Paradoxical insulin-induced increase in gluconeogenesis in response to prolonged hypoglycemia in conscious dogs

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Davis, S. N., R. Dobbins, C. Tarumi, J. Jacobs, D. Neal, and A. D. Cherrington. Paradoxical insulin-induced increase in gluconeogenesis in response to prolonged hypoglycemia in conscious dogs. *Am. J. Physiol.* 268 (*Endocrinol. Metab.* 31): E521–E530, 1995.—The aim of this study was to determine the effects of differing insulin concentrations on the gluconeogenic response to equivalent prolonged hypoglycemia. Insulin was infused intraportally, for 3 h, into normal 18-h fasted conscious dogs at 2 (lower, $n = 6$) or 8 $mU \cdot kg^{-1} \cdot min^{-1}$ (high, $n = 7$) on separate occasions. This resulted in steady-state arterial insulin levels of 80 ± 8 and 610 ± 55 μU/ml, respectively. Glucose was infused during high dose to maintain the hypoglycemic plateau (50 ± 1 mg/dl) equivalent to lower. Epinephrine (806 ± 180 vs. 2,589 ± 260 pg/ml), norepinephrine (303 ± 55 vs. 535 ± 60 pg/ml), cortisol (5.8 ± 1.2 vs. 12.1 ± 1.5 μg/dl), and pancreatic polypeptide (598 ± 250 vs. 1,198 ± 150 pg/ml) were all increased ($P < 0.05$) in the presence of high-dose insulin. Net hepatic glucose production increased significantly from 2.2 ± 0.3 to 3.8 ± 0.5 $mg \cdot kg^{-1} \cdot min^{-1}$ ($P < 0.05$) during high-dose infusion but remained at basal levels (2.3 ± 0.4 $mg \cdot kg^{-1} \cdot min^{-1}$) during lower-dose insulin. During the 3rd h of hypoglycemia, gluconeogenesis accounted for between 42 and 100% of glucose production during high-dose infusion but only 22–52% during lower-dose insulin. Intrahepatic gluconeogenic efficiency, however, increased similarly during both protocols. Lipolysis, as indicated by arterial blood glycerol levels, increased by a greater amount during high- compared with lower-dose insulin infusion. Six hyperinsulinemic euglycemic control experiments (2 or 8 $mU \cdot kg^{-1} \cdot min^{-1}$, n = 3 in each) provided baseline data. Gluconeogenesis remained similar to basal levels, but lipolysis was significantly suppressed during both series of hyperinsulinemic euglycemic studies. In summary, these data suggest that *1*) the important counterregulatory processes of gluconeogenesis and lipolysis can be significantly increased during prolonged hypoglycemia despite an eightfold increase in circulating insulin levels and *2*) the amplified gluconeogenic rate present during the hypoglycemic high-dose insulin infusions was caused by enhanced substrate delivery to the liver rather than an increase in intrahepatic gluconeogenic efficiency.

catecholamines; lipolysis; counterregulation

EFFECTIVE COUNTERREGULATION against prolonged hypoglycemia involves limitation of peripheral glucose utilization and sustained hepatic glucose production (HGP) (12). The continued production of glucose, during hypoglycemia, is essential for survival due to the central nervous system's dependence on glucose as a fuel. The source of the glucose released by the liver during prolonged hypoglycemia has recently been clarified (3, 19, 25). During the initial 1–2 h, hepatic glycogenolysis provides most (67–88%) of the glucose released, but by the 3rd h gluconeogenesis provides 48–88% of HGP (19).

Recently we have demonstrated that insulin per se can amplify the counterregulatory response to hypoglycemia in both normal humans and conscious dogs (10, 11). During high-dose insulin infusion studies, HGP increased significantly compared to baseline values (10, 11). This "paradoxical" increase in HGP is interesting, bearing in mind that hepatic insulinemia was approximately eightfold greater in the high- compared with lower-dose insulin infusion. The increased insulin levels present during high-dose infusions would be expected to reduce the activation of phospho*enol*pyruvate carboxykinase (PEPCK) and therefore limit gluconeogenesis (16). The fact that HGP increased by a greater amount during the high-dose insulin infusions suggests that the amplified counterregulatory response (sympathetic nervous system and cortisol) was probably able to overcome the effects of insulin at the liver. However, the source of glucose release during our earlier study (i.e., glycogenolysis or gluconeogenesis) was not reported. This becomes relevant, since direct sympathetic nervous system innervation of the liver results in glycogenolysis (33) and direct parasympathetic hepatic innervation causes an inhibition of gluconeogenesis (33). Thus, because our previous high-dose insulin infusion studies (10) demonstrated an amplified sympathetic and parasympathetic nervous system response together with an increase of HGP, it may be predicted that the elevated HGP resulted from glycogenolysis rather than gluconeogenesis. This would be compatible with in vitro data demonstrating that insulin inhibits gluconeogenesis (16) but would be inconsistent with in vivo reports of the source of glucose produced during hypoglycemia (3, 19, 25). Therefore the aim of this study was to determine whether the relative contribution of gluconeogenesis to hepatic glucose production is modified by differing insulin levels during prolonged equivalent hypoglycemia in overnight-fasted conscious dogs.

MATERIALS AND METHODS

Animals and surgical procedures. Experiments were carried out on 19 mongrel dogs (weight 20.1–24.7 kg, mean 22.1 ± 0.8 kg) of either sex fed a meat and laboratory food diet (31% protein, 52% carbohydrate, 11% fat, 6% fiber; Kal Kan Meat and Wayne Dog Chow) once daily for 3–4 wk before the experiments. The glucose turnover and hormone data from these experiments have been previously published (10). The dogs were housed in a surgical facility that met American Association for the Accreditation of Laboratory Animal Care guidelines, and the protocols were approved by the University Animal Care Facility. At least 2 wk before an experiment, catheters were inserted into a hepatic vein, the portal vein, and a femoral artery under general anesthesia as described elsewhere (5). Silastic catheters were also placed in a splenic and a jejunal vein. In eight of the dogs Doppler flow probes (Instru-

mentation Development Laboratories, Baylor College of Medicine, Houston, TX) were placed around the portal vein and the hepatic artery. The catheters were filled with saline containing heparin (200,000 U/l; Abbott, North Chicago, IL); their free ends were knotted and with the free ends of the Doppler leads were placed in subcutaneous pockets so that complete closure of the incisions was possible. On the experimental day, after an 18-h overnight fast, the catheters and Doppler leads were exteriorized from their subcutaneous pockets (under local anesthesia, 2% lidocaine; Astra, Worcester, MA). The contents of each catheter were aspirated, and catheters were flushed with saline. Blood was sampled from the arterial, portal vein, and hepatic vein catheters, and insulin was given through the jejunal and splenic vein catheters. An Angiocath (18 gauge, Abbott) was inserted percutaneously into a cephalic vein for infusion of indocyanine green and radioactive tracers.

On the day before the experiment, blood was drawn to determine the leukocyte count and the hematocrit of the animal. Only dogs that had *1*) a leukocyte count $<18{,}000/mm^3$, *2*) a hematocrit $>35\%$, *3*) a good appetite (consuming all their daily ration), and *4*) normal stools were used in an experiment.

Experimental design. Each experiment consisted of a tracer-equilibration period (−120 to −40 min), a control period (−40 to 0 min), and an experimental period (0–180 min). A priming dose of [3-^{3}H]glucose (50 μCi) was given at *time −120 min,* followed by a constant infusion of [3-^{3}H]glucose (0.67 μCi/min), [U-^{14}C]alanine (0.67 μCi/min), and indocyanine green ($0.1\ mg \cdot m^{-2} \cdot min^{-1}$). In the first protocol insulin was infused intraportally, into separate dogs, at a rate of either 2 ($n = 6$) or 8 $mU \cdot kg^{-1} \cdot min^{-1}$ ($n = 7$). The rate of fall of glucose and hypoglycemic nadir were matched in the two groups by a modification of the glucose-clamp technique (14). A second protocol was used to characterize the hormonal and glucose kinetic responses that occurred when insulin was given at the same rates but glucose was infused to maintain euglycemia (6 dogs were studied separately, $n = 3$ with each insulin infusion rate). In this manner, the effects of differing hyperinsulinemia alone (without accompanying hypoglycemia) could be estimated. Arterial blood samples were taken every 10 min throughout the control period and every 15 min during the experimental period. Portal and hepatic vein blood samples were taken at 20-min intervals throughout the control period and at 30-min intervals during the experimental period.

Collection and processing of samples. The collection and processing of blood samples have been described elsewhere, as have the methods of column chromatography used for the determinations of plasma alanine levels and [^{14}C]alanine and [^{14}C]lactate specific activities (6).

Plasma glucose concentrations were measured in quadruplicate by using the glucose oxidase method in a Beckman Glucose Analyzer II (Fullerton, CA). Whole blood lactate, alanine, glycerol, and 3-hydroxybutyrate concentrations were determined in samples deproteinized with 4% (wt/vol) perchloric acid (PCA; 1 ml blood + 3 ml PCA) with the method developed by Lloyd et al. (27) for the Technicon AutoAnalyzer. Blood acetoacetate levels were determined from the above supernatant with a spectrophotometric assay (30). Plasma nonesterified fatty acid (NEFA) concentrations were determined according to the method of Ho (22). Immunoreactive glucagon was measured according to the method of Aguilar-Parada et al. (1) with an interassay coefficient of variation (CV) of 15%. Immunoreactive insulin was measured as described previously (37) with an interassay CV of 11%. Catecholamines were determined by high-performance liquid chromatography (16) with an interassay CV of 17% for epinephrine and 14% for norepinephrine. Two modifications in the procedure for catecholamine determination were made: *1*) a five rather than one point standard calibration curve was used and *2*) aliquots of the initial and final samples of plasma with known amounts of epinephrine and norepinephrine were taken so that accurate identification of the respective catecholamine peaks could be made. Cortisol was assayed by using the Clinical Assays Gamma Coat radioimmunoassay kit with an interassay CV of 6%. Pancreatic polypeptide was measured by using the method of Hagopian et al. (20) with an interassay CV of 8%.

Materials. [3-^{3}H]glucose (New England Nuclear, Boston, MA) was used as the glucose tracer (11.5 mCi/mmol), and [U-^{14}C]alanine (ICN, Irvine, CA) was used as the labeled gluconeogenic precursor (150 mCi/mmol). Insulin was purchased from Eli Lilly (Indianapolis, IN). The insulin infusion solution was prepared with normal saline and contained 3% (vol/vol) of the dog's own plasma. Glucagon ^{125}I tracer was obtained from NOVO (Bagsvaerd, Denmark), and glucagon for the standard curves was purchased from Sigma (St. Louis, MO). D-50W (Abbott) was used for infusion into a peripheral vein when necessary. Indocyanine green was purchased from Hynson, Westcott & Dunning (Baltimore, MD).

Tracer methods and calculations. The net hepatic balance of each substrate (blood lactate, alanine, glycerol, acetoacetate, 3-hydroxybutyrate, plasma glucose, and NEFA) was calculated with the formula [HV − (0.2A + 0.8P)]Q, where A, P, and HV are the femoral artery, portal vein, and hepatic vein concentrations, and Q is the flow (blood or plasma as required) to the liver as determined by indocyanine green. The proportion of the hepatic blood supply provided by the hepatic artery was assumed to be 20% based on mean data obtained from Doppler flow probes placed on the hepatic artery and portal vein ($n = 8$ dogs). The increase in hepatic blood flow during hypoglycemia in the present experiments was due to proportional changes in both portal vein (+24 ± 5%) and hepatic artery flows (+28 ± 6%), consistent with previous findings (24).

The rates of tracer determined glucose appearance (R_a) and utilization (R_d) were calculated according to the methods of Wall et al. (36). Total R_a for glucose is comprised of both endogenous glucose production and the exogenous glucose infused to maintain euglycemia or a given level of hypoglycemia during the glucose clamp. By subtraction of the total amount of exogenous glucose infused from total R_a, hepatic glucose production can be derived. It is now recognized, however, that this model is not fully quantitative, since underestimates of total R_a and R_d can be obtained. The use of a highly purified tracer and measurements made under steady-state conditions minimizes most of these problems. The hepatic [^{14}C]glucose production rate, which is a measure of the overall gluconeogenic rate, was determined using the tracer technique as described elsewhere (8).

The efficiency of the hepatic conversion of alanine to glucose, which reflects the intrahepatic gluconeogenic process, was calculated by dividing the [^{14}C]glucose production by the rate of net [^{14}C]alanine and [^{14}C]lactate uptake by the liver. The measurements of gluconeogenic efficiency using [^{14}C]alanine and [^{14}C]lactate are minimum estimates, because of isotope-dilution within the hepatic oxaloacetate pool and diversion of gluconeogenic precursors to glycogen rather than glucose (19). Calculation of the absolute rate of gluconeogenesis from alanine would require the determination of a correction factor described by Hetenyi (21) or the use of several tracers and specific activity determination of the glucose skeleton as discussed by Katz (23).

The overall gluconeogenic rate ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) can be estimated in two ways. One can assume that all of the

Table 1. *Effect of intraportal insulin infusion on arterial plasma levels of insulin and plasma glucose in overnight-fasted conscious dogs*

Plasma Levels	Insulin Infusion Rate, mU·kg^{-1}·min^{-1}	Control Period, min	Duration of Expt 1 Period, min					
			30	60	90	120	150	180
			Hypoglycemia					
Insulin μU/ml	2	14 ± 4	73 ± 10*	85 ± 12	80 ± 10	82 ± 80	78 ± 8	81 ± 7
	8	14 ± 4	430 ± 32†	548 ± 56†	623 ± 53†	613 ± 46†	620 ± 61†	601 ± 47†
Glucose, mg/dl	2	107 ± 2	77 ± 5*	55 ± 4	52 ± 3	51 ± 2	50 ± 1	49 ± 1
	8	109 ± 2	75 ± 3*	56 ± 1	52 ± 3	52 ± 1	49 ± 1	49 ± 1
			Euglycemia					
Insulin, μU/ml	2	8 ± 2	58 ± 8*	65 ± 4	69 ± 4	74 ± 2	74 ± 15	80 ± 17
	8	15 ± 1	516 ± 72*†	662 ± 99†	640 ± 69†	668 ± 35†	633 ± 47†	678 ± 102†
Glucose, mg/dl	2	108 ± 4	110 ± 7	102 ± 8	94 ± 5	105 ± 3	106 ± 5	102 ± 6
	8	105 ± 5	107 ± 12	99 ± 6	109 ± 3	109 ± 4	105 ± 5	105 ± 5

Values are means ± SE; $n = 6$ for 2 mU·kg^{-1}·min and $n = 7$ for 8 mU·kg^{-1}·min^{-1}. Control period values are average of 2 measurements for insulin and 5 measurements for glucose made during basal period in each dog. Data previously published in Ref. 10. *Initial time point when values are significantly ($P < 0.05$) different from control period mean. †Values for 8 mU·kg^{-1}·min^{-1} are significantly increased ($P < 0.05$) compared with values for 2 mU·kg^{-1}·min^{-1}.

gluconeogenic precursors extracted by the liver are completely converted to glucose and thereby calculate a maximal estimate of this process. To do this in the present study, the net uptake of pyruvate was assumed to be 1/10 that of lactate and the net uptake of gluconeogenic amino acids other than alanine was assumed to be equivalent to that of alanine (19). Alternatively, the net hepatic uptake of all gluconeogenic precursors can be multiplied by the calculated gluconeogenic efficiency to give a minimal estimate of the process. The contribution of gluconeogenesis to overall glucose production can then be calculated by dividing either of the above by 2 to account for the incorporation of the C-3 precursors into the C-6 glucose molecule and then dividing this quotient by the net hepatic glucose balance (μmol·kg^{-1}·min^{-1}) and multiplying by 100. In this way quantitative brackets can be given to the gluconeogenic process.

Statistical analysis. Data are expressed as means ± SE unless otherwise stated and analyzed using standard parametric two-way analysis of variance with a repeated measures design. This was coupled with the paired Student's *t*-test to delineate at which time statistical significance was reached. A value of $P < 0.05$ indicated significant difference.

RESULTS

Insulin, glucose, and counterregulatory hormone levels during hypoglycemic and euglycemic studies. Insulin levels during the high- and lower-dose hypoglycemic and euglycemic studies are shown in Table 1. Plasma glucose fell at an identical rate (Table 1) during both hypoglycemic protocols but was maintained at 50 ± 1 mg/dl during high-dose insulin by an infusion of glucose (1.8 mg·kg^{-1}·min^{-1}). Plasma glucose was maintained constant at baseline levels during both series of euglycemic protocols (Table 1).

In response to hypoglycemia the plasma levels of epinephrine, norepinephrine, cortisol, glucagon, and pancreatic polypeptide increased compared with baseline. By the 3rd h of hypoglycemia, plasma epinephrine, norepinephrine, cortisol, and pancreatic polypeptide were significantly increased in the presence of high- compared with lower-dose insulin infusion, respectively (Table 2). With the exception of epinephrine (which did

Table 2. *Effects of intraportal insulin infusion and resulting hypoglycemia (50 mg/dl) on arterial hormone concentrations in overnight-fasted conscious dogs*

Hormone	Insulin Infusion Rate, mU·kg^{-1}·min^{-1}	Control Period	Duration of Hypoglycemia, min					
			30	60	90	120	150	180
Glucagon, pg/ml	2	68 ± 12	88 ± 18	107 ± 15	120 ± 33	97 ± 23	86 ± 32	76 ± 28
	8	75 ± 10	75 ± 10	93 ± 22	123 ± 27	105 ± 26	76 ± 13	56 ± 9
Epinephrine, pg/ml	2	81 ± 17	164 ± 31*	444 ± 138	781 ± 44	805 ± 165	769 ± 170	817 ± 178
	8	88 ± 14	121 ± 14*	852 ± 84†	2,803 ± 543†	2,871 ± 401†	2,731 ± 258†	2,279 ± 276
Norepinephrine, pg/ml	2	121 ± 10	164 ± 27	216 ± 33*	278 ± 35	284 ± 49	302 ± 57	323 ± 51
	8	111 ± 11	169 ± 25*	374 ± 38†	553 ± 86†	547 ± 88†	545 ± 60†	551 ± 66†
Cortisol, μg/dl	2	2.4 ± 0.6	3.1 ± 1.1	6.1 ± 1.3*	7.5 ± 1.8	7.8 ± 1.1	6.3 ± 1.2	3.9 ± 1.2
	8	2.6 ± 0.6	3.9 ± 0.6	7.7 ± 1.0*	9.6 ± 0.5†	11.8 ± 1.4†	12.8 ± 1.3†	11.1 ± 1.7
Pancreatic polypeptide, pg/ml	2	117 ± 15	232 ± 76	307 ± 88*	675 ± 235	565 ± 158	609 ± 251	611 ± 291
	8	245 ± 91	334 ± 178	1,200 ± 341*†	1,450 ± 148†	1,111 ± 132†	1,106 ± 152†	1,025 ± 189

Values are means ± SE. Control period values are average of 2 measurements made during basal period in each dog. Data previously published in Ref. 10. *Initial time point when values are significantly ($P < 0.05$) different from control period mean. †Values at 8 mU·kg^{-1}·min^{-1} are significantly increased ($P < 0.05$) compared with values at 3 mU·kg^{-1}·min^{-1}.

not change from baseline), insulin in the presence of euglycemia had effects on counterregulatory hormone concentrations. By the 3rd h plasma glucagon and pancreatic polypeptide levels were suppressed compared with control period values during both series of insulin infusions (Table 3). Plasma cortisol and norepinephrine levels, on the other hand, increased significantly compared with control period values during high- but not lower-dose insulin infusions (Table 3).

Glucose production, utilization, and hepatic blood flow during hypoglycemic and euglycemic studies. Tracer determined HGP increased significantly from baseline (2.6 ± 0.2 to 4.6 ± 0.4 mg·kg ^{1}·min ^{1}; $P < 0.01$) by the 3rd h of the high-dose hypoglycemic studies but remained at control period values during the lower-dose group (3.0 ± 0.3 to 3.0 ± 0.2 mg·kg^{-1}·min^{-1}). Net hepatic glucose balance measurements in both protocols confirmed the tracer data (Fig. 1). Hepatic blood flow was similar during the control period of both hypoglycemic protocols (32 ± 3 vs. 31 ± 5 ml·kg^{-1}·min^{-1}, respectively). Hepatic blood flow increased significantly ($P < 0.01$) to 46 ± 3 ml·kg^{-1}·min^{-1} during high-dose infusions but remained unchanged during lower-dose infusions (32 ± 5 ml·kg^{-1}·min^{-1}).

When euglycemia was maintained during insulin infusion, tracer determined HGP was suppressed similarly by the 3rd h (2.7 ± 0.2 to 0.7 ± 0.5 mg·kg^{-1}·min^{-1} high dose, 3.0 ± 0.4 to 0.6 ± 0.5 mg·kg^{-1}·min^{-1} low dose). Net hepatic glucose balance measurements confirmed suppression of glucose production but also revealed a small net uptake of glucose during both insulin infusion protocols (Fig. 1). Glucose utilization was significantly increased during high- compared to lower-dose insulin infusion (17.8 ± 3.1 vs. 11.5 ± 1.8 mg·kg^{-1}·min^{-1}). Hepatic blood flow was similar and stable throughout both euglycemic protocols (39 ± 10 ml·kg^{-1}·min^{-1}) and in the high- and lower-dose protocols (40 ± 6 ml·kg^{-1}·min^{-1}), respectively.

Table 3. *Effects of intraportal insulin infusion with euglycemia on arterial hormone concentrations in overnight-fasted conscious dogs*

Hormone	Insulin Infusion Rate, mU·kg^{-1}·min^{-1}	Control Period	Test Period
Glucagon, pg/ml	2	62 ± 8	42 ± 2†
	8	64 ± 8	45 ± 2†
Epinephrine, pg/ml	2	129 ± 10	108 ± 10
	8	160 ± 31	160 ± 112
Norepinephrine, pg/ml	2	171 ± 31	142 ± 41
	8	125 ± 34	278 ± 60*
Cortisol, pg/ml	2	1.5 ± 0.4	1.9 ± 0.3
	8	1.4 ± 0.4	4.9 ± 0.9*
Pancreatic polypeptide, pg/ml	2	239 ± 30	165 ± 9†
	8	404 ± 133	293 ± 46†

Values are means ± SE. Control period values are averages of 2 measurements made during the basal period. Test period values are averages of 3 measurements made during the 3rd h of insulin infusion. *Values are significantly increased ($P < 0.05$) compared with control period mean. †Values are significant reduced ($P < 0.05$) compared with control period mean.

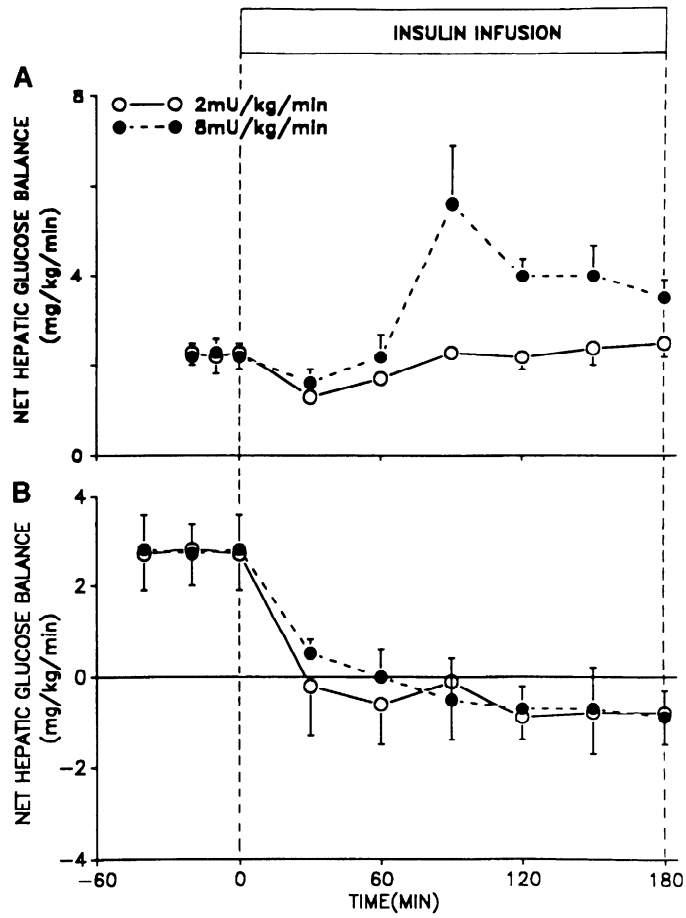

Fig. 1. Effects of intraportally infused insulin (2 and 8 mU·kg^{-1}·min^{-1}) on net hepatic glucose balance during hypoglycemia (*A*) and euglycemia (*B*) in conscious overnight-fasted dogs. During 8 mU·kg^{-1}·min^{-1} hypoglycemia studies, net hepatic glucose balance was initially significantly increased ($P < 0.05$) from control period at 90 min; 8 mU·kg^{-1}·min^{-1} values are significantly increased ($P < 0.05$) compared with 2 mU·kg^{-1}·min^{-1} values. During euglycemic studies, net hepatic glucose balance was initially significantly decreased ($P < 0.05$) from control period at 30 min in both insulin infusion doses.

Gluconeogenic precursor metabolism during hypoglycemic studies. Steady-state arterial blood lactate levels (Fig. 2) were increased by a greater extent ($P < 0.05$) during hypoglycemia in the high-dose insulin group (514 ± 127 to 1,975 ± 250 μM) than in the lower-dose group (799 ± 180 to 1,441 ± 450 μM, $P < 0.05$). The net hepatic production of lactate evident during the control period ceased during hypoglycemia in both groups. By the last hour of hypoglycemia net hepatic lactate uptake was significantly greater ($P < 0.05$) during high (17.2 ± 5.0 μmol·kg^{-1}·min^{-1})- compared with lower-dose insulin infusion (2.0 ± 2.5 μmol·kg^{-1}·min^{-1}).

Arterial blood glycerol levels (Fig. 3) increased by a significantly greater amount ($P < 0.05$) during high (95 ± 14 to 332 ± 58 μM)- than lower-dose insulin (55 ± 14 to 200 ± 30 μM). Net hepatic glycerol uptake was also significantly increased during high (2.0 ± 0.3 to 12.3 ± 2.0 μmol·kg^{-1}·min^{-1})- compared with lower-dose insulin infusion (1.3 ± 0.7 to 3.8 ± 0.6 μmol·kg^{-1}·min^{-1}, $P < 0.01$). Fractional extraction of glycerol remained similar to baseline (60 ± 17%) during both insulin infusion protocols.

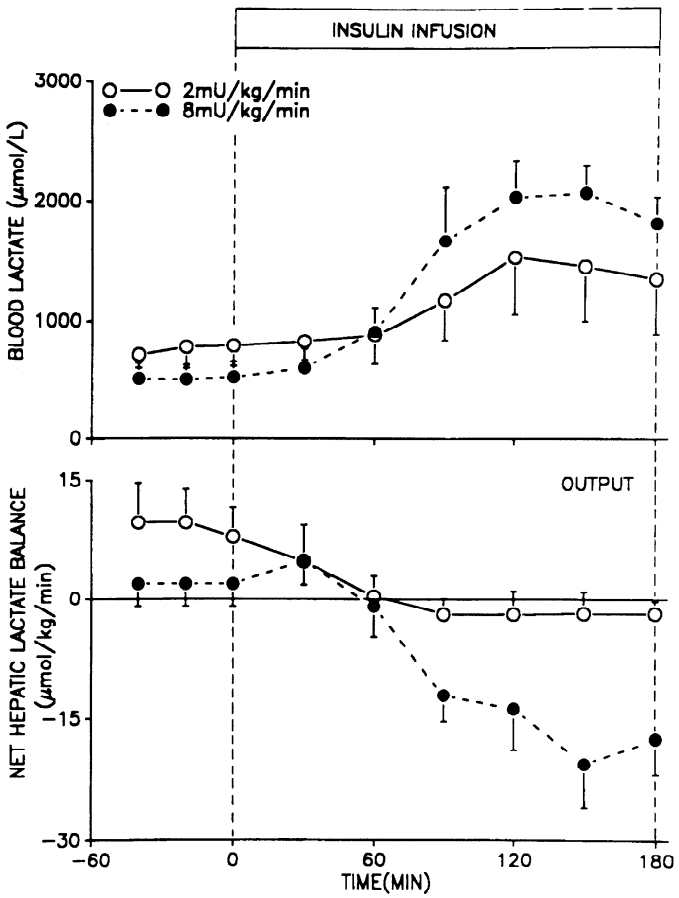

Fig. 2. Effects of intraportally infused insulin (2 and 8 mU·kg^{-1}·min^{-1}) during hypoglycemia on arterial blood lactate and net hepatic lactate balance. Blood lactate level was initially significantly increased ($P < 0.05$) from control period at 90 min during 8 mU·kg^{-1}·min^{-1} infusion but at 120 min for 2 mU·kg^{-1}·min^{-1} infusions. Rise in blood lactate levels are significantly increased ($P < 0.05$) during 8 compared with 2 mU·kg^{-1}·min^{-1} infusion. Net hepatic lactate output switched to uptake at 60 min during both insulin infusion doses. Net hepatic lactate uptake was significantly increased ($P < 0.05$) during 8 compared with 2 mU·kg^{-1}·min^{-1} insulin infusion.

Arterial blood alanine levels (Fig. 4) fell during high (299 ± 35 to 196 ± 16 μM)- and lower-dose insulin infusion (385 ± 25 to 215 ± 33 μM). Tracer determined alanine R_a remained constant during high-dose infusion but fell by a small nonsignificant amount during lower-dose insulin infusion (Table 4). Alanine clearance increased significantly by the 3rd h during high (23.1 ± 4.9 to 40.0 ± 6.6 ml·kg^{-1}·min^{-1}, $P < 0.01$) and lower-dose insulin infusion (25.7 ± 5.0 to 41.0 ± 6.6 ml·kg^{-1}·min^{-1}, $P < 0.01$).

Net hepatic uptake of alanine was significantly increased during high (2.4 ± 0.6 to 5.2 ± 0.7 μmol·kg^{-1}·min^{-1}, $P < 0.01$)- compared with lower-dose insulin infusion (2.8 ± 0.6 to 3.4 ± 0.8 μmol·kg^{-1}·min^{-1}). Fractional extraction of alanine, however, increased similarly during high-dose (23 ± 7 to 45 ± 5%) and lower-dose insulin infusions (20 ± 2 to 37 ± 3%).

Gluconeogenic precursor metabolism during euglycemic studies. Arterial blood lactate levels remained at basal levels during the high-dose insulin infusions (879 ± 248 to 929 ± 208 μM) but fell during the lower-dose insulin studies (793 ± 161 to 527 ± 140 μM). Unlike the hypoglycemic studies, there was net production of lactate throughout both insulin infusion protocols when euglycemia was maintained (Table 5).

Arterial blood alanine levels fell similarly during high (314 ± 76 to 180 ± 46 μM)- and lower-dose insulin infusions (306 ± 63 to 140 ± 20 μM). Net hepatic uptake of alanine remained at basal rates in both protocols. Fractional extraction of alanine increased similarly during high (31 ± 4 to 45 ± 4%)- and lower-dose insulin infusion (22 ± 3 to 41 ± 5%).

Arterial blood glycerol fell significantly during both high (144 ± 28 to 68 ± 14 μM, $P < 0.05$)- and lower-dose insulin infusions (79 ± 16 to 15 ± 6, $P < 0.05$). Net hepatic uptake of glycerol also fell significantly ($P < 0.05$) during both high (4.4 ± 1.5 to 2.5 ± 0.3 μmol·kg^{-1}·min^{-1})- and lower-dose insulin infusion (2.3 ± 0.7 to 0.3 ± 0.1 μmol·kg^{-1}·min^{-1}). Fractional extraction of glycerol remained constant at 67 ± 5% during both insulin infusion protocols.

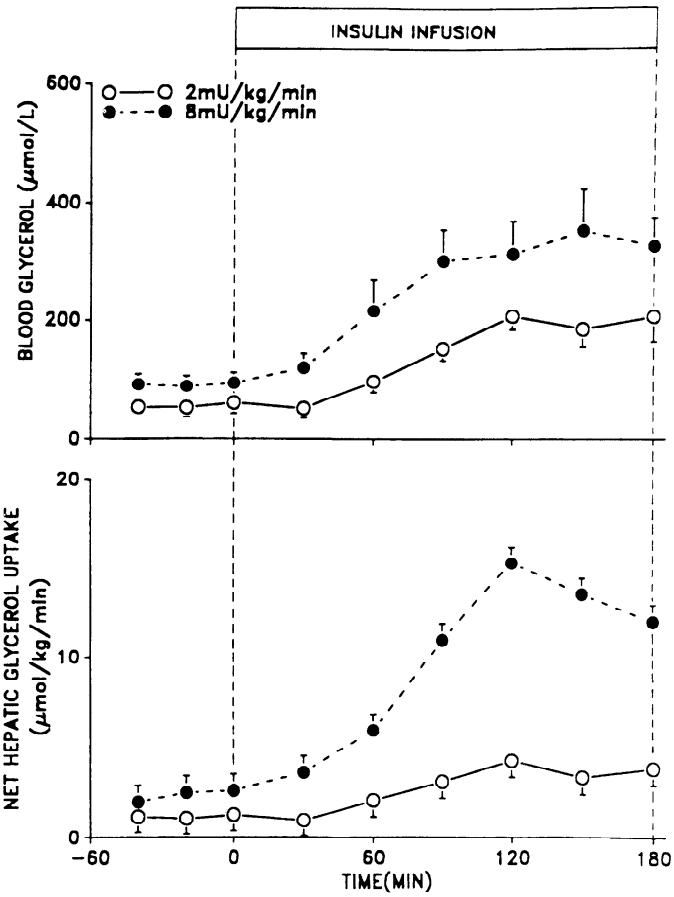

Fig. 3. Effects of intraportally infused insulin (2 and 8 mU·kg^{-1}·min^{-1}) during hypoglycemia on blood glycerol levels and net hepatic glycerol uptake. Blood glycerol levels are initially significantly increased ($P < 0.05$) from control period at 60 min during both infusion doses. Blood glycerol levels are significantly increased ($P < 0.05$) during 8 compared with 2 mU·kg^{-1}·min^{-1} insulin infusions. Net hepatic glycerol uptake was initially significantly increased ($P < 0.05$) from control period at 60 min during 8 mU·kg^{-1}·min^{-1} infusion but at 90 min during 2 mU·kg^{-1}·min^{-1} insulin infusion. Net hepatic glycerol uptake is significantly increased ($P < 0.05$) during 8 compared with 2 mU·kg^{-1}·min^{-1} infusion.

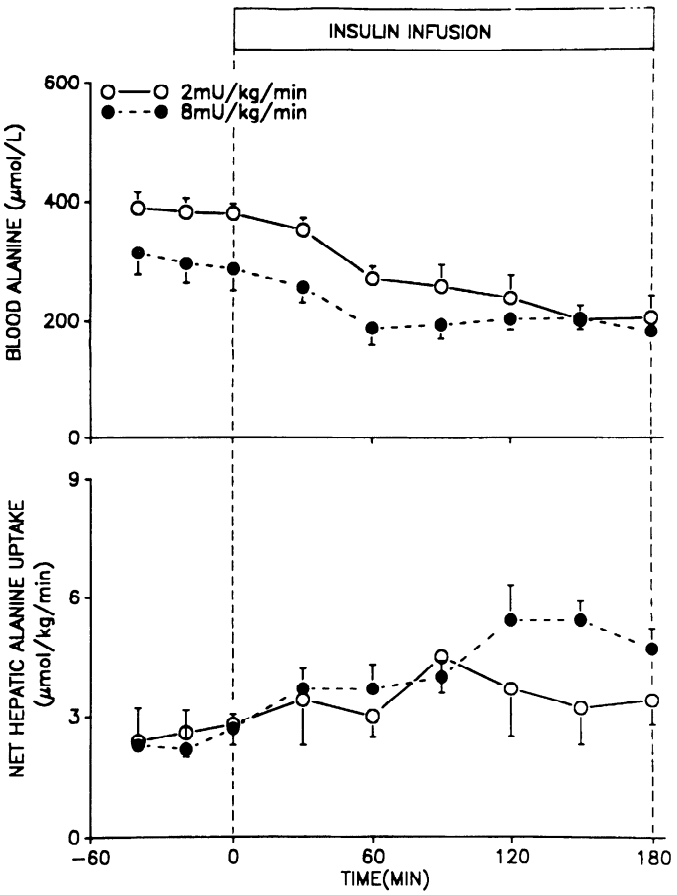

Fig. 4. Effects of intraportally infused insulin (2 and 8 mU·kg^{-1}·min^{-1}) during hypoglycemia on arterial blood alanine and net hepatic alanine uptake. Blood alanine levels were initially significantly reduced ($P < 0.05$) from control period at 60 min during both insulin infusion doses. Net hepatic alanine uptake was initially significantly ($P < 0.05$) increased during 8 mU·kg^{-1}·min^{-1} infusions at 60 min. Net hepatic uptake of alanine is significantly increased ($P < 0.05$) during 8 compared with 2 mU·kg^{-1}·min^{-1} insulin infusion.

Gluconeogenic parameters during hypoglycemic and euglycemic studies. The efficiency with which the liver converted alanine and lactate to glucose under hypoglycemic conditions (Fig. 5) increased similarly during high (22 ± 9 to 43 ± 9%)- and lower-dose insulin infusion (20 ± 11 to 43 ± 11%). During the last hour of the hypoglycemic high-dose insulin infusion, gluconeogenesis contributed minimal and maximal estimates of 1.6 and 3.8 mg·kg^{-1}·min^{-1} of total hepatic glucose production. During the last hour of the hypoglycemic lower-dose insulin infusion these estimates were 0.5 and 1.2 mg·kg^{-1}·min^{-1}, respectively.

Table 5. *Effects of intraportal insulin infusion with euglycemia on arterial blood levels and net hepatic balances of gluconeogenic precursors in overnight-fasted conscious dogs*

	Insulin Infusion Rate, mU·kg^{-1}·min^{-1}	Arterial Level, μM		Net Hepatic Uptake, μmol/kg/min	
		Control period	Test	Control period	Test
Lactate	2	793 ± 161	527 ± 140	−14.1 ± 4.2	−3.5 ± 1.2*
	8	879 ± 248	926 ± 208	−8.0 ± 4.0	−5.0 + 2.0
Alanine	2	306 ± 63	140 ± 20*	2.9 ± 0.6	2.6 ± 0.4
	8	314 ± 76	180 ± 46*	3.8 ± 1.2	3.2 ± 0.4
Glycerol	2	79 ± 16	15 ± 6*	2.3 ± 0.7	0.3 ± 0.1*
	8	144 ± 28	68 ± 14*	4.4 ± 1.5	2.5 ± 0.3*

Values are means ± SE. Control period values are averages of 3 measurements made during basal period. Test period values are averages of 3 measurements made during 3rd h of insulin infusion. Negative rates of net hepatic uptake indicate net hepatic production. *Values significantly reduced ($P < 0.05$) compared with control period values.

The efficiency with which the liver converted alanine and lactate under euglycemic conditions fell during high-dose insulin infusion (28 ± 19 to 15 ± 4%) but remained at basal levels during lower-dose insulin infusion (15 ± 8 to 13 ± 4%). During the last hour of the euglycemic high-dose insulin infusion, gluconeogenesis contributed minimal and maximal estimates of 0.1 and 0.7 mg·kg^{-1}·min^{-1} of total hepatic glucose production. During the last hour of the euglycemic lower-dose insulin infusion, these estimates were 0.1 and 0.5 mg·kg^{-1}·min^{-1}.

Ketone body metabolism and NEFA during hypoglycemia and euglycemia. The plasma arterial NEFA level increased to peak concentrations by 90 min of hypoglycemia during both high (660 ± 130 to 801 ± 130 μM)- and lower (770 ± 180 to 1053 ± 245 μM)-dose insulin infusions. However, during the final hour of hypoglycemia NEFA levels returned toward baseline in both groups. Net hepatic uptake of NEFA increased by similar modest amounts during high (3.7 ± 0.8 to 4.8 ± 1.1 μmol·kg^{-1}·min^{-1})- and lower-dose insulin infusion (3.7 ± 1.0 to 4.3 ± 2.2 μmol·kg^{-1}·min^{-1}).

Table 4. *Effects of intraportal insulin infusion with resulting hypoglycemia on tracer determined alanine appearance, disappearance, and clearance in overnight-fasted conscious dogs*

	Insulin Infusion Rate, mU·kg^{-1}·min^{-1}	Control Period	Duration of Hypoglycemia, min					
			30	60	90	120	150	180
Alanine R_a, μmol·kg^{-1}·min^{-1}	2	9.3 ± 1.5	8.9 ± 1.5	8.4 ± 1.0	7.6 ± 1.4	6.9 ± 1.0	7.0 ± 1.4	7.4 ± 1.1
	8	7.0 ± 1.4	6.3 ± 1.3	6.4 ± 1.4	6.2 ± 1.3	7.0 + 1.4	6.5 + 1.2	6.1 + 1.1
Alanine R_d, μmol·kg^{-1}·min^{-1}	2	10.4 ± 2.6	13.3 ± 1.5	11.5 ± 2.1	10.7 ± 2.1	9.2 ± 1.7	8.6 ± 0.8	7.5 ± 1.6
	8	7.5 ± 1.9	13.4 ± 4.9	13.4 ± 2.9	7.5 ± 2.0	6.6 ± 2.0	8.7 ± 1.9	7.7 ± 1.4
Alanine clearance, μmol·kg^{-1}·min^{-1}	2	25.7 ± 5.0	40.8 ± 6.6*	49.4 ± 15.4*	50.9 ± 13.2*	50.6 ± 17.1*	45.0 ± 7.6*	36.6 ± 5.8*
	8	23.1 ± 4.9	46.3 ± 11.3*	66.9 ± 13.2*	34.4 ± 8.3*	38.2 ± 8.3*	41.6 ± 6.3*	40.3 ± 6.6*

Values are means + SE. Control period values are averages of 3 measurements made during basal period in each dog. R_a, rate of appearance; R_d, rate of disappearance. *Values are significant increased ($P < 0.01$) from control period values.

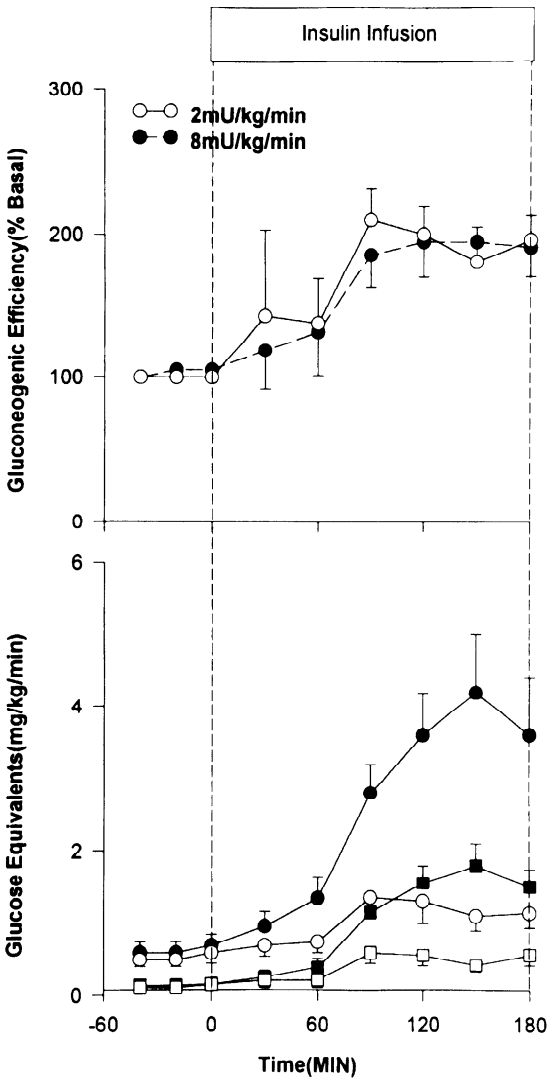

Fig. 5. Effects of intraportally infused insulin (2 and 8 mU·kg^{-1}·min^{-1}) during hypoglycemia on gluconeogenic efficiency and minimal and maximal rates of gluconeogenesis expressed as glucose equivalents. Gluconeogenic efficiency is initially significantly increased ($P < 0.05$) from control period at 90 min during both insulin infusion doses. Minimal (■) and maximal (●) estimates of gluconeogenesis are significantly increased ($P < 0.05$) during 8 compared with minimal (□) and maximal (○) estimates of gluconeogenesis during 2 mU·kg^{-1}·min^{-1} infusions. Minimal and maximal estimates of gluconeogenesis were initially significantly increased ($P < 0.05$) from control period at 60 min during 8 mU·kg^{-1}·min^{-1} infusion but at 90 min during 2 mU·kg^{-1}·min^{-1} insulin infusion.

The blood acetoacetate level and net hepatic acetoacetate production remained unchanged (Table 6) during hypoglycemia in the presence of high- and lower-dose insulin infusions. Similarly, blood 3-hydroxybutyrate and net hepatic 3-hydroxybutyrate production did not change significantly from baseline during either hypoglycemic insulin infusion protocol.

When euglycemia was maintained during insulin infusions (Table 7), plasma NEFA levels fell similarly and significantly in both high (1027 ± 150 to 101 ± 35 μM)- and lower-dose infusion protocols (579 ± 88 to 55 ± 6 μM). Net hepatic uptake of NEFA decreased similarly during high- and lower-dose insulin infusions.

DISCUSSION

Increased rates of gluconeogenesis usually occur when there are low circulating levels of insulin. In response to fasting and prolonged exercise, when the insulin level falls, an increased gluconeogenic rate is observed (7, 37). In underinsulinized diabetic individuals, unrestrained gluconeogenesis contributes significantly to the elevated fasting hyperglycemia (35). These in vivo observations fit hand in hand with the in vitro demonstration that insulin represses the activity of PEPCK, a key glyconeogenic enzyme (17). It is thus clear from both in vivo and in vitro data that hyperinsulinemia usually inhibits gluconeogenesis (reviewed in Ref. 17). One exception to this general rule occurs during prolonged hypoglycemia (3, 19, 25). Studies in humans (3, 25) and dogs (19) thus indicated the importance of increased rates of gluconeogenesis in sustaining HGP during insulin-induced hypoglycemia. These previous studies have demonstrated that some component of the counterregulatory response is able to overcome the repressive effect of insulin on glucose production at the liver. The present study extends those observations by clearly demonstrating that the amplified counterregulatory response attributable to extra insulin in the presence of a given hypoglycemia overrides the local inhibitory effect of insulin on fat and liver so that lipolysis and gluconeogenesis are paradoxically increased.

Data now clearly support the fact that insulin per se can stimulate secretion of glucoregulatory hormones (cortisol and norepinephrine) under euglycemic conditions (19, 31). More recently, the microneurography technique has been used to demonstrate direct increases of sympathetic nervous activity during hyperinsulinemic euglycemia (2). Because of differing experimental designs and problems of statistical power the effects of insulin per se on glucoregulatory hormone secretion during hypoglycemia has been more difficult to resolve. We and others (9–11) have demonstrated that insulin per se can increase epinephrine and/or norepinephrine levels during hypoglycemia. Other studies have reported a nonsignificant increase in catecholamine levels by insulin per se during hypoglycemia (26, 28), and one study has reported a suppression of epinephrine by hyperinsulinemia (15). The explanation for this apparent disparity of results has been discussed elsewhere (10, 11). The counterregulatory hormone data from the present experiments have been previously published (10). Therefore we will only state here that there are clear increases in catecholamines (epinephrine and norepinephrine) and cortisol levels in the presence of equivalent hypoglycemia when the insulin level is raised.

The fact that plasma glucagon levels were equivalent during both insulin dose infusions indicates that this hormone was not responsible for the amplified metabolic responses occurring during high-dose insulin infusions. It would therefore appear that the amplified catecholamine (derived from adrenal glands or synapses) and/or cortisol levels were responsible for the increased metabolic responses evident during high-dose infusions. This assumption appears reasonable, since

Table 6. *Effects of intraportal insulin infusion and resulting hypoglycemia on arterial levels and net hepatic balances of NEFA and ketone bodies in overnight-fasted conscious dogs*

	Insulin Infusion Rate, mU·kg^{-1}·min^{-1}	Control Period	Duration of Hypoglycemia, min					
			30	60	90	120	150	180
			Arterial levels					
NEFA, μM	2	770 ± 180	618 ± 148	828 ± 138	1,053 ± 245	1,030 ± 260	848 + 152	830 ± 151
	8	660 ± 83	362 ± 94	559 ± 99	801 ± 130	768 ± 105	659 ± 149	596 ± 202
Acetoacetate, μM	2	85 ± 10	73 ± 9	84 ± 13	99 ± 13	96 ± 12	93 ± 14	96 ± 16
	8	95 ± 11	92 ± 9	89 ± 11	98 ± 11	83 ± 4	93 ± 13	85 ± 6
3-Hydroxybutyrate, μM	2	23 ± 13	10 ± 4	14 ± 4	18 ± 5	25 ± 4	27 ± 12	27 ± 13
	8	21 + 7	11 ± 3	14 ± 3	18 ± 3	13 ± 3	13 ± 2	13 ± 3
			Net hepatic balance					
NEFA, μmol/min	2	3.8 ± 1.2	1.8 ± 1.3	2.5 ± 0.7	4.3 ± 2.7	5.0 ± 2.5	4.0 + 2.0	3.8 + 2.1
	8	3.7 ± 0.8	1.5 ± 0.5	3.2 ± 1.0	6.0 ± 0.7	6.8 ± 1.3	4.2 ± 1.0	3.4 ± 0.9
Acetoacetate, μmol·kg^{-1}·min^{-1}	2	−0.8 ± 0.2	−0.6 ± 0.2	−0.6 ± 0.3	−1.0 ± 0.3	−0.7 ± 0.2	−0.8 ± 0.2	−1.0 ± 0.4
	8	−1.1 ± 0.4	−1.0 ± 0.2	−1.3 ± 0.4	−1.9 ± 0.3	−1.5 ± 0.2	−1.4 ± 0.3	−1.5 ± 0.2
3-Hydroxybutyrate, μmol·kg^{-1}·min^{-1}	2	−0.8 ± 0.2	−0.5 ± 0.06	−0.4 ± 0.2	−0.8 ± 0.3	−0.8 ± 0.3	−0.7 ± 0.2	−0.8 ± 0.2
	8	−1.0 ± 0.3	−0.5 + 0.7	−0.8 ± 0.2	−0.9 ± 0.2	−0.9 ± 0.2	−0.8 ± 0.2	−1.0 ± 0.2

Values are means ± SE. Control period values are averages of 3 measurements made during the basal period in each dog. Negative rates of net hepatic uptake indicate net hepatic production. NEFA, nonesterified fatty acids.

there are no data to demonstrate that other neuropeptides or neurotransmitters (e.g., vasopressin, corticotropin-releasing hormone, and oxytocin, which were not measured in these studies) have any meaningful metabolic effects during mild to moderate hypoglycemia. Furthermore, the demonstrable effects of increased cortisol levels on liver, muscle, and adipose tissue are somewhat delayed (3–6 h) (13). It thus appears likely that the increased metabolic responses occurring during the high-dose insulin infusion (1–3 h) were caused by either a combined (additive or synergistic) effect of the amplified cortisol and catecholamine responses or solely by the enhanced catecholamine levels.

Despite hepatic insulin levels of >1,000 μU/ml, the increased counterregulatory response resulted in a significant increase (~60%) in HGP during the high-dose infusions. The increased glucose production rate measured by tracer methodology was confirmed by the hepatic arteriovenous difference data. During the last hour of the high-dose insulin infusion, the minimal and maximal estimates of gluconeogenesis were 1.6 and 3.8 mg·kg^{-1}·min^{-1}, respectively, whereas during the last hour of lower-dose infusion these estimates were 0.5 and 1.2 mg·kg^{-1}·min^{-1}. Thus gluconeogenesis was quantitively greater during the high-dose insulin infusions. Intrahepatic gluconeogenic efficiency, however, increased similarly during low- and high-dose insulin infusions. This indicates that the increased gluconeogenesis occurring during the high-dose insulin infusion was primarily due to a greater substrate release by peripheral tissues (muscle and adipose tissue) rather than an effect at the liver per se. The present data are supported

Table 7. *Effects of intraportal insulin infusion with euglycemia on arterial levels and net hepatic balances of NEFA and ketone bodies in overnight-fasted conscious dogs*

	Insulin Infusion Rate, mU·kg^{-1}·min^{-1}	Control Period	Duration of Euglycemia, min					
			30	60	90	120	150	180
			Arterial levels					
NEFA, μM	2	579 ± 92	236 ± 54*	116 ± 21*	91 ± 16*	62 ± 5*	49 ± 6*	54 ± 6*
	8	684 + 200	253 ± 10*	225 ± 50*	180 ± 60*	100 ± 30*	75 ± 30*	52 ± 20*
Acetoacetate, μM	2	100 ± 23	90 ± 20	90 ± 20	100 ± 10	80 ± 20*	90 ± 10*	90 ± 30*
	8	94 ± 6	95 ± 10	79 ± 10	100 ± 12	86 ± 2*	101 ± 23*	65 ± 2*
3-Hydroxybutyrate, μM	2	12 ± 2	5 ± 1*	4 ± 1*	3 ± 1*	3 ± 1*	2 ± 1*	2 + 1*
	8	12 ± 5	6 ± 2*	4 ± 3*	2 ± 1*	3 ± 2*	1 ± 0.3*	1 ± 0.2*
			Net hepatic balance					
NEFA, μmol/min	2	5.0 ± 1.5	1.7 ± 0.7*	0.4 ± 0.2*	0.5 ± 0.1*	0.2 ± 0.1*	0.3 ± 0.1*	0.1 ± 0.1*
	8	8.5 ± 3.0	1.2 + 0.4	1.2 ± 0.3*	1.4 ± 0.3*	0.6 ± 0.3*	0.2 ± 0.1*	0.2 ± 0.1*
Acetoacetate, μmol·kg^{-1}·min^{-1}	2	−1.1 ± 0.2	−0.2 ± 0.1*	−0.2 ± 0.1*	−0.1 ± 0.1*	−0.1 ± 0.1*	−0.1 ± 0.1*	−0.1 ± 0.1*
	8	−1.3 ± 0.6	−1.0 ± 0.6	−0.5 ± 0.3*	−0.5 ± 0.3*	−0.4 ± 0.3*	−0.4 ± 0.4*	−0.5 ± 0.4*
3-Hydroxybutyrate, μmol·kg^{-1}·min^{-1}	2	−1.1 ± 0.3	−1.2 ± 0.3	−0.5 ± 0.1*	−0.4 ± 0.1*	−0.3 ± 0.1*	−0.2 ± 0.1*	−0.2 ± 0.1*
	8	−1.0 ± 0.2	−0.4 ± 0.1*	−0.5 ± 0.2*	−0.3 ± 0.1*	−0.2 ± 0.1*	−0.2 ± 0.1*	−0.1 ± 0.1*

Values are means + SE. Control period values are averages of 3 measurements made during basal period in each dog. Negative rates of net hepatic uptake indicate net hepatic production. *Time points when values are significantly ($P < 0.05$) reduced from control period values.

by other studies in humans and dogs than an effect at the liver per se. The present data are supported by other studies in humans and dogs, indicating that increases in catecholamine level such as occurred in this study have only a modest gluconeogenic effect on the liver directly but have a marked peripheral action (32, 34). The effects of the amplified catecholamine levels evident during hypoglycemia are underscored by the hyperinsulinemic euglycemic clamp data. During the latter studies, when catecholamines remained at basal levels, HGP and gluconeogenesis were greatly reduced. Interestingly, the marked insulinemia present during the high-dose insulin infusion did not suppress gluconeogenesis further than the lower-dose insulin infusion, indicating a maximal effect with the latter.

The effects of the amplified catecholamine levels on peripheral metabolism are evident when hypoglycemic and euglycemic studies are compared. During hyperinsulinemic euglycemia arterial blood lactate levels did not increase and the liver remained a net producer of lactate throughout both series of insulin infusions. During hypoglycemia, despite reduced insulin-mediated glucose uptake into muscle, arterial lactate levels increased significantly from baseline, thereby indicating that an additional source for lactate production was operating over and above a stimulation of glycolysis by insulin. In fact, because both arterial blood lactate and net hepatic uptake of this substrate increased, it is evident that the rate of release from peripheral tissue must have increased dramatically. These findings strongly suggest an increase in muscle glycogenolysis by catecholamines as the explanation of the increased arterial lactate levels.

Blood alanine levels fell during both hypoglycemic protocols. This fall in alanine can be explained by the facts that *1*) extrahepatic supply could not match increased alanine clearance (i.e., hepatic utilization) and *2*) hepatic fractional extraction of alanine increased. Hepatic uptake of alanine was increased during the high- compared with lower-dose insulin infusion despite similar circulating levels of alanine and an equivalent rise in hepatic fractional extraction. This occurred because hepatic blood flow increased by a greater amount compared with lower-dose infusion and extraction also increased during the euglycemic studies. This indicates that system A transporters were stimulated during hypoglycemic and euglycemic protocols. This is intriguing, since during the euglycemic studies glucagon a potent activator of alanine transport was decreased. Furthermore, a high intracellular concentration of pyruvate, parallel to increased lactate production, would result in elevated intracellular alanine levels, which would tend to inhibit transport of alanine into the hepatocyte. This indicates that insulin per se can activate hepatic system A transporters.

Insulin is a potent inhibitor of lipolysis (29). Thus, during both series of euglycemic studies, circulating levels of NEFA and glycerol and net hepatic uptake of these substrates were similarly and significantly reduced. Contrary to these findings, blood glycerol and net hepatic uptake of this substrate increased during both series of hypoglycemic studies. Even more striking was the greater increase in blood glycerol and net hepatic uptake during the high-dose insulin infusions. This further reinforces the increased metabolic effects of the amplified catecholamine response. NEFA levels and hepatic uptake were also increased during the hypoglycemic compared with euglycemic studies. Analysis of NEFA flux is more complex to interpret, since, unlike glycerol, NEFA can undergo reesterification within the adipocyte in the presence of increased insulinemia. The increased lipolytic response evident during the high-dose insulin infusions represents an integral part of the counterregulatory response. The increased blood glycerol delivery to the liver represents an important augmentation of gluconeogenic precursor supply. In fact, it can be calculated that glycerol contributed up to 28% of the available carbon for gluconeogenesis. Furthermore, the increased plasma levels of NEFA provide energy for hepatic gluconeogenesis (18) and restrict peripheral glucose utilization due to substrate competition in muscle.

In summary, we have demonstrated that at equivalent hypoglycemia, an approximately eightfold greater elevation in insulin levels into the pharmacological range can result in increased gluconeogenic and lipolytic responses. These increased metabolic responses are most likely due to the amplified catecholamine levels evident during high-dose insulin infusion. The increase in gluconeogenesis during high-dose insulin-induced hypoglycemia was not due to a difference in intrahepatic gluconeogenic efficiency but rather an increased "push" of substrates to the liver. However, it should be noted that these present results cannot easily be related to clinical practice, since the insulin doses used in this study are higher than those used on a day-to-day basis by diabetic patients. In conclusion, this study demonstrates that during hypoglycemia insulin's effect on amplifying the autonomic-adrenomedullary counterregulatory response allows its usual inhibitory actions at the liver and adipose tissue to be paradoxically reversed.

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