fasting blood glucose levels (between 12.6 and 29.2 mmol/l). The other two diabetic dogs, with only mild glucose intolerance (fasting blood glucose 8–10 mmol/l), showed a greater rise in glucose clearance in response to the increasing insulin concentrations, with marked right shift of their dose-response curves as compared with the control group (Fig. 2). Thus, the magnitude of the reduction of insulin-mediated glucose clearance appeared to parallel the severity of the hyperglycaemia.

An improvement in insulin-mediated glucose disposal occurred in all of the five dogs restudied after 14 days of insulin treatment (Fig. 3). The maximal response of metabolic clearance rate of glucose reached

Fig. 3. A–E Comparison of individual insulin dose-response curves of glucose clearance in diabetic dogs before (■—■) and after (▲—▲) a 10–14 day period of insulin therapy. The shaded areas represent the normal range of insulin dose-response curves

or approached normal in three of the dogs (Fig. 3 B, C and D), but remained low in the other two dogs (Fig. 3 A and E).

Discussion

This study demonstrates that alloxan-induced, insulin-deficient diabetes in the dog is characterised by increased basal hepatic glucose production and a marked defect in glucose disposal. However, increased basal hepatic glucose production is readily suppressed during low-dose insulin infusion, as noted previously [15, 16], whereas the defect of glucose disposal persists despite very high levels of exogenous insulin. Thus it appears that, in hyperglycaemic diabetic dogs under basal conditions, hepatic production of glucose fails to be suppressed by the elevated blood glucose levels [15–18]. It is not certain from the present study to what extent the insulin deficiency contributed to the failure of the hyperglycaemia to suppress basal hepatic glucose production [17, 19]. However, the ready suppressibility of the raised glucose production by the small incremental rises of insulin during the insulin infusion ($25 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$), also noted by other workers [15, 16, 20], suggests that, in the untreated diabetic dogs, basal hepatic glucose overproduction is mainly due to insulin deficiency rather than hepatic insulin resistance. Interestingly, the increased basal hepatic glucose production correlated with fasting blood glucose, suggesting that hepatic glucose overproduction is important in the development of the fasting hyperglycaemia.

Concurrent with the increased hepatic glucose production, untreated diabetic dogs had a marked defect in glucose disposal [22]. This was highlighted when glucose utilization in the hyperglycaemic diabetic dogs was compared with that during matched hyperglycaemia in

normal dogs (Fig. 1). Thus, in diabetic dogs, glucose utilization remains normal only in the presence of hyperglycaemia [21]. The fact that glucose utilization rose only slightly (by 25%–50%) during the two low-dose insulin infusions (25 and $30 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) is consistent with previously published reports for man [16, 19] and dog [15, 20]. This also implies that low-dose infusions of insulin lower blood glucose predominantly by suppressing hepatic glucose production, and not significantly by stimulating peripheral glucose uptake [16, 20]. In order to define more clearly the defect in glucose disposal, glucose clearance was assessed at euglycaemia by insulin dose-response curves calculated from the metabolic clearance rate of glucose, as the previously validated parameter of insulin action [5, 23–26]. A marked defect of glucose disposal was again noted in the untreated diabetic dogs, at both physiological and very high levels of insulin (Fig. 2). The degree of insulin unresponsiveness closely paralleled the severity of the diabetes. The most hyperglycaemic dogs showed little or no response to insulin, while a moderate response was seen in the two mildly hyperglycaemic dogs (Fig. 2).

When glucose turnover was re-examined after 10–14 days of insulin therapy, the increased hepatic glucose production had fallen (Fig. 1) and a clear improvement of glucose disposal had occurred (Fig. 3). Whether a more prolonged period of insulin therapy or a more precise insulin replacement regimen could have led to a normalisation of glucose turnover and glucose clearance remains to be proven. Nevertheless, the present data are in accord with the original suggestion of Reaven et al. that alloxan-induced hypoinsulinaemia leads directly to the development of an insulin-resistant state [3]. These data are therefore consistent with the view that chronic insulin deficiency leads to a reduction in those insulin-dependent systems responsible for the maintenance of normal glucose disposal [27].

However, several other factors may have contributed to the development of the insulin resistance. Elevated levels of non-esterified fatty acids and ketone bodies, which are known to inhibit insulin action and glucose uptake, may have been a contributing factor [28, 29]. In addition the anti-insulin stress hormones, (counter-regulatory hormones), glucagon and adrenaline, may have played a role in the development of the insulin resistance [30]. However, glucagon levels were similar in all groups and were readily suppressed by the low-dose insulin infusion, and adrenaline levels are usually normal or suppressed by general anaesthesia [31]. On the other hand, adrenaline levels may rise during acute normalisation of blood glucose from hyperglycaemia [32, 33]. However, the rises are usually relatively small and short-lived [32, 33], and therefore unlikely to have inhibited peripheral glucose disposal to the degree observed in the diabetic dogs [34]. Thus, the above mechanisms probably did not play a major rôle in the development of the insulin resistance of these diabetic dogs.

In conclusion, the alloxan-diabetic dog is characterised by marked impairment of glucose disposal and peripheral insulin resistance, as well as an increase in basal hepatic glucose production. The insulin resistance parallels the severity of the hyperglycaemia and is largely reversed by a period of insulin treatment. These studies lend support to current clinical practice, aimed at achieving 'ideal' metabolic control in our diabetic patients.

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