The Acute Metabolic Effects of Glucagon and Its Interactions with Insulin in Forearm Tissue

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Summary. The acute effects of glucagon (mol. wt. 3500) and its interactions with insulin were studied in the forearm during eight studies in seven normal, post-absorptive males. The protocol consisted of a 2 h baseline, 1 h glucagon perfusion (mean glucagon increment, 691 ± 50 pg/ml), 1 h perfusion of both insulin and glucagon (mean insulin increment of 105 ± 13 mU/l) and a 30 min recovery period. Simultaneous arterial (A), deep venous (DV), and superficial venous (SV) blood samples were obtained at 30 min intervals. Perfusion of glucagon resulted in a decrease in (A–DV) non-esterified fatty acids of -0.128 ± 0.057 mmol/l ($n = 7$, $p < 0.05$) and (A–SV) non-esterified fatty acids of -0.081 ± 0.036 mmol/l ($n = 7$, $p < 0.05$), as well as a change in deep compartment uptake of glycerol after 60 min of -0.044 ± 0.019 µmol/min/100 ml of forearm tissue ($n = 6$, $p < 0.05$), indicating increased lipolysis. There was also a decrease in net glucose uptake as reflected by a change in (A–DV) of -0.24 ± 0.09 mmol/l ($n=7$, $p < 0.025$) and (A–SV) of 0.10 ± 0.05 mmol/l ($n=7$, $p < 0.05$). There was also a net decrease in deep arteriovenous differences of potassium in six of seven subjects. Insulin levels, similar to those found after a meal, rapidly reversed the effects of glucagon on non-esterified fatty acid, glucose and potassium. These effects persisted throughout the recovery period.

Key words: Forearm perfusion, serum insulin, plasma glucagon, glucose, plasma non-esterified fatty acids, blood ketone bodies, blood glycerol, glucagon, extrahepatic effects.

Glucagon levels are reported to be elevated in a number of pathological states in man, including renal and [1] hepatic disease [2], stress [3], sepsis [4] and diabetic ketoacidosis [5, 6]. These elevations may be due in part to non-bioactive glucagon [7]. The contribution of bioactive glucagon (mol. wt. 3500) to the metabolic abnormalities of such states remains a matter of conjecture.

Significant hepatic effects of glucagon have been observed in all species. These include stimulation of glycogenolysis, gluconeogenesis, and ketogenesis [8]. In non-primates physiological concentrations of glucagon enhance lipolysis in vitro. However, such studies must be applied to man with caution because of marked interspecies differences [9, 10, 11]. Systemic administration of supraphysiological doses of glucagon leads to insulin and adrenaline release [12], thus clouding the interpretation of such experiments. Recent investigations during which somatostatin was infused suggest that abnormalities in glucagon secretion may worsen metabolic control in insulin deficient diabetes mellitus [13, 14].

The intent of these experiments was to examine the local effects of glucagon both in the absence and presence of increments in insulin. Using the forearm perfusion technique glucagon increments could be achieved without perturbing systemic metabolism. Glucagon concentrations were increased in the forearm venous circulation to levels similar to those occurring in ketoacidosis [5, 6], and insulin to venous levels similar to those seen after a meal in normal individuals.

Materials and Methods

Subject Selection

day^{-1}. Informed consent was obtained prior to each experiment. All procedures were performed in the Thorndike Clinical Research Center of Boston University Medical Center at Boston City Hospital. Perfusions were begun at 0900 h, after an overnight fast of 12–14 h, and continued until approximately 1330 h. The experiments were performed in accordance with the principles of the Declaration of Helsinki.

Forearm Technique

These studies used a minor modification of the forearm perfusion technique of Andres and Zierler previously described [15, 16]. Deep venous and superficial venous catheters were inserted in a retrograde manner. The brachial artery was cannulated with an 18 g, thin wall Cournand needle. A double-lumen Lilienthal adaptor fitted to the arterial needle allowed sampling of incoming arterial blood via a central stainless steel cannula and perfusion of the forearm distal to the arterial sampling site. The circulation of the arm was perfused at 0.10 ml/min. Five minutes prior to and throughout each sampling period the hand circulation was occluded at the wrist by a paediatric blood pressure cuff to prevent mixing between venous beds [17]. Simultaneous arterial and venous specimens were obtained. Evans Blue (150 µg/min) was perfused throughout the experiment to monitor blood flow as calculated by a modified Fick principle [16].

Experimental Protocol

The protocol used consisted of a 2 h baseline period, 1 h of glucagon perfusion alone, 1 h of glucagon and insulin, and a 30 min recovery period. Samples were obtained at 30 min intervals throughout. The baseline and recovery perfusates consisted of 0.125 g human serum albumin and 75 mg Evans Blue in 50 ml of 0.154 mol/l saline. Glucagon and glucagon-insulin perfusates contained saline, human serum albumin, Evans Blue and the designated hormone(s). The glucagon perfusion was calculated to give a forearm venous plasma increment of 400 pg/ml based on a mean forearm plasma flow of 30 ml/min. Glucagon (lyophilised; USP, Eli Lilly, lot number 9PB59A) was used. For the insulin-glucagon period single component pork insulin (Eli Lilly lot number XX0240AMX/-C2Ct3016-4A) was added to a 25 ml aliquot of the glucagon perfusate. The dose of insulin was calculated to give an average venous plasma increment of 100 mU/l. Four sets of samples, arterial (A), deep venous (DV), and superficial venous (SV) were obtained during the baseline period. Two sets were obtained during the glucagon perfusion, two during glucagon plus insulin and one during the recovery period. Glucagon and insulin were gifts of Dr. John Galloway of Eli Lilly Laboratories.

Sample Handling

Blood samples of 14 ml were obtained from each sampling site simultaneously. Four ml of whole blood was quickly mixed with 4 ml of chilled 30% (v/v) perchlorate and kept at −20 °C until later analysis for lactate, pyruvate, glycerol, 3-hydroxybutyrate, and acetoacetate. Frozen filtrates (kept at −20 °C until needed) for substrate measurements were neutralized on the day of analysis (within 30–60 days of sampling). The maximum loss of substrate during such storage was 20% and all samples from a given study were analyzed in the same assay. Two ml of whole blood was placed in a tube containing 1000 International Units of Trasylol (FBA Pharmaceuticals), spun at 4 °C and the plasma removed and frozen for later glucagon determination. One ml was placed in a disposable Wintrobe tube for measurement of plasmacrit (1-haematocrit). The remainder of the sample was spun twice at 4 °C.

This plasma was used for determination of dye, non-esterified fatty acids (NEFA), glucose and potassium concentrations. Concentrations were corrected for heparin-filled syringe dead space.

Analytical Methods

NEFA, glucose, glycerol, lactate, pyruvate, ketone bodies and hormone levels were determined in duplicate. Potassium concentrations were assessed in triplicate. Glucose was analysed by a glucose oxidase, colorimetric method, and glycerol, 2-hydroxybutyrate, acetoacetate, pyruvate and lactate were analysed by enzymatic techniques [18]. The coefficients of variation (CV) for these methods are 0.7, 4.8, 6.2, 3.4, 1.1, and 1.2% respectively. Potassium concentrations were measured by internal standard flame photometry. NEFA concentrations were measured by a modification of the method of Novak, CV 7% [19]. Plasma insulin concentrations were determined by radioimmunoassay using Wright antibody lot number 496 and a charcoal separation technique previously described [15]. Plasma glucagon concentrations were determined by radioimmunoassay using 30K antibody, lot number 33, pool number 2 supplied by Dr. Roger Unger of Texas Southwest Medical School. Insulin and glucagon standards were a gift of Dr. Mary Root of Eli Lilly Laboratories.

Data Handling

Arteriovenous differences across the deep vein (A–DV) and superficial vein (A–SV) reflect ongoing metabolism in the two forearm compartments. Previous conventions concerning data handling during unstable flow periods were observed [15]. Uptake or output estimates (plasma flow or whole blood flow x arteriovenous difference = Q$\cdot$) were obtained for the deep compartment tissues but could not be estimated accurately for the superficial tissues of the forearm because of individual variability of flow across this compartment [20].

Repeated measurements within a particular observation period were pooled for each experiment and group means were obtained. A positive arteriovenous difference or Q$\cdot$ reflects net uptake or decreased output of a particular constituent across a venous bed. A negative difference or Q$\cdot$ reflects either output or decreased uptake across that compartment. Prior to interperiod analysis, data were compared using repeated measure analysis of variance. Inter-period differences were examined only when the analysis of variance was significant ($p < 0.05$). Within period means were evaluated to determine if a mean was significantly positive or negative using one tailed Student t tests. The hypotheses posed were that glucagon would have effects opposite to that of insulin (one tailed questions). Inter-period means were compared by paired analysis. Data are presented as mean ± SEM or mean ± the standard error of the difference (SED) [21]. Data were analysed from seven subjects by pooling two sets of experimental results and from the single individual who was studied twice. All data analysis was performed for the seven individuals except for hormone concentrations.

Results

Plasma Flow

Plasma flow was stable in all but one subject with an increase of less than 20% between the baseline and experimental periods for all subjects. In subject 7 there was an increase in flow during sampling period 3. The flow then plateaued at a new level.

Table 1. Mean baseline period arteriovenous differences of plasma constituents

Constituent	(A–DV)±SEM	P	(A–SV)±SEM	P	Deep versus superficial
Glucose	+0.46 ±0.07 (7)	<0.01	+0.48 ±0.05 (7)	<0.01	NS
Potassium	+0.09 ±0.05 (7)	NS	+0.17 ±0.03 (7)	<0.01	NS
Lactate	−0.098±0.058 (5)	NS	−0.214±0.041 (5)	<0.01	NS
NEFA	+0.082±0.048 (7)	NS	−0.081±0.050 (7)	NS	<0.05
Glycerol	−0.007±0.008 (6)	NS	−0.028±0.015 (6)	NS	<0.05
Acetoacetate	+0.056±0.023 (6)	<0.05	+0.041±0.018 (6)	<0.05	<0.05
3-hydroxybutyrate	+0.097±0.026 (6)	<0.01	+0.122±0.038 (6)	<0.025	NS
Pyruvate	0.000±0.006 (6)	NS	−0.015±0.004 (6)	<0.01	<0.025

Results are shown in mmol/l. A = arterial, DV = deep venous and SV = superficial venous. The numbers in parentheses indicate the number of subjects analysed. In general, four observations contributed to an individual mean during the baseline period for each experiment. P = the probability that a particular mean is significantly negative or positive is given. Significance was assessed by a single tail t test. The last column is the probability that paired mean deep and superficial arteriovenous differences are negative or positive (single tail t test)

Table 2. Effects of glucagon and glucagon + insulin perfusion on metabolite uptake across the deep venous bed in μ mol/min/100 ml of forearm tissue

Period

Period	Baseline[a]	Glucagon[b]	Glucagon+insulin[c]	Recovery[d]
Glucose	+0.90 ±0.15 (7)	+0.64 ±0.14 (7)	+2.16 ±0.40 (7)	+2.29 ±0.39 (7)
Potassium	+0.18 ±0.08 (7)	−0.02 ±0.12 (7)	+0.63 ±0.14 (7)	+0.72 ±0.18 (7)
NEFA	+0.143±0.076 (7)	−0.053±0.102 (7)	+0.315±0.077 (7)	+0.590±0.155 (7)
Glycerol	+0.000±0.025 (6)	−0.064±0.043 (6)	+0.011±0.109 (6)	+0.091±0.053 (6)
Lactate	−0.394±0.298 (6)	−0.604±0.184 (6)	−0.795±0.200 (6)	−0.453±0.286 (6)
Pyruvate	+0.003±0.004 (6)	+0.034±0.038 (6)	+0.079±0.076 (6)	−0.002±0.028 (6)
Acetoacetate	+0.177±0.053 (6)	+0.153±0.049 (6)	+0.250±0.040 (6)	+0.168±0.084 (6)
3-hydroxybutyrate	+0.264±0.056 (6)	+0.242±0.141 (6)	+0.297±0.054 (6)	+0.794±0.224 (6)

The numbers in parentheses refer to the number of subjects analysed. The mean of experimental means is given. In general, there were four observations each for baseline, two for glucagon and glucagon plus insulin and one for recovery period
[a] Significant within period uptake or output: glucose, p < 0.01; potassium < 0.05; acetoacetate < 0.01; 3-hydroxybutyrate < 0.01
[b] Significant within period uptake or output: glucose, p < 0.01; lactate < 0.01; acetoacetate < 0.025. The following differed from the baseline period: glucose < 0.05 and NEFA < 0.05
[c] Significant within period uptake or output: glucose, p < 0.01; potassium < 0.01; lactate < 0.01; NEFA < 0.01; acetoacetate < 0.01; 3-hydroxybutyrate < 0.01. The following were greater or less than the baseline period: glucose < 0.025; potassium < 0.05; and lactate < 0.05. The following were significantly different from the glucagon period: glucose < 0.01; potassium < 0.01; and NEFA < 0.01
[d] Significant within period uptake or output: glucose, p < 0.01; potassium < 0.01; NEFA < 0.01; acetoacetate < 0.05; 3-hydroxybutyrate < 0.01. The following were different from the baseline period: glucose < 0.01; potassium < 0.05; NEFA < 0.01; glycerol < 0.05 and 3-hydroxybutyrate < 0.05

Arterial Concentrations of Metabolites and Potassium

Concentrations of potassium and all metabolites except for acetoacetate were stable throughout. Acetoacetate concentrations rose slightly during the study but were significantly elevated above control at only one observation point (the first sample of the glucagon plus insulin period, p < 0.05).

Baseline Characteristics of the Deep and Superficial Beds

Arteriovenous differences of various plasma constituents measured across forearm tissues in the baseline period are shown in Table 1. Deep

arteriovenous differences reflected a net uptake of glucose, acetoacetate, and 3-hydroxybutyrate.

Uptake or output across the deep tissues (Q˙DV) is shown in Table 2 and was qualitatively similar to that of arteriovenous differences alone.

Arteriovenous differences across superficial tissues (Table 1) indicated a significant net fractional output of lactate and pyruvate. Five of seven subjects had negative (A–SV) NEFA during the baseline period (Table 4). The ratio of NEFA to glycerol was highly variable from individual to individual, ranging from 2.8 to 8.1. Net uptake of glucose, potassium, acetoacetate and 3-hydroxybutyrate were also indicated.

Table 3. Plasma hormone concentrations

Perfusion period	A	DV–A	SV–A
Insulin (μ U/ml)			
Basal	7.3±1.8	−0.7±0.6	−0.4±0.6
Glucagon	8.2±2.1	−0.3±0.9	−1.1±0.8
Glucagon and insulin	13.1±3.7	106.0±26.8	103.4±26.2
Recovery	8.3±1.4	3.4±1.7	2.5±1.9
Glucagon (pg/ml)			
Basal	117±22	−9±7	−12±11
Glucagon	167±23	688±50	700±64
Glucagon and insulin	224±62	713±90	718±92
Recovery	164±22	29±15	13±22

Each number is the mean for eight experiments. For the purposes of this analysis, the two experiments in subject 1 were not pooled. In an individual experiment four observations were made during baseline, two during glucagon and glucagon plus insulin, and one during recovery periods
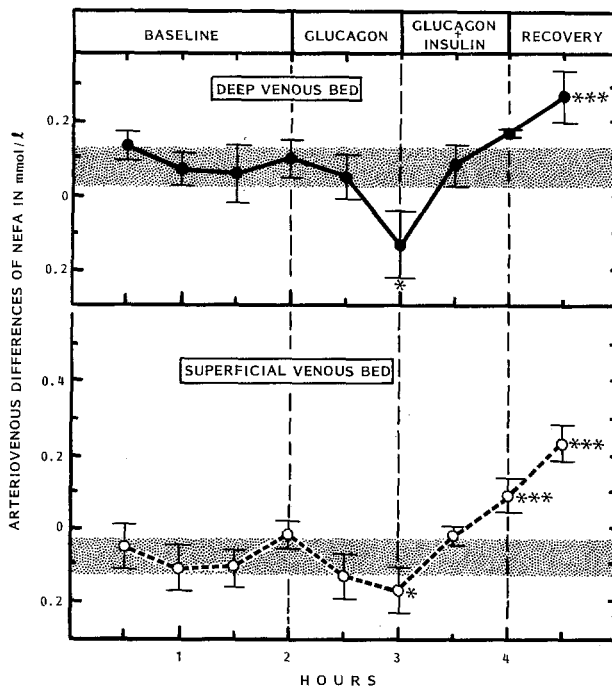
Fig. 1. Mean arteriovenous differences of NEFA (mmol/l, ±SEM) in the human forearm during glucagon infusion. The shaded area indicates the baseline arteriovenous differences ± SEM. Data from seven individuals are shown. Significant differences relative to baseline are indicated by an asterisk(s): *, $p < 0.05$, **, $p < 0.025$, and ***, $p < 0.01$

Deep and superficial beds are compared with respect to arteriovenous differences in Table 1. These differences indicated relatively more output from the superficial bed of NEFA, glycerol, and pyruvate.

Hormone Levels (Table 3)

Mean arterial levels of insulin were stable (7.3 ± 1.8 mU/l) until insulin perfusion was begun. There was then a small rise in mean systemic insulin concentrations (13.1 ± 3.7 mU/l). Perfusion of insulin into the brachial artery resulted in a mean increment in forearm plasma insulin concentrations of 104.5 mU/l (mean of deep and superficial venous increments). Increases in insulin levels ranged from 40 to 200 mU/l. Concentrations returned to baseline levels during the recovery period.

Arterial levels of glucagon in the post-absorptive state were 117 ± 22 pg/ml. Systemic levels rose during glucagon perfusion alone to a mean of 167 ± 23 pg/ml and continued to rise during perfusion of glucagon and insulin to a final level of 224 ± 62 pg/ml. During recovery, systemic levels fell to a mean of 164 ± 22 pg/ml. The mean increment in forearm plasma glucagon levels during glucagon perfusion was 691 pg/ml, and during perfusion of glucagon and insulin, 715 pg/ml. During the recovery period significant arteriovenous differences of glucagon were no longer detectable. There was a large intersubject variation in venous plasma glucagon increments achieved, with a range between 200–1300 pg/ml.

Glucagon Effects upon Lipolysis

Perfusion of glucagon stimulated lipolysis significantly in both the deep and superficial tissue compartments as indicated by increased release of NEFA (Fig. 1, Table 4). Mean deep arteriovenous differences of NEFA which were positive during the baseline period, became negative at 60 min. The change in A–DV relative to baseline at 60 min of perfusion was −0.226 ± 0.101 mmol/l, $p < 0.05$. The change in deep compartment Q˙ NEFA for the entire glucagon period was −0.196 ± 0.088 μmol min^{-1} 100 ml^{-1} of forearm tissue, $p < 0.025$ (Table 2) indicating either decreased uptake of NEFA or increased output. The within period relative arteriovenous differences of NEFA across the superficial compartment became negative in six of seven subjects at 30 min of perfusion and significantly negative at 60 min (Table 4). The net difference from baseline at 60 min was −0.117 ± 0.049 mmol/ml ($p < 0.05$, n=7).

In the six subjects in whom glycerol was measured, changes in arteriovenous differences of glycerol paralleled those of NEFA, with significant effects seen at 60 min (net change of −0.022 ± 0.010, $p < 0.05$, n=6, Table 5). When flow was taken into account the change in glycerol output across deep tissues at 60 min was −0.044 ±

Table 4. Net change in arteriovenous differences of NEFA[a]

Subject	Baseline	Glucagon[b] 30 min	60 min	Glucagon + insulin[b] 90 min	120 min	Recovery[b] 150 min
		Deep venous				
1	−0.125	−0.113	−0.267	0.194	0.348	0.297
2	0.102	0.049	−0.046	0.002	0.073	0.073
3	0.039	−0.087	−0.040	−0.011	0.136	0.071
4	0.222	−0.068	−0.797	−0.376	−0.086	0.013
5	0.170	0.030	−0.130	0.223	−0.035	0.322
6	0.195	−0.046	−0.230	−0.101	−0.028	0.264
7	−0.029	0.027	−0.074	0.068	0.191	0.153
Mean	−	−0.030	−0.226	0.000	0.096	0.187
SED	−	0.024	0.101	0.076	0.055	0.052
p	−	NS[c]	<0.05[c]	NS[d]	NS[d]	<0.01[d]
		Superficial venous				
1	−0.184	−0.076	−0.114	0.154	0.284	0.395
2	−0.084	−0.060	−0.058	0.106	−0.023	0.042
3	0.008	−0.048	0.004	−0.028	0.127	0.171
4	−0.317	−0.043	−0.043	0.303	0.303	0.466
5	−0.063	−0.120	−0.318	0.104	0.194	0.338
6	0.124	−0.084	−0.281	−0.247	0.063	0.278
7	−0.048	0.121	−0.008	0.058	0.319	0.189
Mean	−	−0.044	−0.117	0.064	0.181	0.268
SED	−	0.029	0.049	0.064	0.050	0.055
p	−	NS[c]	<0.05[c]	NS[d]	<0.001[d]	<0.01[d]

[a] Results given in mmol/l
[b] The column headings indicate minutes after beginning glucagon perfusion
[c] Probability that a value is more negative than baseline
[d] Probability that a mean value is more positive than baseline

0.019 μmol min^{-1} 100 ml^{-1} of forearm tissue, (p < 0.050, n=6). Changes in superficial bed arteriovenous differences were not significant.

Glucagon Effects on Glucose Uptake and Potassium Movement

There was a decrease in glucose arteriovenous differences across the deep and superficial beds with glucagon (Fig. 2). Decreased deep differences were found in all subjects, whereas decreases in the superficial differences were seen in five of seven subjects. Net changes in deep and superficial arteriovenous differences of glucose were maximal 60 min after glucagon perfusions were begun, −0.24 ± 0.09 and −0.10 ± 0.05 mmol/l, (p < 0.025 and < 0.05 respectively). The overall change in Q̇ DV glucose during glucagon was −0.25 ± 0.13 μmol min^{-1} 100 ml^{-1} of forearm tissue (p < 0.05, Table 2).

In six of seven subjects mean (A–DV) potassium decreased during glucagon by −0.11 ± 0.05 mmol/l (p < 0.05, n=6, Fig. 3 and Table 5) and Q̇ DV by −0.29 ± 0.14 μmol min^{-1} 100 ml^{-1} of forearm tissue (p < 0.05, n=6, Table 3). In subject 6,

arteriovenous differences increased relative to baseline, while in the others, decreased differences were observed for the 30 min period and for four of seven at the 60 min period (Table 5). There were no significant effects upon potassium movement in the superficial compartment.

Ketone Body Utilisation, Pyruvate and Lactate (Table 2)

Glucagon caused no significant alterations in utilisation or output of these substrates.

The Simultaneous Perfusion of Insulin and Glucagon (Figs. 1, 2 and 3; Tables 2, 4, and 5)

Simultaneous perfusion of insulin with glucagon resulted in a reversal of the effects of glucagon. Inhibition of glucagon mediated lipolysis across the superficial tissues (Fig. 1 and Table 4) was indicated by a net increase in (A–SV) NEFA relative to the glucagon and baseline periods. Similar alterations were observed across the deep tissues of the forearm (Fig. 2, Tables 2 and 4). (A–DV) NEFA which were
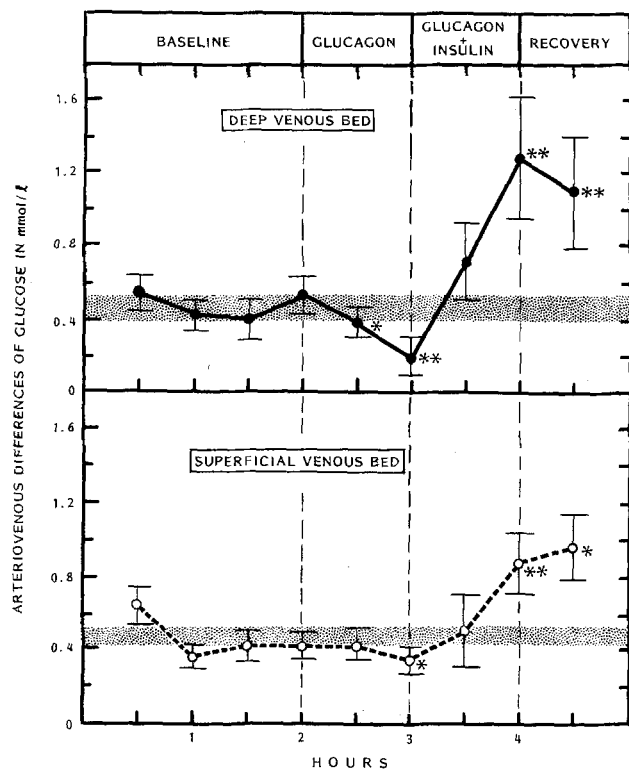
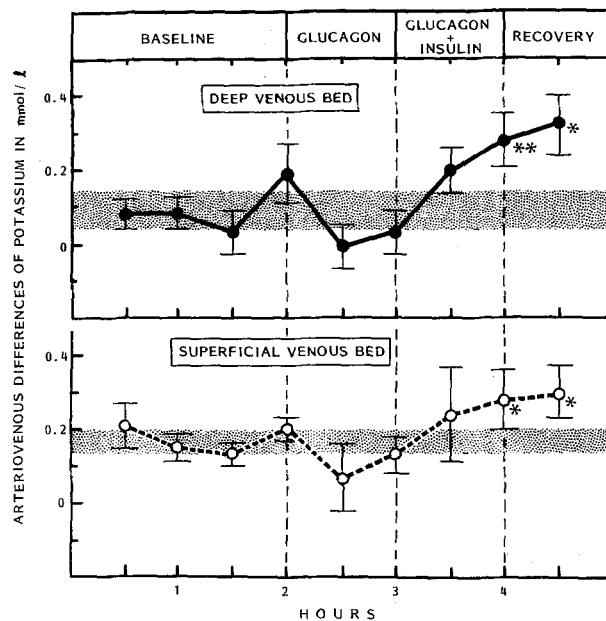**Fig. 2.** Mean arteriovenous differences of glucose. Details as in Figure 1

Fig. 3. Mean arteriovenous differences of potassium. Details as in Figure 1

Table 5. Net change in deep arteriovenous differences of glycerol and potassium relative to baseline[a]

| Subject | Baseline | Glucagon[b] | | Glucagon + insulin[b] | | Recovery[b] |
		30 min	60 min	90 min	120 min	150 min
Glycerol						
1	−0.037	−0.039	−0.060	0.001	0.022	0.026
2	0.013	−0.003	−0.023	0.048	0.168	−0.003
3	0.013	−0.033	−0.033	−0.033	−0.023	0.047
4	0.005	−0.035	−0.025	0.015	0.025	0.055
5	0.013	0.037	0.007	0.017	−0.003	0.027
6	−	−	−	−	−	−
7	−0.010	0	0	0.020	0	0
Mean	−	−0.014	−0.022	0.011	0.032	0.025
SED	−	0.013	0.010	0.011	0.028	0.010
p	−	NS[c]	<0.05[c]	NS[d]	NS[d]	<0.05[d]
Potassium						
1	0.15	−0.06	−0.11	0.20	0.25	0.31
2	0.03	−0.03	−0.07	0.18	0.48	0.08
3	0.10	−0.14	−0.30	−0.18	0.07	0
4	0.24	−0.21	−0.22	−0.03	0.28	0.06
5	−0.14	−0.11	0.06	0.38	0.61	0.065
6	0.07	0.14	0.24	0.30	0.46	0.55
7	0.15	−0.28	−0.14	−0.06	−0.14	0
Mean	−	−0.10	−0.06	0.11	0.29	0.24
SED	−	0.05	0.07	0.08	0.11	0.10
p	−	NS[c]	NS[c]	NS[d]	<0.025[d]	<0.05[d]

[a] Results given in mmol/l
[b] The column headings indicate minutes after beginning glucagon perfusion
[c] Probability that a value is more negative than baseline
[d] Probability that a value is more positive than baseline

significantly negative relative to baseline during glucagon became positive with respect to the glucagon period (0.172 ± 0.043 μmol/ml, p < 0.01) and were no longer distinguishable from baseline values.

The effects of insulin on glucose and potassium (Figs. 2 and 3, Tables 2 and 5) were opposite to those of glucagon. (A–SV) and (A–DV) glucose were increased significantly during perfusion relative to baseline and glucagon periods (also observed for Q DV glucose). Changes in A–SV and A–DV glucose relative to the glucagon periods were 0.41 ± 0.14 and 0.73 ± 0.20 μmol/ml for both (p < 0.025. Mean (A–SV) potassium levels were increased over baseline by insulin at 60 min of perfusion by 0.15 ± 0.06, (p < 0.05). (A–DV) and Q DV potassium were significantly increased over both baseline and glucagon perfusion periods by insulin.

Recovery Period (Figs. 1, 2 and 3; Tables 2, 4 and 5)

During recovery significant insulin effects upon glucose, potassium, and NEFA were discernible in the deep and superficial tissues, relative to baseline.

Discussion

The objectives of these experiments were to determine whether pathophysiological concentrations of glucagon at the top end of the range found in ketoacidosis would stimulate lipolysis, affect substrate utilisation and/or interfere with insulin action. In vitro and in vivo human studies have been contradictory [10, 14, 22, 23, 24] concerning the lipolytic and contra-insulin effects of glucagon. Enhanced insulin secretion in individuals during the infusion of glucagon probably accounts for much of the confusion concerning in vivo human studies [25, 26], since the lipolytic response to glucagon could be blocked with insulin concentrations as low as 25 mU/l [26].

Blockade of the expected increase in glucagon during acute insulin withdrawal in insulin dependent diabetics results in a substantial reduction of NEFA concentrations and ketone bodies [13, 27]. However, somatostatin therapy in established ketoacidosis does not reverse the metabolic disturbances implying that glucagon worsens but does not initiate the metabolic disturbance [28]. Glucagon concentrations frequently exceed 1000 pg/ml in ketoacidosis [6]. Further, Raskin and Unger have shown that when glucagon was suppressed in insulin dependent diabetics by a 48 h infusion of somatostatin, metabolic abnormalities were normalized [29].

In the studies described in this paper, increases in glucagon levels of approximately 700 pg/ml were

associated with a significant release of NEFA across both the deep and superficial venous compartments of the forearm. Mean glycerol A–DV also became more negative than baseline and output across deep tissue increased significantly. However, significant glycerol output from superficial tissues was not observed. This was in keeping with the small magnitude of changes observed in lipolysis, the lesser blood flow and tissue mass in the superficial venous compartment and the relatively large capacity for lipolytic activity of the muscle containing deep venous compartment [30, 31]. Despite significant increases in systemic glucagon levels, arterial insulin, NEFA, and glycerol levels did not change. This demonstrated that systemic lipolysis was not induced by a doubling of glucagon in the presence of normal post-absorptive concentrations of insulin and is consistent with results reported by others [32].

The results presented in this paper apparently conflict with the findings of Pozefsky et al. [33], who were unable to demonstrate lipolytic effects of glucagon using a similar technique. These workers studied both post-absorptive and 50 h fasted individuals. In the latter case, endogenous glucagon levels, systemic lipolysis, as well as intracellular levels of NEFA are maximal [22]. Despite the significant decrease in insulin levels associated with prolonged fasting, the peak glucagon effects may already have been present making additional effects difficult to demonstrate. In the former studies, NEFA data were not shown. Because lipolytic effects were small in magnitude, measurements of NEFA may be required to detect these effects, since NEFA: glycerol ratios exceed 3:1 during lipolysis in man [31].

Glucagon-enhanced lipolysis was associated with decreased glucose uptake across both venous beds. This finding was reminiscent of the competitive process between fatty acid and glucose utilisation first described by Randle et al. [34]. Evidence in the literature suggested that the intracellular rather than extracellular concentrations of NEFA were important in the regulation of glucose uptake [35]. Lindsey and Madison have previously shown that glucagon administration in the dog led to a decrease in peripheral glucose disposal by up to 40% [36]. In the experiments we have presented, acute elevations in glucagon concentrations were associated with a greater than 30% decrease in net glucose arteriovenous differences across both the superficial and deep venous beds in association with enhanced lipolysis. Unfortunately, we could not perform respiratory quotient measurements during these studies and cannot comment on the utilisation of tissue glycogen in the presence of glucagon increments. Glucagon may have exerted its effects upon glucose uptake by

increasing intracellular concentrations of NEFA and thereby NEFA utilisation. This effect of glucagon may contribute to glucose sparing during periods of active lipolysis.

The effects of glucagon upon potassium movement tended to parallel those on glucose. A hyperkalaemic effect of glucagon was first observed by Wolffson and Ellis [37]. Galansino et al. reported that glucagon perfusion across liver resulted in increased potassium output, and more recently, evidence has accumulated that glucagon contributes to renal potassium loss [38, 39]. Thus, glucagon may contribute to potassium depletion in insulin deficiency by direct tissue loss and enhancement of renal excretion.

Insulin concentrations similar to those observed in the post-prandial state readily reversed the lipolytic effects of glucagon, despite the continued presence of increased glucagon concentrations. In addition to blockade of lipolysis, insulin also promoted a rapid and complete reversal of glucagon effects upon glucose and potassium uptake. While a significant portion of the effects of insulin on glucose uptake are due to direct enhancement of glucose uptake by tissues, insulin may also act indirectly by blocking glucagon stimulated lipolysis. The anti-glucagon effects of insulin which were demonstrated during the perfusion of both hormones make it likely that glucagon's effects upon peripheral tissues are of importance only during states of relative or absolute insulin deficiency.

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