Istituto di Medicina Clinica
Divisione di Gerontologia e Malattie del Ricambio
Università di Padova

METABOLIC EFFECTS OF GLUCAGON IN ENDOGENOUS HYPERTRIGLYCERIDEMIA *

ROMANO NOSADINI GIUSEPPE SOLDÀ FLORA DE BIASI
ANTONIO TIENGO

In man, glucagon has a stimulating action on hepatic glucose production [22], ketogenesis and lipolysis [20], as well as a hypotriglyceridemic effect. The metabolic effects of glucagon on lipolysis, ketogenesis and gluconeogenesis seem dependent on prehepatic insulin levels. In fact, in insulin-dependent diabetics, these effects are enhanced [21], while in hyperinsulinemic obese patients they are absent [19]. In endogenous hypertriglyceridemia, which is frequently associated with obesity and diabetes and increased insulin secretion, these glucagon-induced metabolic effects have not yet been studied.

While it is known that prolonged treatment with pharmacological levels of glucagon induces a reduction in triglyceride levels [2] even in human hypertriglyceridemia, it has recently been postulated that resistance to the metabolic effects of the hormone may constitute one of the concurring elements in the development of hyperlipemia [6]. This glucagon resistance is suggested by the finding of high fasting and post-arginine plasma glucagon levels in man and in rats with experimental hyperlipemia [5,23]. In order to confirm or disprove a reduced metabolic glucagon action in human hyperlipemia, as demonstrated by EATON [5] in rats with experimental hyperlipemia, we have studied the effects of glucagon in a group of subjects with endogenous hypertriglyceridemia. Glucagon was administered in pharmacological doses apt to determine plasma concentration similar to the physiological levels present in the portal vein, and its effects on lipolysis, ketogenesis and endogenous production of glucose and triglycerides were studied.

Key-words: Endogenous hypertriglyceridemia; Free fatty acids; Glucagon effects: Glucagon injection; Insulin/glucagon interrelationship; Ketogenesis; Lipolysis.

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MATERIALS AND METHODS

Seventeen subjects ranging in age from 29 to 64 years (average ± SEM: 43 ± 2) with hyperprebetalipoproteinemia were studied. The patients were classified as Type IV or V (according to Fredrickson) on the basis of electrophoretic separation of lipoproteins on agarose gel [16], and the determination of basal triglyceride and cholesterol values. The 3 patients originally considered Type V were reclassified as Type IV during hospitalization.

The patients were subdivided into two groups on the basis of their glucose tolerance: group 1, normal tolerance; group 2, impaired glucose tolerance according to the criteria of FAJANS and CONN [10]. Average weight (± SEM) of these patients was 112 ± 3% of their ideal body weight (according to the Metropolitan Life Insurance Co. Tables, 1959) (group 1: 111 ± 4%; group 2: 116 ± 4%) (tab. 1).

A group of normolipemic, metabolically normal subjects was chosen as controls. The average age of this group was 33 ± 3 years (range: 18-50 years) and average weight was 100 ± 2% of ideal body weight.

All tests were carried out at 09^{00} following a 13-h overnight fast. For the two weeks preceding the study all subjects followed a free diet containing at least 250 g/*die* of carbohydrates and not more than 500 ml of wine (11-12% alcohol).

No patient was insulin-dependent or under treatment with oral hypoglycemic or hypolipidemic agents.

One mg of glucagon, furnished by the Eli Lilly Co., was dissolved in 250 ml physiological saline. The dose of 1 μg/kg was injected i.v. over 60 sec in patients and controls. The plastic syringe was washed with refluxed venous blood following injection. A 21 gauge needle, inserted in the median brachial vein of the contralateral arm, was kept pervious with slow dripping physiological saline. Blood samples were taken 15 min and immediately before glucagon injection, and then at 5, 10, 20, 30, 40, and 60 min after. The samples were collected in heparinized test tubes, placed immediately in an ice water bath, and centrifuged at 3-4 °C within 30 min from the end of the test. Glucose, insulin (IRI), free fatty acids (FFA), 3-β-hydroxybutyrate (β-OH), alanine, and triglycerides were determined in every sample. Glucose was determined enzymatically according to HUGGETT and NIXON [12], insulin according to HERBERT et al. [11], FFA according to DUNCOMBE [4], and β-hydroxybutyrate according to MELLANBY and WILLIAMSON [15]. Triglyceride determinations were carried out according to EGGSTEIN and KREUTZ [7] and alanine according to WILLIAMSON [24]. The concentration of the various metabolites and insulin was expressed as the absolute change from basal values and as the increment and decrement area with respect to basal values.

Student's *t*-test was employed to compare the statistical significance in the same group by paired data analysis. In the comparison between the results obtained in the various groups probability values were calculated by means of the Mann-Whitney test. This test is robust against outliers and does not demand a normal distribution.

RESULTS

Basal values (tab. 1) - Both hyperlipemic groups showed slight but significant overweight as compared to the controls. Both groups also showed significantly higher fasting triglyceride and cholesterol levels as compared to the controls. Glucose values,

parameter / groups	age (years)	body weight (% ideal weight)	plasma triglycerides (mg/100 ml)	plasma cholesterol (mg/100 ml)	plasma glucose (mg/100 ml)	plasma IRI (μU/ml)	plasma FFA (μEq/l)	plasma β-hydroxybutyrate (μmol/100 ml)	plasma alanine (μmol/100 ml)
hyperlipemics (no. = 9)	40 (29-57)	111* (100-115)	440** (170-1,836)	295* (163-544)	86 (75-99)	5 (1-8)	428 (244-700)	23 (10-50)	561 (404-940)
diabetic hyperlipemics (no. = 8)	46 (35-64)	116** (97-119)	699** (170-2,048)	301* (168-544)	95* (81-122)	12°° (4-19)	655 (450-1,200)	17 (13-20)	535 (420-820)
controls (no. = 11)	33 (23-50)	100 (95-105)	100 (50-148)	189 (70-238)	82 (70-96)	3 (1-12)	502 (335-1,124)	24 (15-45)	430 (300-740)

The mean of the parameters was determined using the basal values reported before i.v. glucagon injection (1 μg/kg) (range in parentheses)
Significance of differences between fasting values of hyperlipemics with or without diabetes and controls calculated by the Mann-Whitney test: * = $p<0.05$; ** = $p<0.01$
Significance of differences between fasting values of hyperlipemics with and without diabetes calculated by the Mann-Whitney test: °° = $p<0.01$

Tab. 1 - Characteristics of the groups studied.

parameter / groups	area (min)	controls	hyperlipemics	diabetic hyperlipemics
plasma glucose (g/min)	60	1.007 (415 - 1.605)	1.445 (350 - 2.200)	2.002** (530 - 3.245)
plasma FFA (mmol/min)	60	−3.641 (−15.170 - +1.510)	−5.530 (1.665 - −13.895)	−12.544 (2.390 - −14.930)
	20	1.142 (555 - 1.900)	−0,098 (2.235 - −2.240)	−2.360* 2.830 - −9.250)
plasma β-hydroxybutyrate (μmol/min)	60	−199 (390 - −675)	−113 (980 - −1.010)	−91 (1.000 - −1.210)
	20	64 (115 - −200)	132 (170 - 25)	140 (−60 - 192)
plasma IRI (μU/min)	60	390 (92 - 755)	465 (100 - 990)	472 (125 - 1.030)
plasma triglycerides (g/min)	60	−0.666 (425 - −2.005)	−1.396 (3.000 - −11.285)	−4.350 (330 - −20.560)
plasma alanine (mmol/min)	60	−3.010 (440 - −8.550)	−5.088 (650 - −15.450)	−1.840 (4.850 - −7.910)

Significance of differences between area of hyperlipemics with or without diabetes and controls calculated by the Mann-Whitney test: * = $p<0.05$; ** = $p<0.01$

Tab. 2 - Increment and decrement area from basal values of the metabolites and hormones after i.v. glucagon injection (1 μg/kg). The values are the means of each group with ranges in parentheses.

as compared to the controls, and insulin levels, as compared to the other two groups, were significantly higher in the diabetic hyperlipemic group. FFA, β-OH and alanine fasting levels were not significantly different in the three groups.

Glucagon injection (tab. 2, figs 1 and 2) - Glucagon induced a significant glucose increase in the two hyperlipemic groups as well as in the controls. Glucose increase in the hyperlipemic (diabetic or not) groups was significantly higher than that of the controls. Glucose increment area was significantly increased in the diabetic group as compared to the controls. There was a simultaneous decrease of basal alanine levels throughout the 60 min following hormone injection in both normal and hyperlipemic patients. The glucagon-induced insulinopoietic effect was marked but not significantly different in the three groups. The lipolytic glucagon effect (fig. 1) was evaluated by means of the variation in plasma FFA concentrations. The significant FFA increase observed in the control group, as compared to basal values ($p<0.01$) in the first 20 min following i.v. glucagon, was not reported in the two hyperlipemic groups. The absolute values and area increase in FFA within 20 min were significantly higher

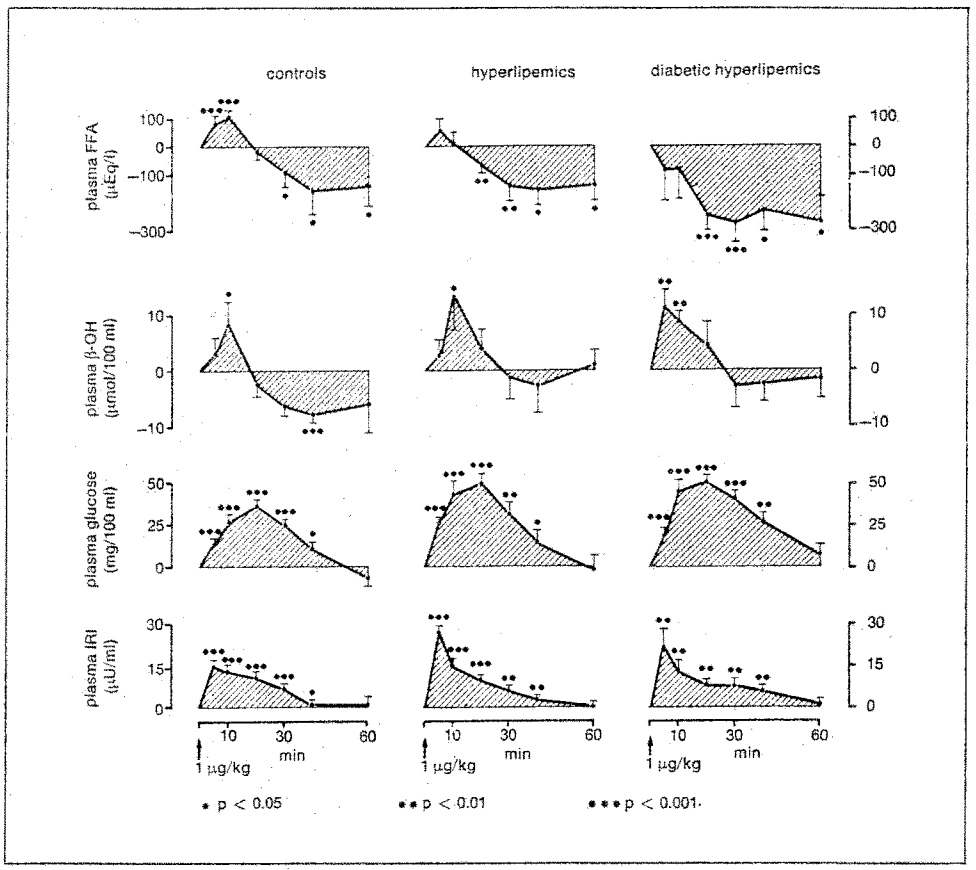

Fig. 1 - Absolute changes from basal values in plasma concentrations of FFA, β-hydroxybutyrate, glucose and IRI following i.v. glucagon injection in normal and hyperlipemic subjects with or without diabetes.

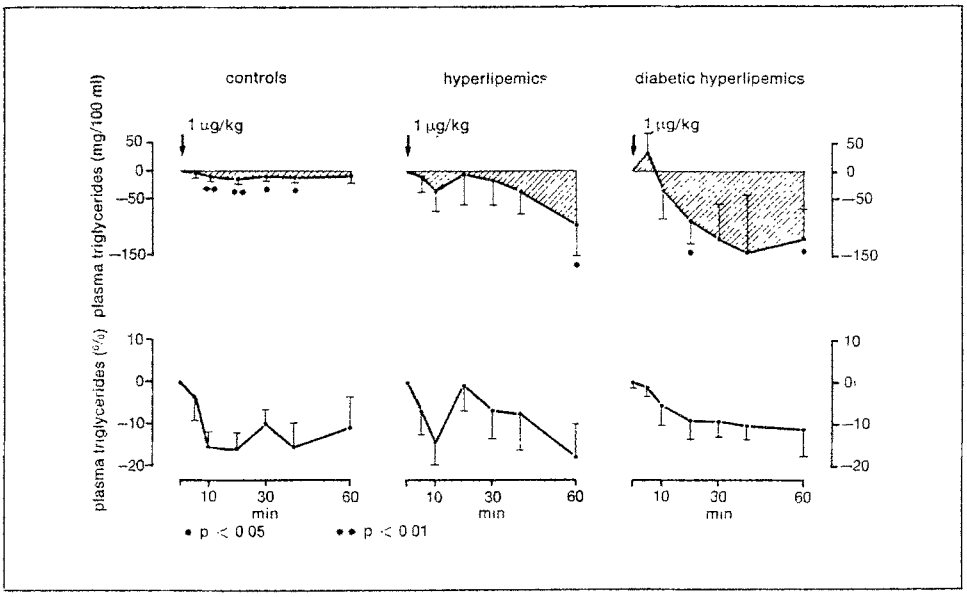

Fig. 2 - Absolute and percentual decrement of plasma triglycerides below basal values following i.v. glucagon injection in the three groups studied.

in the control group compared to the study groups. The subsequent decrease in FFA after 20 min was significant in the three groups, while no significant intergroup variations were observed.

The ketogenic effect of glucagon was evaluated by means of the increase in β-OH. This metabolite increased significantly but not differently in all three groups. A significant decrease in triglyceride levels was observed in all the groups. Maximum percentage and absolute decreases were not significantly different in the three groups.

Correlations (fig. 3) - A 1% negative correlation was found between fasting insulin levels and area of FFA increase within the first 20 min following glucagon injection ($r = -0.535$, $p<0.01$).

There was no significant correlation between fasting insulin and area of β-OH increase.

DISCUSSION

From our result, it can be concluded that pharmacological doses of glucagon infused into hypertriglyceridemic subjects induce:

— an enhanced hyperglycemic effect;
— a lack of lipolytic effect;
— a preserved ketogenic and hypotriglyceridemic effect.

The hyperglycemic effect of glucagon, which was observed in all three groups but most markedly in the hyperlipemics, is brought about by the stimulation not only of glycogenolysis but also of hepatic gluconeogenesis from aminoacids and lactate [9]. The fall in plasma alanine observed after glucagon is the expression of enhanced

alanine uptake and utilization induced by glucagon, as clearly shown in man by CHIASSON et al. [3]. The lack of FFA mobilization in glucagon-treated hyperlipemics may be the expression of the prevalence of the insulin-induced antilipolytic action over the lipolytic effect as a result of the inhibition of the adenylcyclase-cAMP system which modulates lipase activation in adipocytes.

In fact, in hyperlipemic subjects with normal or reduced glucose tolerance, a condition of relative hyperinsulinism exists analogous to that observed in obesity and maturity-onset diabetes. EATON and SCHADE [19] have reported a missing lipolytic glucagon effect in hyperinsulinemic obesity. Moreover, both hyperlipemic groups showed slight but significant overweight. In our hypertriglyceridemic patients, a negative correlation was found between fasting basal insulin levels and FFA mobilization within the first 20 min. On the contrary, within the first minutes of its administration glucagon showed a lipolytic effect in normal subjects. Our results are in agreement with those of POZZA et al. [18] in forearm tissue and of SCHADE and EATON [20] in intact man and are in contrast with the reports of LILJENQUIST et al.[13] and POZEFSKY et al.[17].

A significant β-OH increase was observed in normal subjects with even higher values in hyperlipemics. This had already been observed in normal subjects [20] following administration of equal doses in rapid bolus injection. This ketogenic effect in hyperlipemic subjects does not seem to be dependent upon increased hepatic FFA afflux, secondary to the lipolytic effect of the hormone since mobilization of FFA was not observed in the hyperlipemics.

The hypotriglyceridemic effect of glucagon was demonstrated both in normals and in hyperlipemics. This same effect was observed in hyperlipemic patients but at much higher doses [1, 2, 8].

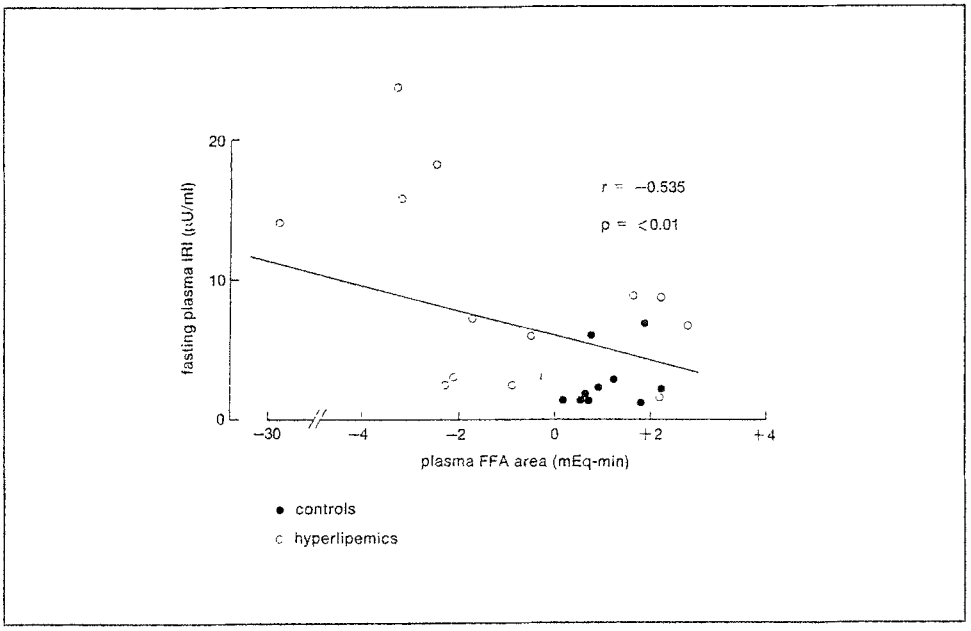

Fig. 3 - Correlation between fasting IRI and FFA increment area in control and hyperlipemic subjects.

This hypotriglyceridemic action could in part explain increased intrahepatic FFA utilization of ketone body production at the cost of FFA esterification [14]. The persistence of the hypolipidemic effect of glucagon invalidates the hypothesis of a resistance to exogenous glucagon and tends to negate the possibility of reduced hormonal activity in hyperlipemia.

SUMMARY

The metabolic effects of glucagon, administered i.v. in doses of 1 μg/kg, were evaluated in two groups of patients with endogenous hypertriglyceridemia (Types IV and V according to Fredrickson) with normal and reduced glucose tolerance and in a control group. Glucagon had a lipolytic effect, evaluated as the plasma increase of free fatty acids (FFA) during the first 20 min in normal subjects, but not in the two hyperlipemic groups. A negative correlation was observed between fasting IRI level and FFA mobilization. The ketogenic and hypotriglyceridemic effects of glucagon were demonstrated in normal and hyperlipemic groups. It would seem, therefore, that at the pharmacological doses injected, there is no resistance to the hypotriglyceridemic effect of glucagon in endogenous hypertriglyceridemia.

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Requests for reprints should be addressed to:

ROMANO NOSADINI
Istituto di Medicina Clinica
Divisione di Gerontologia e Malattie del Ricambio
Policlinico
Via Giustiniani, 2, 35100 Padova - Italy