

Effect of glucagon infusion on some plasma metabolites and hormones in obese children*

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The metabolic and hormonal effects of glucagon infusion (6 µg/kg/h) for three hours were studied in obese children. Glucagon caused a sustained hyperglycaemia and hyperinsulinaemia and a lower than normal (non-obese) growth hormone response. Plasma triglycerides, cholesterol, glycerol and the majority of the free amino acids showed a significant decrease in comparison with the controls, while free fatty acids showed a moderate decrease.

Glucagon administration revealed some hormonal and metabolic abnormalities of obesity. The effect of glucagon-induced insulin secretion and the action of pharmacologic doses of glucagon have, however, to be considered in the interpretation of the metabolic effect of glucagon.

Glucagon is considered a glucogenic hormone, and recent investigations (12) have shown that glucagon also modulates lipid metabolism and has important metabolic effects on the three principal lipid substrates in the blood, i.e. free fatty acids, ketone bodies and triglycerides. Glucagon has also been shown to stimulate the secretion of a number of hormones including growth hormone and insulin (9, 11).

The present investigation was undertaken to study the metabolic and hormonal effects of glucagon infusion in obese children.

MATERIAL AND METHODS

The investigations were carried out in 24 non-obese and 35 obese children weighing more than 20% in excess of their ideal body weight.

Blood glucose concentration was estimated by the glucose oxidase method. Plasma triglyceride, glycerol and cholesterol levels were determined enzymatically using Boehringer kits. Immunoreactive insulin and growth hormone levels were measured by double radioimmunoassay using commercially available kits of Radiochemical Centre Amersham for insulin and of Biodata for growth hormone determination. Plasma free amino acids were measured by automatic ion exchange chromatography (Beckman Multichrom). Lactate, pyruvate and free fatty acid determinations were made by standard methods described in a previous paper (10).

The mean, standard error and the correlation coefficient were calculated with standard methods. Statistical significance of the difference between the means of various groups were evaluated according to Student's *t*-test.

In this series of investigations postabsorptive venous blood sample after an overnight fast was taken prior to glucagon infusion for baseline metabolite and hormone estimation. Glucagon (Cristalline Glucagon, Eli Lilly, Indianapolis) was

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infused into an antecubital vein at a rate of $6 \mu\text{g}/\text{kg}/\text{h}$ for 180 min, by means of a constant infusion pump. Venous blood samples were drawn at 60, 120 and 180 min during the infusion.

RESULTS

Blood glucose (Fig. 1). Fasting blood glucose concentration was similar in the control and obese group (76.9 ± 3.6 vs. 83.4 ± 2.9 mg/dl). After 60 min of glucagon infusion, blood glucose increased by nearly 50 mg/dl in both groups ($p < 0.001$), then it tended to fall during the 2nd and 3rd h in both control and obese children and in the control group it returned to the pre-infusion level. The fall of blood glucose was less marked in the obese children and they remained significantly hyperglycaemic throughout the whole infusion period.

Plasma immunoreactive insulin. The fasting plasma insulin level was significantly higher in the obese group (26 ± 5.1 vs. $0.5 \pm 3.9 \mu\text{U}/\text{ml}$). Glucagon infusion induced endogenous insulin secretion in both groups with peak values at 60 min, after which a continuous and significant decline was seen. But insulin secretion as judged by the plasma insulin levels was much higher in the obese group during the whole infusion period (Fig. 1).

Growth hormone (Fig. 1). Basal plasma growth hormone levels were similar in both groups (9.6 ± 3.8 vs. 2.5 ± 0.8 ng/ml). The hormone levels tended to increase during the infusion in both groups. The increment, however, reached the level of significance only at 180 min in the control group and at 120 and 180 min in the obese group.

Blood lactate, pyruvate and lactate/pyruvate ratio. Basal levels were

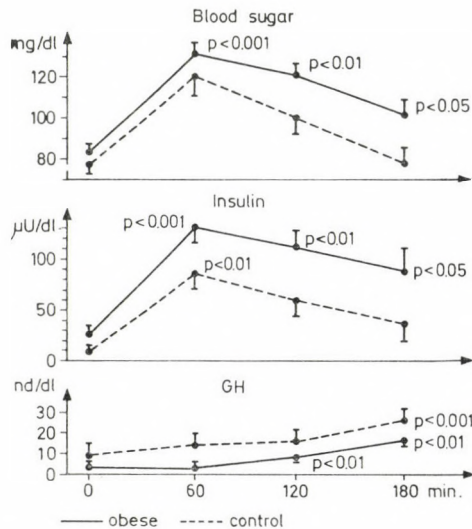


FIG. 1. Blood glucose, plasma insulin and growth hormone levels during glucagon infusion

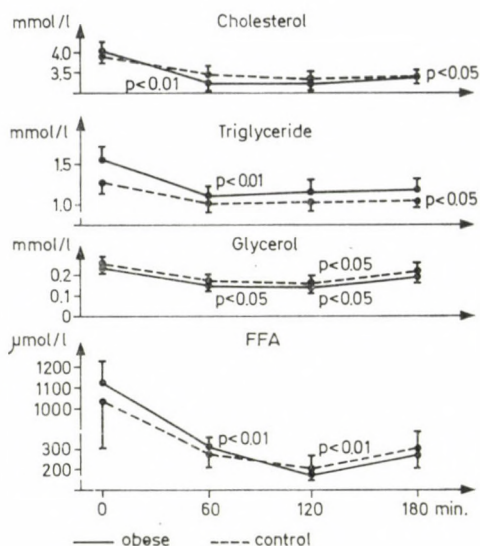


FIG. 2. Effect of glucagon infusion on plasma cholesterol, triglyceride, glycerol and FFA levels

similar in both groups and remained practically unchanged during the 3 h glucagon infusion.

Plasma free fatty acids (Fig. 2). There was no significant difference in fasting FFA levels between the control and obese children. Plasma FFA levels significantly decreased during the first and second hour of glucagon infusion in both groups with no apparent change in the third hour.

Cholesterol (Fig. 2). Baseline cholesterol levels were similar in the two groups. Cholesterol showed a tendency to fall during the first hour of glucagon infusion, but the decrease was significant only in the obese group. The decrease of plasma cholesterol reached significance at 120 min in the control group with no change thereafter. Plasma cholesterol remained unchanged between 60 and 180 min in the obese children.

Triglycerides (Fig. 2). Plasma fasting triglyceride levels were higher in the obese group (1.55 ± 0.1 vs. 1.25 ± 0.1 mmol/l). There was a significant fall during the first hour of infusion in the obese group with no change thereafter. There was also a tendency to decrease in the control group, but only the 180 min value was significantly below the starting level.

Glycerol (Fig. 2). The fasting levels were similar in both groups. Significantly lower levels could be observed at 120 min in the control group and at 60 and 120 min in the obese group.

Plasma amino acids (Fig. 3 and Table I). Fasting total free amino acid levels were somewhat but not significantly higher in the obese group. Among the individual free amino acids the concentration of alanine and lysine were significantly

elevated, but a number of other free amino acids also tended to increase (Fig. 4 and Table I).

More than half of the individual free amino acids measured showed a significant decline during the glucagon infusion, especially during the second and third hour. As far as the total free amino acid levels are concerned, the changes were similar in both groups but more pronounced

in the obese children. Among the individual free amino acids, glycine, alanine, valine, isoleucine, leucine, tyrosine, lysine, ornithine and arginine decreased significantly in the obese group. The same amino acids showed a significant decline during glucagon infusion in the control group except for alanine, lysine and ornithine, the levels of which did not change significantly.

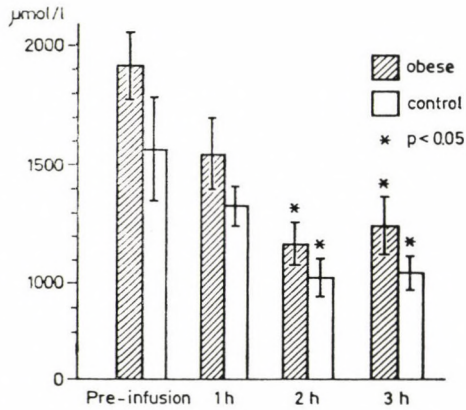


FIG. 3. Plasma total free amino acid levels during glucagon infusion

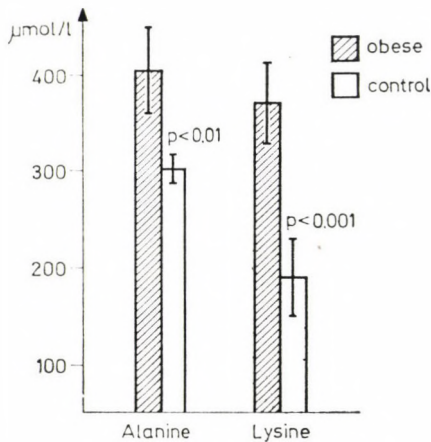


FIG. 4. Plasma alanine and lysine levels in obese and control children

TABLE I

Effect of exogeneous hyperglucagonaemia (continuous infusion of glucagon) on individual plasma amino acid levels in obese children ($M \pm SE$)

		Pre-infusion	1 h	2 h	3 h
Taurine	Obese	66 ± 11	45 ± 10	46 ± 8	39 ± 9
	Control	51 ± 18	56 ± 9	29 ± 9	87 ± 14
Aspartate	O	34 ± 5	24 ± 2	28 ± 4	25 ± 4
	C	27 ± 9	35 ± 8	15 ± 8	19 ± 5
Glycine	O	214 ± 16	147 ± 10	112 ± 7***	101 ± 10***
	C	178 ± 37	151 ± 34	114 ± 35	92 ± 15*
Alanine	O	402 ± 42	310 ± 24	264 ± 18**	252 ± 18**
	C	303 ± 19	275 ± 44	215 ± 38	230 ± 40
Cystine	O	85 ± 19	106 ± 9	78 ± 25	100 ± 20
	C	178 ± 101	137 ± 7	81 ± 18	74 ± 12
Valine	O	267 ± 21	221 ± 16	167 ± 13***	187 ± 17**
	C	252 ± 44	233 ± 40	169 ± 24	142 ± 19*
Methionine	O	10 ± 4	11 ± 3	4 ± 1	33 ± 8
	C	11 ± 1	11 ± 1	4 ± 1	33 ± 8
Isoleucine	O	68 ± 8	48 ± 3*	33 ± 3***	37 ± 21
	C	50 ± 5	44 ± 8	28 ± 1*	28 ± 2*
Leucine	O	137 ± 9	108 ± 9	78 ± 10***	87 ± 11**
	C	119 ± 19	105 ± 26	57 ± 7	76 ± 4
Tyrosine	O	64 ± 14	44 ± 5	28 ± 3*	23 ± 4*
	C	44 ± 9	40 ± 4	21 ± 1*	26 ± 1*
Phenylalanine	O	66 ± 11	47 ± 4	56 ± 11	53 ± 9
	C	66 ± 10	34 ± 1	20 ± 1	36 ± 1
Lysine	O	376 ± 39	300 ± 45	203 ± 26**	221 ± 29**
	C	182 ± 47	134 ± 4	203 ± 23	128 ± 37
Ornithine	O	64 ± 8	70 ± 22	31 ± 5**	39 ± 11
	C	36 ± 13	28 ± 17	29 ± 1	21 ± 4
Arginine	O	61 ± 6	48 ± 5	36 ± 5**	34 ± 4**
	C	63 ± 22	30 ± 7	20 ± 5*	25 ± 2
Total	Obese	1914 ± 122	1529 ± 138	1164 ± 102*	1234 ± 112*
	Control	1560 ± 182	1310 ± 88	1005 ± 83*	1017 ± 96*

*0.05; **p < 0.01; *** p < 0.001.

DISCUSSION

One of the most striking effects of the continuous glucagon infusion was a marked insulin secretory response in both groups, especially in the obese children. The glucagon-induced insulin secretion greatly modified the effect of glucagon. The plasma FFA curve was the mirror image of that of the insulin curve (Fig. 2): the higher the insulin, the lower the FFA; in fact, the lipolytic effect of glucagon (11) seemed to be completely suppressed by the glucagon-induced insulin secretion.

Insulin is lipogenic, and there is a fall in FFA during insulin infusion, e.g. during the insulin treatment of diabetic ketoacidosis and during glucose tolerance tests. Although plasma glucagon levels were not measured during the infusion, they probably were high during the entire period, so it is possible that the ratio of these two antagonistic hormones have determined the overall metabolic effects. Some of the metabolites measured may also have been more "sensitive" to glucagon rather than to insulin levels. Thus, the fall in triglycerides may have been primarily a glucagon effect since glucagon may reduce the circulating plasma triglyceride concentration (11), and oral glucose administration results in a definite increase in plasma triglycerides (1).

Felig et al. (5, 6) were the first to report significantly higher levels of branched-chain amino acids, tyrosine and phenylalanine in obese adults.

The hyperaminoacidaemia was interpreted as a manifestation of the insulin ineffectiveness characteristic of obesity. Furthermore, it was speculated that the hyperaminoacidaemia might have provided the feedback signal to the beta cells through which insulin resistance was accompanied by an appropriately augmented secretory rate of insulin. The increment of branched-chain amino acids and that of tyrosine and phenylalanine was not significant in our material. The difference may be explained by comparing the fasting plasma insulin levels of the two series of investigations. Fasting plasma insulin was only moderately increased in our obese children compared with the overweight adults of Felig et al. (5).

The recent date of Felig et al. (6) showed significantly higher alanine levels in a group of obese adults. Interestingly, the first paper of the same group (5) reported essentially similar alanine levels in obese and in control subjects.

Chaussain et al. (4) also found identical plasma alanine levels in obese and control children. In contrast, in the present study, alanine was one of the two amino acids which showed a significantly higher fasting level in obese children. The cause of hyperalaninaemia is far from clear. Since among the metabolic hormones regulating plasma amino acid levels glucagon seems to be the most important, the question arises whether or not the increased alanine level was due to hypoglucagonaemia. Fast-

ing plasma glucagon levels have not been measured in our patients, but alpha-cell function, as judged by basal levels and by responses to glucose and protein administration, was found to be normal in obese persons (7).

It is interesting that the hyperalaninaemia in the obese adult patients of Felig et al. (6) was accompanied by an increased splanchnic uptake of alanine. This association probably reflects an increased rate of gluconeogenesis.

The hypoaminoacidaemia induced by bolus injection or infusion of glucagon is well documented (2, 3, 8). In interpreting this metabolic effect, two important factors must be considered. The dose of glucagon infused was pharmacological (8) and the glucagon stimulated insulin secretion may greatly modify the effect of glucagon. Endogenous insulin secretion as seen after the administration of glucose (5), significantly reduced the levels of branched-chain amino acids, tyrosine and phenylalanine.

In conclusion, glucagon infusion had a profound effect on the plasma level of most investigated metabolites. Plasma triglycerides, cholesterol, glycerol and the majority of the amino acids decreased significantly, while free fatty acids showed a moderate decrease in comparison with the controls.

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