Originals

Cardiovascular sequelae of endotoxin shock in diabetic dogs

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Summary. Diabetic patients exhibit a higher incidence of post-surgical sepsis, as well as a higher rate of mortality from sepsis, than their non-diabetic counterparts. This may be a result of cardiovascular deterioration associated with diabetes mellitus. This study was designed to characterize the cardiovascular sequelae associated with endotoxin shock in a canine model of diabetes. Diabetes was induced with alloxan (50 mg/kg) and streptozotocin (30 mg/kg) in dogs weighing 19–25 kg. Thirty days later, anaesthetized dogs were instrumented to obtain blood pressures, blood samples, left ventricular chamber diameter, circumflex arterial blood flow, and aortic blood flow. Metabolic parameters were calculated according to the Fick principle, and myocardial inotropic state assessed with the end-systolic pressure-diameter relationship. After stable baseline measurements, *Escherichia coli* endotoxin (1 mg/kg) was infused over 1 h, and measurements were obtained every 30 min. After endotoxin administration diabetic dogs became more hypoten-

sive than the non-diabetic dogs. Cardiac performance parameters were also depressed to a greater degree. These changes could be attributed to depressions in vascular resistance and myocardial inotropic state in diabetic dogs. Cardiac dysfunction occurred in association with a relative decrease in the supply to demand ratio for oxygen in the diabetic dogs, suggesting functional ischaemia. Data indicating a decrease in pre-load and vascular resistance in the diabetic group suggest a greater degree of vascular collapse, vascular pooling, or extravasation of fluid than occurred in the non-diabetic group. These data support the hypothesis that the cardiovascular system of diabetic subjects cannot tolerate a septic insult as well as their non-diabetic counterparts.

Key words: Diabetes mellitus, endotoxin, shock, ventricular function, end-systolic pressure-dimension relationship, pepsis.

In a study by Lewis et al. [1] it was reported that diabetic patients have a higher rate of mortality from sepsis than their non-diabetic septic counterparts. As a further complication it had been noted that septic shock following surgical procedures occurred with greater frequency in diabetic than non-diabetic patients [2]. It is our contention that the cardiomyopathies of diabetes mellitus contribute to this increased mortality during sepsis.

Myocardial function and metabolism are altered during diabetes mellitus. In addition to reduced myocardial glucose utilization [3, 4] Type 2 (non-insulin-dependent) diabetes results in myocardial triglyceride and cholesterol accumulation, decreased diastolic compliance, and reduced cardiac function in both man [5] and an experimental canine model [6]. Myocardial dysfunction has also been characterized in Type 1 (insulin-dependent) diabetes in man [7] and in animal models of diabetes [3, 8] and may explain the increased incidence of non-

atherosclerotic-related heart failure in diabetic patients [9].

McDonough and co-workers [10] recently reported that myocardial depression (examined ex vivo in a Langendorff preparation) occurred in streptozotocin-diabetic rats after intraperitoneal injection of live *Escherichia coli* at concentrations that did not produce a similar depression in hearts from non-diabetic rats. While this strongly suggests an intrinsic contractile defect, there are problems inherent in isolated perfused organ systems that prevent accurate extrapolation to the environment in vivo. Thus, in reviewing the literature pertinent to this problem, it is evident that there is a need for a systematic in vivo analysis of myocardial function during septic shock in a model of diabetes. We chose to examine this problem using an *E. coli* endotoxin model of septic (endotoxin) shock. This study was undertaken to characterize the cardiovascular sequelae and components responsible for dysfunction as-

sociated with endotoxin challenge in a canine model of diabetes.

Materials and methods

Diabetes was induced in mongrel dogs of either sex (19–25 kg), according to the methods of Stevenson and Parsons [11]. After a 16 h fast, mannitol (0.5 g/kg) was injected intravenously (i. v.). Twenty minutes after the mannitol injection streptozotocin (30 mg/kg) and alloxan monohydrochloride (50 mg/kg) were administered i. v. followed by 400–500 ml 10% glucose in water subcutaneously (s. c.). Experiments were performed 30 days after induction of diabetes. Glucose concentrations were monitored daily for one week, then at least every third day and insulin (NPH) was administered (1 IU/kg, s. c.) as needed to maintain blood glucose between 5.6–8.4 mmol/l (plasma glucose 11.1–16.7 mmol/l). Haematocrit levels were determined periodically. No insulin was given within 24 h prior to initiating any experimental protocol. Dogs were considered diabetic if plasma glucose values remained greater than 11.1 mmol/l for the 30-day period of diabetes. During the 30-day period of diabetes blood samples for determination of non-esterified fatty acids, β-hydroxybutyrate, and insulin were centrifuged at 1500 g for 10 min at 4°C and the serum was stored at − 20°C for later assay.

On the day of the experiment dogs were anaesthetized with sodium pentobarbital (30 mg/kg i. v.), intubated, and ventilated with room air against 3–5 cm H_2O positive end-expiratory pressure using a Harvard respirator. After administering succinyl choline (1 mg/kg, i. v.) a left thoracotomy was performed in the fifth intercostal space, and the heart exposed. A silastic cannula was placed into the coronary sinus via the right atrial appendage and was used to obtain coronary venous blood samples. A Gould electromagnetic flow probe was placed around the circumflex artery to measure blood flow with a Gould SP 2202 flowmeter. A femoral arterial cannula was advanced into the abdominal aorta to measure arterial blood pressure and obtain arterial blood samples. Central venous pressures were obtained through a thoracic venous cannula introduced via a femoral vein. On-line haemodynamic measurements were recorded on a Gould 2800s physiological recorder.

Details of the methods used to examine cardiac performance and contractility have been described elsewhere [12, 13]. To obtain instantaneous pressure a P-7 Konigsberg transducer was inserted into the left ventricle (LV) via an apical stab incision and was closed with a purse string suture. LV pressure was electronically differentiated to obtain the first derivative of left ventricular pressure with respect to time (LV dP/dt). The maximum positive value of this parameter, LV dP/dt_{max}, was used as an index of cardiac systolic performance. The LV minor axis diameter was measured using the ultrasonic transit time method [14]. A balloon occluder was placed around the ascending aorta to vary the loading conditions, thus obtaining a large range of pressure-diameter loops. This was done by briefly (<7 s) inflating the balloon to partially occlude the aorta. These data were used to assess the end-systolic pressure-dimension relationship, an index that has been suggested to measure myocardial contractility independent of changes in pre-load [15, 16] and after-load [16, 17]. All measurements were recorded on a Nicolet 4094 digital storage oscilloscope and transferred to a HP-9845B computer for analysis of E_{es}, the slope of the end-systolic pressure-dimension relationship, and the X^2 coefficient of a second order polynomial of the end-systolic pressure-dimension relationship [18]. End-systole was defined as the time when maximum pressure occurred at minimum diameter on each differentiated loop [19]. Any loops representing extra-systolic beats were excluded from the analysis of the end-systolic pressure-dimension relationship.

After surgical instrumentation, animals were allowed to stabilize for a minimum of 60 min. Respirator volumes and rates were set by careful monitoring of arterial blood gases and pH status prior to initiating the temporal protocol. Immediately after this stabilization period basal metabolic, and haemo- and cardiodynamic profiles were determined. After basal measurements had been made the endotoxin group dogs received an i. v. infusion of *E. coli* endotoxin

(17 $\mu g \cdot kg^{-1} \cdot min^{-1}$; 5 mg/ml solution; Difco, Detroit, Mich., USA) for 1 h. Dogs in the non-shock group received an equivalent volume of 0.9% NaCl. Measurements of metabolic and inotropic parameters were then made every 30 min after initiating endotoxin infusion. Haemodynamics and LVdP/dt were monitored on line.

Since a large number of animals in the diabetic group died after 1.5 h we analysed data up to that time to avoid problems associated with loss of animals from the group blocks. Any animals that lived past 4 h were killed by the rapid i. v. injection of saturated KCL solution. The circumflex area of the heart (to which blood flow was being measured) was stained with monastral blue while maintaining an equal or greater pressure KCl infusion into the left anterior descending artery. All myocardial metabolic data were standardized per 100 g heart weight by dividing the substrate uptake values obtained from each dog by its circumflex perfused area and multiplied by 100.

Arterial and coronary sinus blood gases, pH and oxygen content were determined with a Radiometer Acid-Base laboratory (ABL-3; Copenhagen, Denmark). Concentrations of glucose and lactate were determined with YSI 23A and 23L analysers, respectively (Yellow Springs Instruments, Yellow Springs, Ohio, USA). Myocardial uptake of substrates was calculated as the product of the arterio-coronary sinus concentration difference and circumflex blood flow, and normalized to tissue weight. Serum non-esterified fatty acids were determined colorimetrically using a commercially available kit (Wako Pure Chemicals Indust., Ltd, Asaka, Japan). Serum insulin was determined with radioimmunoassay, using a commercially available kit (Cambridge, Inc., Billerica, Mass., USA). Serum β-hydroxybutyrate was measured spectrophotometrically with a commercially available kit (Sigma Chemical Co., St. Louis, Mo., USA).

Statistical analysis

Data are presented for each time period, and as change from baseline (pre-endotoxin) values (see figure inserts). All data underwent Bartlett's test for homogeneity of variance, followed by two-way analysis of variance (ANOVA) in a completely randomized design to examine differences between groups and across time. Transformation by common logarithm was necessary prior to ANOVA for some of the data that exhibited heteroscedasticity. Means were compared using the Least Significant Difference test. The data for E_{es} and X^2 coefficients were inherently heterogeneous. For these data the change from baseline was calculated and the Kruskall-Wallis test used to determine differences between groups. For all analyses α was set at 0.05.

Results

Characterization of diabetes

Twenty-four hours after induction of diabetes serum glucose concentrations were significantly elevated compared to the pre-diabetic level (Fig. 1; mean > 20 mmol/l) and non-diabetic animals (data not shown). This is similar to values reported by others [11]. A few of the diabetic dogs required insulin (regular or protamine zinc, as required) during the first week to maintain blood glucose below 25 mmol/l. By day 7, and for the remaining portion of the 30-day period, serum glucose fell to a lower (as compared to days 1–3) hyperglycaemic level (17 > mean > 14). In all but two diabetic dogs serum insulin concentrations were consistently below assay detection. In the two diabetic dogs that demonstrated detectable levels, concentrations did not exceed 10 μU/ml. These two dogs remained hyperglycaemic. One was tested with an i.v. bolus of glucose (see below) and did not respond to of insulin secretion to glucose. Non-esterified fatty acid concentrations were
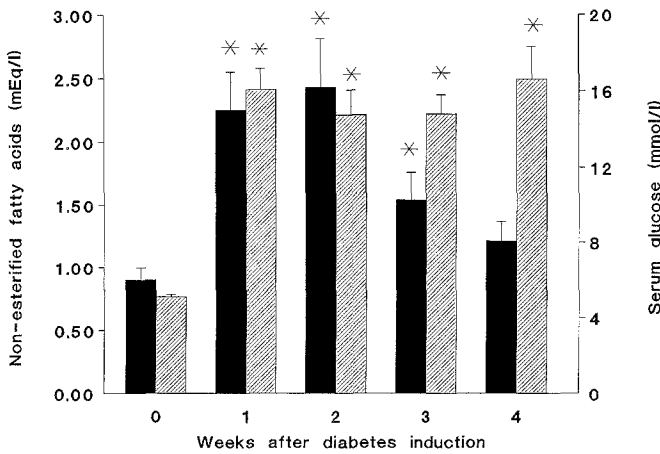
Fig. 1. Non-esterified fatty acids in serum (■) and serum glucose (▨) before, and at various times during the 30 days after induction of diabetes. Values = means ± SEM; $n = 13$. * Significant difference from day 0; $p < 0.05$

also elevated during the first 3 weeks (Fig. 1), but fell to concentrations not significantly different from pre-diabetes concentrations. All of the diabetic dogs lost weight, falling from 23.6 ± 0.6 to 18.7 ± 0.4 kg ($p < 0.05$). The non-diabetic dogs weighed 22.8 ± 0.6 and 22.4 ± 1.1 kg in control and endotoxin shock groups, respectively. The diabetic animals in control and endotoxin shock groups weighed 18.6 ± 0.5 and 18.7 ± 0.4 kg, respectively. Despite these differences in body weight, there were no significant differences in heart weights between the diabetic and non-diabetic dogs (133.2 ± 10.0 vs 156.2 ± 9.0 g, respectively). There were no significant differences in haematocrit or β-hydroxybutyrate concentrations between diabetic and non-diabetic animals.

At the end of experiments in three control non-diabetic and three control diabetic animals changes in serum insulin concentrations in response to an i.v. bolus of 30 mg/kg glucose were determined. The non-diabetic dogs responded with characteristic increases in serum insulin, while the insulin concentrations of the diabetic dogs remained below detection.

Effects of endotoxin

A. Cardiovascular parameters. A slow infusion of endotoxin (lipopolysaccharide; LPS) caused a progressive hypotension in all dogs (Fig. 2). Mean arterial blood pressure (MAP) values were not significantly different between groups prior to LPS infusion, but the MAP in diabetic dogs was significantly lower than non-diabetic dogs at 1.0 and 1.5 h after starting LPS infusion. Reductions in peak left ventricular (LV) pressure were similar to those for MAP (data not shown). No significant differences were seen in central venous pressures at any time.

Cardiac index values (CI) fell after beginning LPS (Fig. 3). At 1.5 h the mean cardiac output in diabetic endotoxic animals was significantly lower than in their non-diabetic counterparts. The means presented in Figure 3 reflect data from all but one animal in the non-diabetic/LPS group, and two in the diabetic/LPS group,
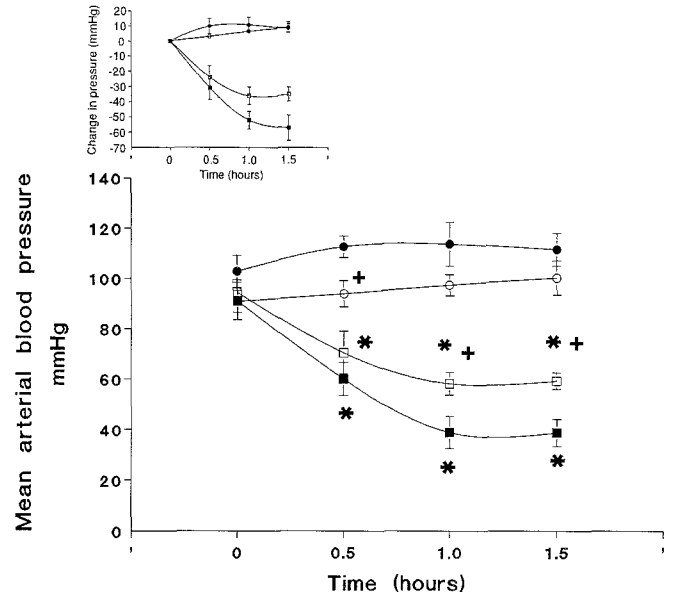

Fig. 2. Mean arterial blood pressure before and 0.5, 1.0, and 1.5 h post-endotoxin/0.9% NaCl. Inset graph is change in mean arterial blood pressure. Groups represented are Control/Non-diabetic (C/ND; open circles; $n = 5$), Control/Diabetic (closed circles; $n = 4$), Endotoxin shock/Non-diabetic (open squares; $n = 7$), and Endotoxin shock/Diabetic (closed squares; $n = 6$). Values = means ± SEM. * Significant difference from pre-endotoxin value; $p < 0.05$; + Significant difference from corresponding diabetic group; $p < 0.05$
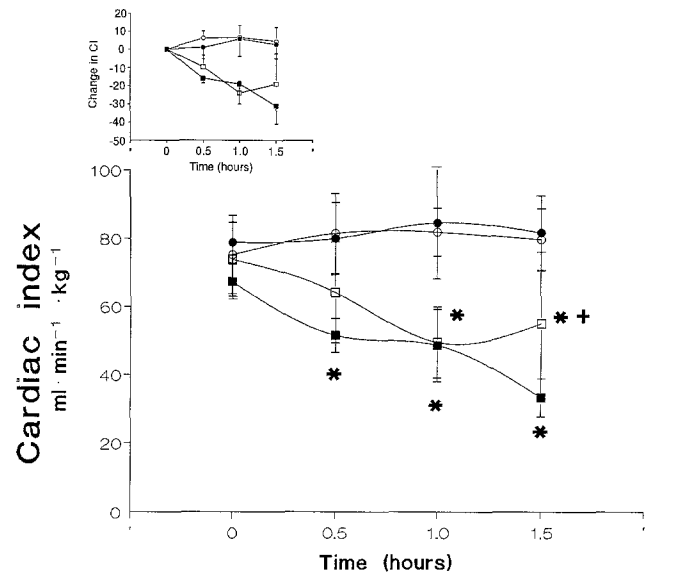

Fig. 3. Cardiac index (CI; ml·min⁻¹·kg⁻¹) before and 0.5, 1.0, and 1.5 h post-endotoxin/0.9% NaCl. Inset graph is change in CI. Groups represented are Control/Non-diabetic (open circles; $n = 5$), Control/Diabetic (closed circles; $n = 4$), Endotoxin shock/Non-diabetic (open squares; $n = 6$), and Endotoxin shock/Diabetic (closed squares; $n = 4$). Values = means ± SEM. * Significant difference from pre-endotoxin value; $p < 0.05$; + Significant difference from corresponding diabetic group; $p < 0.05$

due to loss of reliable cardiac output signals in these three animals after initiating LPS infusion. Coincidentally, these three animals demonstrated the lowest MAP values in their groups by 0.5 h after initiating LPS infusion. This suggested to us that the electromagnetic flow probe electrodes lost contact with the surface of the

aorta, owing to a reduction in aortic diameter with this rapid loss in MAP.

Two-way analysis of variance indicated main differences in heart rate between the non-LPS and LPS animals in non-diabetic and diabetic groups, and between the diabetic and non-diabetic groups that received LPS, but no specific time point differences were indicated. Values for stroke volume followed a pattern similar to CI (data not shown).

In diabetic dogs endotoxin shock was associated with a decrease in end-diastolic diameter (Table 1). This was not seen in the non-diabetic endotoxic group. By 1.5 h the end-diastolic diameter was significantly different between the diabetic and non-diabetic endotoxin groups. From those animals in which CI could be obtained, systemic vascular resistance index was calculated (Fig. 4). There were significant (main) differences between diabetic and non-diabetic groups prior to receiving LPS, the diabetic groups exhibiting higher systemic vascular resistance index values. Accordingly, the pre-LPS values in diabetics were different from the non-diabetic animals, but, after LPS infusion, the systemic vascular resistance index fell significantly in the diabetic group, until there was no longer any significant difference between them and the non-diabetic group receiving LPS. The fall in systemic vascular resis-

Table 1. Myocardial blood flow and metabolic parameters

		Time post-endotoxin (h)			
		0.0	0.5	1.0	1.5
End-diastolic diameter (mm)					
Non-diabetic	Control	30.8 ± 3.0	31.0 ± 2.9	31.1 ± 3.0	30.9 ± 3.1
	Endotoxin	33.8 ± 4.8	32.1 ± 4.9	31.6 ± 5.3	31.0 ± 5.2[b]
Diabetic	Control	26.7 ± 1.4	27.5 ± 1.7	27.2 ± 2.0	27.6 ± 1.6
	Endotoxin	24.7 ± 3.4	23.6 ± 3.4	23.0 ± 3.4	21.4 ± 3.6[a]
Circumflex arterial blood flow (ml · min^{-1} · 100 g^{-1})					
Non-diabetic	Control	98.8 ± 11.6	95.9 ± 13.1	105.7 ± 12.6	100.8 ± 14.2
	Endotoxin	89.8 ± 11.8	67.5 ± 14.8	48.5 ± 8.2[a]	58.1 ± 14.6[a]
Diabetic	Control	80.1 ± 14.5	81.8 ± 15.0	82.6 ± 15.5	83.8 ± 13.3
	Endotoxin	92.3 ± 25.2	52.0 ± 19.0[a]	33.9 ± 9.8[a]	40.0 ± 8.8[a]
Myocardial oxygen uptake (ml · min^{-1} · 100 g^{-1})					
Non-diabetic	Control	12.0 ± 1.6	12.1 ± 1.8	14.4 ± 2.6	12.9 ± 1.0
	Endotoxin	11.5 ± 1.6	8.6 ± 1.2	6.5 ± 0.8[a]	6.9 ± 0.9[a]
Diabetic	Control	14.3 ± 2.2	13.2 ± 2.1	14.7 ± 3.0	15.1 ± 2.3
	Endotoxin	13.0 ± 3.0	8.8 ± 3.2[a]	6.1 ± 1.9[a]	7.2 ± 1.6[a]
Myocardial oxygen delivery (ml · min^{-1} · 100 g^{-1})					
Non-diabetic	Control	18.8 ± 2.5	17.9 ± 2.6	20.9 ± 3.0	19.1 ± 2.5
	Endotoxin	19.3 ± 2.5	14.6 ± 2.6	11.4 ± 1.6[a]	13.0 ± 2.4[a]
Diabetic	Control	17.6 ± 2.9	16.4 ± 2.7	18.5 ± 3.8	19.4 ± 3.4
	Endotoxin	16.8 ± 3.4	10.8 ± 4.2	7.3 ± 2.3[a]	9.2 ± 2.3[a]
Myocardial glucose uptake (mg · min^{-1} · 100 g^{-1})					
Non-diabetic	Control	7.2 ± 0.9	8.3 ± 2.3	7.2 ± 1.9	6.8 ± 1.9
	Endotoxin	5.8 ± 1.1	4.8 ± 1.1	4.0 ± 1.3	2.6 ± 0.4
Diabetic	Control	3.6 ± 0.8	4.7 ± 1.8	3.4 ± 1.5	4.6 ± 2.5
	Endotoxin	2.2 ± 0.4	3.0 ± 2.3	3.9 ± 0.3	3.4 ± 1.4
Arterial blood glucose concentration (mmol · l^{-1})					
Non-diabetic	Control	3.7 ± 0.2[b]	3.7 ± 0.2[b]	3.7 ± 0.1[b]	3.6 ± 0.1[b]
	Endotoxin	3.4 ± 0.3[b]	3.5 ± 0.3[b]	3.8 ± 0.5[b]	3.0 ± 0.3[b]
Diabetic	Control	7.4 ± 0.8	7.9 ± 0.7	6.7 ± 0.7	6.4 ± 0.7
	Endotoxin	8.7 ± 1.4	8.5 ± 1.4	8.3 ± 0.4	7.7 ± 0.8
Arterial blood lactate concentration (mmol · l^{-1})					
Non-diabetic	Control	1.16 ± 0.25	1.16 ± 0.26	1.13 ± 0.25	1.10 ± 0.21
	Endotoxin	1.11 ± 0.12	1.45 ± 0.17	2.37 ± 0.29[a]	2.35 ± 0.29[a, b]
Diabetic	Control	1.34 ± 0.25	1.35 ± 0.24	1.39 ± 0.24	1.41 ± 0.25
	Endotoxin	1.00 ± 0.14	1.72 ± 0.23	3.36 ± 0.98[a]	4.63 ± 1.1[a]
Serum insulin (μU/ml)					
Non-diabetic	Control	10 ± 1.4	13 ± 3.0	10 ± 2.6	10 ± 2.4
	Endotoxin	9.7 ± 1.8	18 ± 6.8	43 ± 31	20 ± 12.3
Diabetic	Control	4.7 ± 1.3	4.6 ± 1.2	4.8 ± 1.3	4.1 ± 1.1
	Endotoxin	2.8 ± 0.3	5.1 ± 2.4	13 ± 8.5	47 ± 28

End-diastolic diameter, myocardial blood flows and metabolic parameters before and at 0.5, 1.0, and 1.5 h post-endotoxin. Groups represented are control/non-diabetic ($n = 5$), control/diabetic ($n = 4$), endotoxin shock/non-diabetic ($n = 7$), and endotoxin shock/diabetic dogs ($n = 6$). Values are means ± SEM

[a] Significant difference from pre-endotoxin value; $p < 0.05$
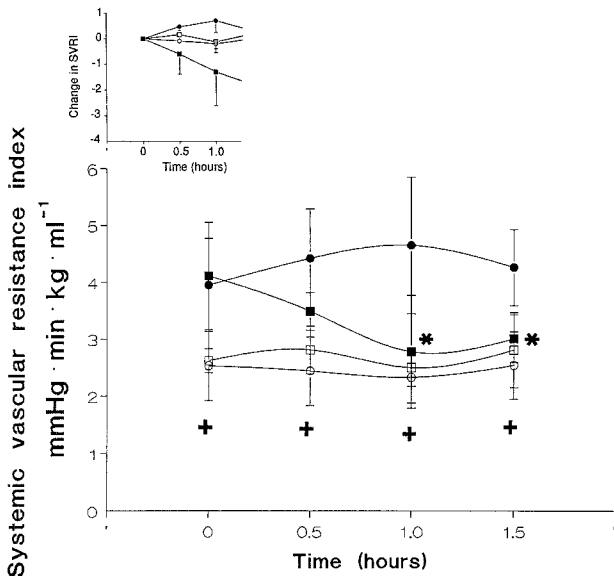[b] Significant difference from corresponding time in diabetic group; $p < 0.05$

Fig. 4. Systemic vascular resistance index (SVRI; $mmHg \cdot min \cdot kg \cdot ml^{-1}$) before and 0.5, 1.0, and 1.5 h post-endotoxin/0.9% NaCl. Inset graph is change in SVRI. Groups represented are Control/Non-diabetic (open circles; $n = 5$), Control/Diabetic (closed circles; $n = 4$), Endotoxin shock/Non-diabetic (open squares; $n = 6$), and Endotoxin shock/Diabetic (closed squares; $n = 4$). Values = means ± SEM. * Significant difference from pre-endotoxin value; $p < 0.05$; + Significant difference from corresponding diabetic group; $p < 0.05$

tance index and lower CI values in diabetic dogs would account for the greater depression in MAP in this group.

The maximum value of the first derivative of LV pressure with respect to time ($LVdP/dt_{max}$; Fig. 5) was significantly depressed at 1.0 h after beginning LPS infusion in non-diabetic dogs. In diabetic dogs that received LPS $LVdP/dt_{max}$ was reduced relative to its non-shock control group, and the non-diabetic shock group at 0.5, 1.0 and 1.5 h. The absolute values for maximum negative value of $LVdP/dt$ ($LVdP/dt_{min}$; Fig. 6), an index of LV relaxation rate, fell significantly by 1.0 and 1.5 h after starting LPS in non-diabetic dogs. In diabetic dogs that receive LPS the absolute values for dP/dt_{min} were reduced relative to its non-shock control group, and the non-diabetic shock group at 0.5, 1.0 and 1.5 h.

Circumflex artery blood flows of LPS groups fell to levels significantly different from their respective control groups (Table 1). Coronary blood flow in the diabetic LPS group was significantly depressed at an earlier time, first showing a depression at 0.5 h. There were no significant differences in circumflex arterial vascular resistances.

Table 2 lists the changes from baseline in E_{es} and the X^2 coefficient for the end-systolic pressure-dimension relationship in the various groups. There was a significant increase in E_{es} in the diabetic group that received LPS, as compared to its non-shock control. However, the change in X^2 coefficients in both shock groups were significantly positive relative to their respective non-shock groups, indicating reduced inotropic state. In addition, the diabetic group that received LPS demonstrated significantly greater increases in values of the X^2 coefficients than the non-diabetic/LPS group. The effect of this type of change

in the X^2 coefficient of the end-systolic pressure-dimension relationship is evident in Figure 7, taken from a representative diabetic animal.

B. Metabolic parameters. Myocardial oxygen uptake (MVO_2; Table 1) was significantly depressed after LPS administration. Supply of oxygen (Table 1) also fell in both LPS groups. Significant (main) differences in myocardial supply to demand ratio (SDR; Fig. 8) for oxygen existed between all diabetic and non-diabetic dogs. Specific differences between diabetic and non-diabetic dogs in endotoxic shock were at 0.5 and 1.0 h.

As mentioned before, diabetic dogs had elevated blood glucose concentrations. LPS administration had no significant effect of blood glucose concentrations by 1.5 h (Table 1). Blood lactate concentrations rose after LPS infusion, significantly more in the diabetic dogs (Table 1). No significant differences in glucose uptakes were seen (Table 1). LPS caused no statistically significant changes in RIA-measured insulin concentrations (Table 1), but it should be noted that in some diabetic dogs that received LPS, the RIA used to measure insulin indicated periods of detectable concentrations after beginning LPS.

Discussion

Twenty-four hours after injection of streptozotocin and alloxan dogs displayed characteristics associated with diabetes: serum glucose was greater than 11 mmol/l, non-esterified fatty acids were elevated, and, in all but one animal, serum insulin was not detected (the exception being one animal that occasionally demonstrated serum insulin concentrations greater than 2.5 but less than 5 μU/ml).
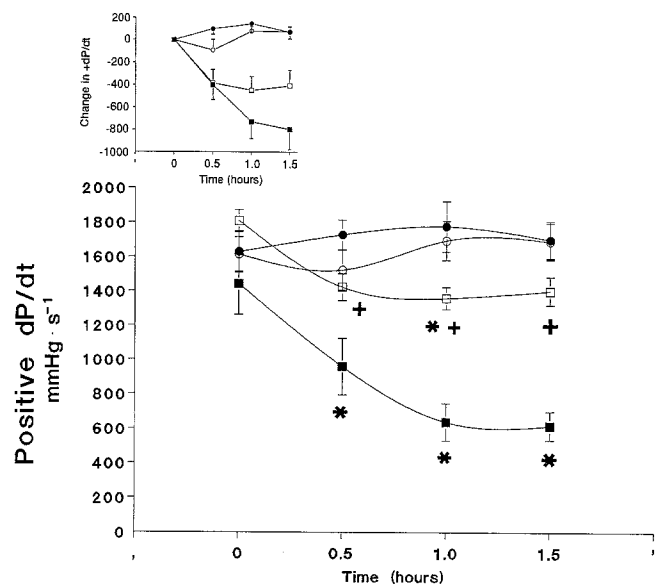

Fig. 5. Maximum positive value of the first derivative of left ventricular pressure with respect to time ($LVdP/dt_{max}$) before and 0.5, 1.0, and 1.5 h post-endotoxin/0.9% NaCl. Inset graph is change in $LVdP/dt_{max}$. Groups represented are Control/Non-diabetic (open circles; $n = 5$), Control/Diabetic (closed circles; $n = 4$), Endotoxin shock/Non-diabetic (open squares; $n = 7$), and Endotoxin shock/Diabetic (closed squares; $n = 6$). Values = means ± SEM. * Significant difference from pre-endotoxin value; $p < 0.05$; + Significant difference from corresponding diabetic group; $p < 0.05$

Table 2. Changes from baseline in E_{es} and X^2 coefficient values for the end-systolic pressure-dimension relationship

		Time post-endotoxin (h)		
		0.5	1.0	1.5
E_{es} (mm Hg/mm)				
Non-diabetic	Control	-0.075 ± 1.2	-1.10 ± 1.9	2.43 ± 1.7
	Endotoxin	2.38 ± 0.97	2.56 ± 1.4	2.06 ± 3.3
Diabetic	Control	0.025 ± 1.0	-1.73 ± 2.2	-3.25 ± 2.2
	Endotoxin	2.58 ± 2.6	9.9 ± 3.7	11.9 ± 9.3[a]
X^2 coefficient				
Non-diabetic	Control	-0.37 ± 0.75	-0.59 ± 0.71	-0.79 ± 0.48
	Endotoxin[a, b]	1.37 ± 0.83	1.18 ± 0.97	0.72 ± 0.3
Diabetic	Control	-0.02 ± 0.39	-0.03 ± 1.45	-1.18 ± 0.79
	Endotoxin[a]	4.97 ± 2.97	3.81 ± 1.88	52.6 ± 49.0

Values are means $\pm$ SEM of the absolute change from baseline (pre-endotoxin) in E_{es} and the X^2 coefficient for the end-systolic pressure-dimension relationship. Groups represented are control/non-diabetic ($n = 5$), control/diabetic ($n = 4$), endotoxin shock/ non-diabetic ($n = 7$), and endotoxin shock/diabetic dogs ($n = 6$). Statistical analysis was performed using the Kruskall Wallis test.
[a] Significant difference over time within group; $p < 0.05$
[b] Significant difference from corresponding diabetic group; $p < 0.05$

During the 30 days of diabetes prior to beginning the experimental protocol the dogs were generally demonstrating hyperphagia, polyuria (qualitative observations), and weight loss, while serum glucose concentrations remained elevated and serum insulin concentrations, with the noted exception, remained below detection. Non-esterified fatty acid concentrations fell to levels not different from pre-diabetic levels by 30 days. We believe this can be attributed, in part, to the loss of adipose weight during this period of time. The weight loss experienced by these dogs did not appear to affect heart weights, since there were no significant differences in heart weights between the diabetic and non-diabetic groups. Haematocrit values in diabetic dogs were not significantly different from non-

Fig. 6. Maximum negative value of the first derivative of left ventricular pressure with respect to time ($LVdP/dt_{min}$) before and 0.5, 1.0, and 1.5 h post-endotoxin/0.9% NaCl. Inset graph is change in $LVdP/dt_{min}$. Groups represented are Control/Non-diabetic (open circles; $n = 5$), Control/Diabetic (closed circles; $n = 4$), Endotoxin shock/Non-diabetic (open squares; $n = 7$), and Endotoxin shock/ Diabetic (closed squares; $n = 6$). Values = means $\pm$ SEM. * Significant difference from pre-endotoxin value; $p < 0.05$; + Significant difference from corresponding diabetic group; $p < 0.05$

diabetic dogs, suggesting that inadequate hydration was not a problem. Similar serum β-hydroxybutyrate concentrations among diabetic and non-diabetic dogs indicated absence of ketoacidosis. In toto, these data indicate a stable diabetic group, with hyperglycaemia, insulin below detectable levels, and no complications associated with ketoacidosis or low circulating blood volumes.

Slow endotoxin infusion resulted in cardiovascular depression in all animals. This depression was more pronounced in diabetic dogs, as evidenced by the lower blood pressures and cardiac outputs. In five out of six diabetic dogs mean arterial blood pressure fell below 50 mm Hg by 1.5 h after initiating infusion. This did not occur in the non-diabetic dogs. Greater elevations in blood lactate in the diabetic group may also suggest more severe perfusion abnormalities in this group.

In assessing the effects of endotoxin in diabetic animals, we focussed attention on parameters that would allow us to determine the components of the cardiovascular system that contributed to the greater degree of cardiovascular depression observed in this group. In diabetic dogs endotoxin shock was associated with a decrease in end-diastolic diameter which is probably attributable to decreased venous return. Because systemic vascular resistance index values indicated that diabetic animals were less able to maintain peripheral vascular tone during endotoxin shock, blood pooling may be responsible for decreased venous return. However, the contribution of extravasation was not assessed, and may also be a factor in these differences between diabetic and non-diabetic dogs.

The larger decrease in cardiac index in diabetic dogs can be attributed, in part, to decreased venous return, but evidence also argues for contribution by myocardial contractile depression. Changes in positive and negative LVdP/dt indicated that both systolic and diastolic cardiac performance were depressed to a greater degree in the diabetic dogs that received endotoxin. In the past, these indices have been used to evaluate changes in inotropic state, but in vivo these are misleading. These indices are very load sensitive, and loading conditions were obviously altered during endotoxin shock. We used the end-systolic pressure-dimension relationship to determine changes in
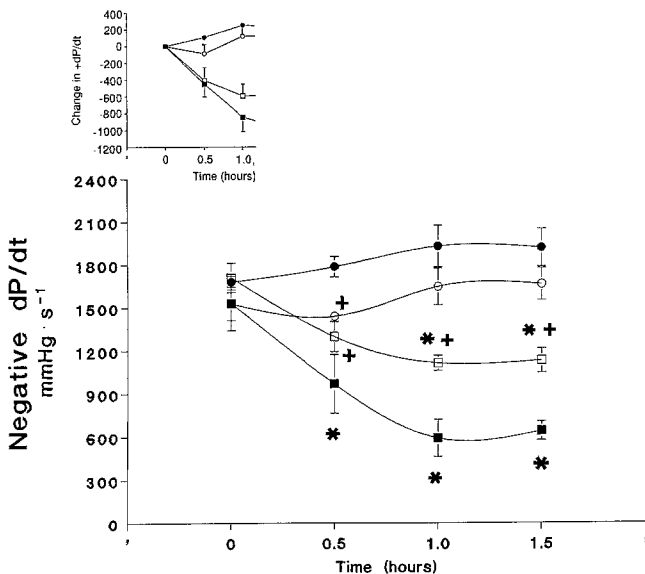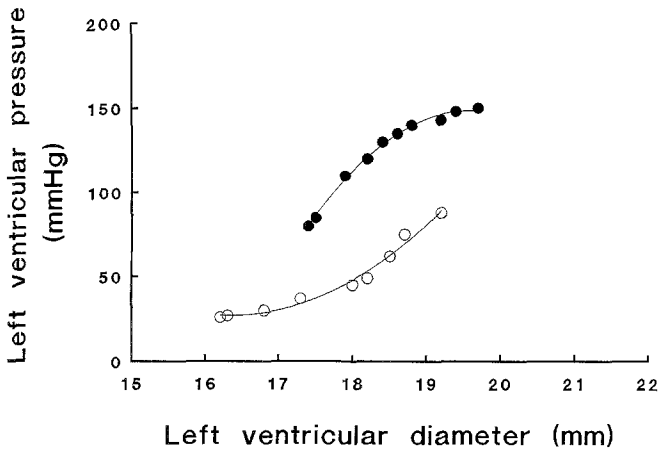
Fig. 7. End-systolic points from a representative diabetic dog are plotted, and the line fitted to the second order polynomial for these points, are illustrated. Closed circles are points generated before endotoxin administration. Open circles were obtained 1.0 h after endotoxin administration. The change in curvature between 0 and 1.0 h (change from concave to the x axis to convex, the X^2 coefficient from negative to positive) is representative of a decrease in cardiac contractility (inotropic state)
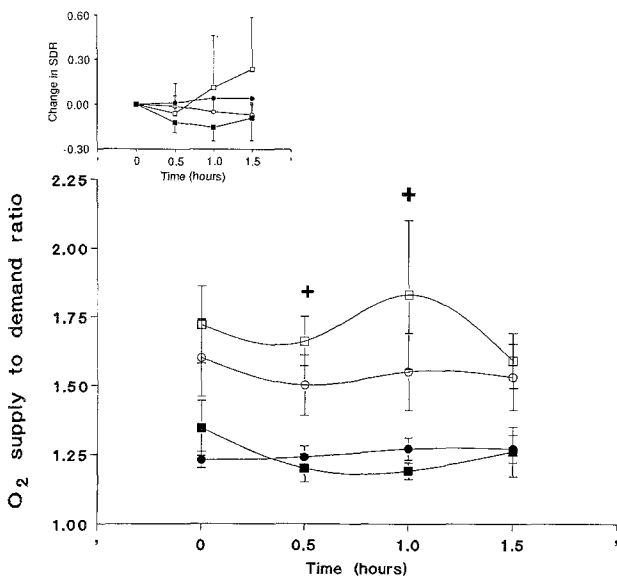

Fig. 8. Myocardial oxygen supply to demand ratio before and 0.5, 1.0, and 1.5 h post-endotoxin/0.9% NaCl. Inset graph is change in supply to demand ratio. Groups represented are Control/Non-diabetic (open circles; $n = 5$), Control/Diabetic (closed circles; $n = 4$), Endotoxin shock/Non-diabetic (open squares; $n = 7$), and Endotoxin shock/Diabetic (closed squares; $n = 6$). Values = means ± SEM. * Significant difference from pre-endotoxin value; $p < 0.05$; + Significant difference from corresponding diabetic group; $p < 0.05$

myocardial contractility. This index has been suggested for measurements of myocardial contractility in vivo and is suggested to be independent of changes in pre-load [15,16] and after-load [16, 17]. Complete analysis of the end-systolic pressure-dimension relationship (including second order polynomial fit) indicated depression in inotropic state by 1.5 h in all dogs that received endotoxin. Although we have reported increased contractility during endotoxin shock in previous studies [12,13], the model used was quite different. The current model employs slow endotoxin infu-

sion, which does not produce the initial sudden, profound hypotensive episode so characteristic of bolus endotoxin administration [12,13,20]. The results of the present study agree with work of others in which slow infusion was used [21] or wherein endotoxin infusion was guarded to prevent initial, severe hypotension (< 50 mm Hg) [22].

The inotropic depression was more severe in the diabetic group, as indicated by the significant increase in the value of the X^2 coefficient for the end-systolic pressure-dimension relationship as fit to a quadratic equation. The diabetic endotoxin group did demonstrate a significant increase in E_{es}, the slope of the end-systolic pressure-dimension relationship, but the X^2 coefficient of these points when examined for fit to a second order polynomial became more positive, as it did in the non-diabetic endotoxin group. Although an increase in E_{es} has been interpreted exclusively as an increase in inotropic state, Burkhoff et al. warned that measurements of the end-systolic pressure-dimension relationship in vivo must include curvilinear analysis to avoid misinterpretation. Our data indicated that after LPS infusion the E_{es} values in diabetic dogs reflected the slope of only the steep portion of a second order polynomial with positive X^2 coefficient. This can occur with severe myocardial inotropic depression [12,13,18].

There are several possible causes of this depression of myocardial inotropic state. Ischaemia is one such case. Evidence indicates that myocardial ischaemia is not a problem in human sepsis [23] or animal models of septic shock [24, 25], but these conditions do not typically press the limits of coronary autoregulation. We found significantly lower values for the oxygen supply to demand ratio in diabetic compared to non-diabetic animals. The decrease in mean arterial blood pressure below 50 mm Hg in the majority of diabetic dogs creates a dangerous condition in which the autoregulatory capabilities of the coronary circulation can be compromised. Indeed, the significant differences we found in the supply to demand ratio for oxygen between diabetic and non-diabetic dogs in endotoxin shock suggests that myocardial ischaemia may have been a complication in the diabetic dogs. Conclusive evidence may be found in future studies that examine transmural blood flow or metabolic changes in diabetic endotoxic animals.

Adrenergic influences have been shown to be vitally important to vascular and myocardial compensatory responses during endotoxin shock [13, 26]. Halmagyi et al. [26] demonstrated that adrenergic blockade unmasked the need for increased adrenergic vascular tone during endotoxin shock in dogs. Law et al. [13] demonstrated the importance of increased beta-adrenergic tone in the heart in endotoxin shock. Any impairment of these responses would likely be deleterious. The cardiovascular systems of diabetic humans and animals have been shown to demonstrate reduced responsiveness or sensitivity to adrenergic stimuli [27–29]. This may well have contributed to the observed decrease in vascular tone and myocardial depression in diabetic dogs that received endotoxin. Experiments have been designed to determine the validity of this hypothesis in the future.

Toxicity from alloxan or streptozotocin did not appear to be a problem. None of the diabetic dogs used in this study demonstrated any signs of illness, outside of those associ-

ated with diabetes. Indeed, this method of diabetes induction, using lower doses of both alloxan and streptozotocin, was chosen to reduce the toxic effects of either substance. It is also unlikely that 30 days after diabetes induction acute toxic effects of these substances would be manifested.

Although there were no significant changes in insulin concentrations, the RIA for insulin suggested measurable quantities in some of the diabetic dogs after LPS challenge. On this basis one might suspect the efficacy of the diabetes induction methods. However, diabetic animals challenged with glucose did not respond with elevations in serum insulin, as did the non-diabetic dogs. We must, therefore, consider the possibility that endotoxin shock leads to the evolution of non-insulin substances in the dog that are cross-reactive or interfere with the RIA used.

Endotoxin administration to diabetic dogs resulted in more severe cardiovascular dysfunction than in non-diabetic animals. The cardiovascular collapse can be attributed to both cardiac and vascular functional deficits. As suggested by evidence in the literature, we believe that changes in adrenergic sensitivity associated with diabetes mellitus may be responsible for some of these changes. These potential mechanistic considerations warrant further exploration.

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