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Abnormal glucagon response to arginine and its normalization in obese hyperinsulinaemic patients with glucose intolerance: importance of insulin action on pancreatic Alpha cells

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Summary. An excessive glucagon secretion to intravenous arginine infusion was found in obese hyperinsulinaemic patients with glucose intolerance. This study was designed to determine whether the glucagon hyperresponsiveness to arginine in these patients would improve by insulin infused at a high enough dose to overcome insulin resistance. By infusing high dose insulin during arginine infusion, the previously exaggerated glucagon response to arginine could be normalized. To normalize the abnormal glucagon response, insulin doses of 4.2 ± 0.7 and 3.8 ± 0.5 IU were required during arginine infusion in obese hyperinsulinaemic patients with impaired glucose tolerance and Type 2 (non-insulin-dependent) diabetes mellitus, respectively. This achieved plasma peak insulin levels 3 to 4 times higher than those observed in non-obese healthy subjects. Furthermore, we clarified whether or not the effect of normalizing insulin action and/or glycaemic excursions contributed to normalizing the exaggerated glucagon response to arginine in these patients. Blood glucose was clamped while high dose insulin was infused at the same levels as observed during the arginine infusion test with no insulin infusion. As a result, normalization of the exaggerated plasma glucagon response was achieved, whether hyperglycaemia existed or not. These results clearly demonstrate that, similar to non-obese hypoinsulinaemic Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetic patients, the exaggerated Alpha-cell response to arginine infusion in obese hyperinsulinaemic patients with glucose intolerance is secondary to the reduction of insulin action on the pancreatic Alpha cell, and that the expression of insulin action plays an important part in normalizing these abnormalities.

Key words: Obese hyperinsulinaemic patient, glucagon, Alpha cell, insulin resistance, arginine infusion, artificial endocrine pancreas.

It is well known that an exaggerated rise in plasma glucagon during intravenous arginine infusion is one of several characteristic abnormalities in both Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) hypoinsulinaemic diabetic patients.

Kawamori et al. demonstrated that when the plasma insulin response to arginine in hypoinsulinaemic diabetic patients simulated those of healthy subjects with the aid of an artificial endocrine pancreas, the abnormal plasma glucagon responses were normalized, whether hyperglycaemia existed or not. Thus, it has been clearly proven that the exaggerated response of the pancreatic Alpha cell to arginine in hypoinsulinaemic non-obese diabetic patients is secondary to insulin deficiency [1, 2].

In obese hyperinsulinaemic patients, a similar exaggerated plasma glucagon response to arginine has been reported [3]. However, Raskin et al. [4] reported that the glucagon hyperresponsiveness to arginine in Type 2 diabetic patients was not improved by supra-physiological doses of insulin. In their studies, four out of the six patients were obese and probably insulin resistant. Therefore, it is not yet clear whether this abnormality in obese hyperinsulinaemic diabetic patients simply represents the consequences of deficient insulin action or reflects an independent and primary disturbance of Alpha-cell function. The effect of insulin on glucagon secretion should be clarified after careful examination of obese hyperinsulinaemic Type 2 diabetic patients.

In this report, therefore, we evaluated the glucagon responses to arginine in five groups of subjects: non-obese healthy subjects, obese normoinsulinaemic patients, and obese hyperinsulinaemic patients with normal glucose tolerance, impaired glucose tolerance and glucose intolerance (Type 2 diabetes). To clarify the mechanism of abnormal glucagon responses in the obese hyperinsulinaemic patients, we also investigated the effect of insulin infused at a high enough dose to overcome insulin resistance or the exaggerated glucagon response. Furthermore, we determined which factors, physiological glycaemic excursion and/or insulin action, contributed to the

Table 1. Clinical characteristics of the non-obese healthy subjects, obese normoinsulinaemic patients and obese hyperinsulinaemic patients

	No.	Age (years)	Sex (M/F)	BMI (kg/m^2)	ΣIRI (OGTT) (mU/l)	Glucose metabolized (M) ($mg \cdot kg^{-1} \cdot min^{-1}$)
Non-obese healthy subjects	10	39.0 ± 11.5	6/4	21.2 ± 0.4	173.0 ± 18.3	9.9 ± 0.7
Obese normoinsulinaemic patients	6	38.4 ± 5.3	3/3	29.6 ± 3.3[a]	184.5 ± 28.3	7.8 ± 1.1
Obese hyperinsulinaemic patients NGT	6	37.5 ± 7.1	3/3	31.6 ± 3.7[a]	479.9 ± 57.3[b]	5.4 ± 0.6[b]
IGT	6	36.4 ± 6.7	3/3	30.6 ± 3.2[a]	466.0 ± 42.8[b]	2.3 ± 0.6[b]
Type 2 diabetes	6	40.8 ± 7.5	2/4	31.2 ± 2.9[a]	488.1 ± 30.2[b]	2.8 ± 0.6[b]

BMI, body mass index; ΣIRI, integrated IRI values of five sampling points from 0 to 120 min during oral glucose tolerance test (OGTT). NGT, normal glucose tolerance; IGT, impaired glucose tolerance. Results are expressed as mean ± SEM.
[a] $p < 0.05$, [b] $p < 0.01$ as compared with non-obese healthy subjects

normalization of the exaggerated glucagon responses in these groups.

Materials and methods

Ten non-obese healthy subjects, six obese normoinsulinaemic patients and 18 obese hyperinsulinaemic patients were investigated. Body mass index of the obese patients was equal to or higher than 27 kg/m^2. In this study, hyperinsulinaemic patients have been defined as those whose plasma immunoreactive insulin (IRI) responses, expressed as the integrated IRI value of five sampling points from 0 to 120 min (ΣIRI) during a 75 g oral glucose tolerance test (OGTT), were above 2 SD of the mean value of those from non-obese healthy subjects. All subjects gave informed consent.

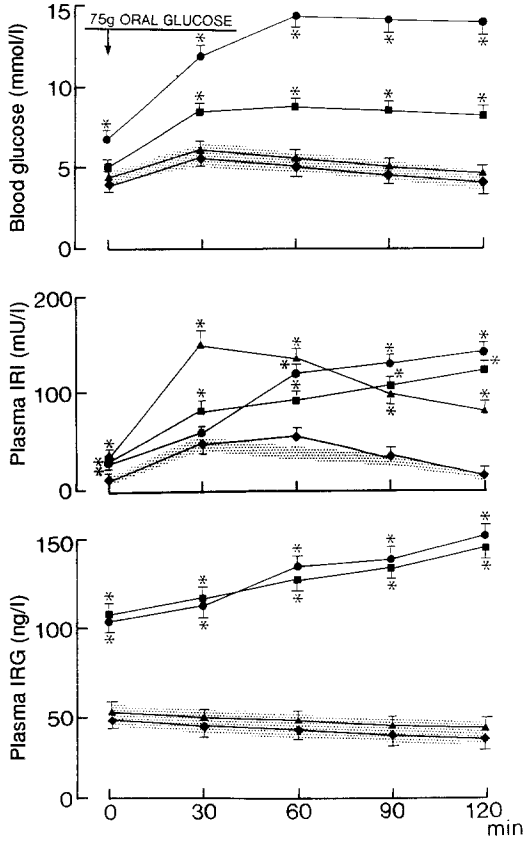

Fig. 1. Blood glucose, plasma immunoreactive insulin (IRI) and glucagon (IRG) responses in obese normoinsulinaemic patients ($n = 6$, ◆—◆) and obese hyperinsulinaemic patients with normal glucose tolerance ($n = 6$, ▲—▲), with impaired glucose tolerance ($n = 6$, ■—■) and Type 2 (non-insulin-dependent) diabetes mellitus ($n = 6$, ●—●) during a 75 g oral glucose tolerance test *(arrow)*. The mean values in 10 non-obese healthy subjects are also depicted (shaded area). Mean ± SEM, * $p < 0.05$ vs non-obese healthy subjects

The obese hyperinsulinaemic patients were divided into three groups according to the results of the OGTT [5]: obese hyperinsulinaemic with normal glucose tolerance (obese hyperinsulinaemic NGT), impaired glucose tolerance (obese hyperinsulinaemic IGT) and Type 2 diabetes mellitus (obese hyperinsulinaemic Type 2 diabetes).

All the diabetic patients had been treated with diet alone, and had shown no islet cell antibodies on testing or had a history of ketosis. Sex, age, the degree of obesity and ΣIRI values during OGTT in these five groups are shown in Table 1. The sex distribution and mean age were not significantly different among the five groups, and the degrees of obesity and hyperinsulinaemia were not statistically different among the three obese hyperinsulinaemic groups.

Experimental protocol

Euglycaemic clamp test. To estimate the degrees of insulin resistance, ten non-obese healthy subjects, six obese normoinsulinaemic patients and 18 patients consisting of the three obese hyperinsulinaemic groups (six patients in each group) underwent the euglycaemic clamp test.

After overnight fasting, each patient was connected to an artificial endocrine pancreas (AEP, Model STG-22, Nikkiso, Tokyo, Japan). A priming infusion of insulin (semisynthetic human short-acting insulin, Novo, Denmark, 800 mU over 10 min) was given, followed by a constant infusion at 40 $mU \cdot m^2 \cdot min^{-1}$ for 110 min. Glucose was infused to keep blood glucose levels between 4.4 and 5.6 mmol/l for 120 min with the amount of glucose metabolized being calculated from the amount of glucose infused during the 20–120-min period. During this test, the mean coefficient of variation of the glucose values was 4.7 ± 0.6%. A quantitative estimate of insulin resistance was obtained by glucose metabolized (M) [6].

Intravenous arginine infusion test. Patients were studied on three separate days within a 2-week period. In all subjects, after overnight fasting, 0.5 g of arginine/kg of body weight (10% arginine in distilled water) was infused for 30 min.

To determine the mechanism of the exaggerated glucagon responses in obese hyperinsulinaemic patients, the following two experiments were performed.

Experiment (1): Infusion of insulin at a dose high enough to overcome insulin resistance during intravenous arginine infusion test

Each patient from the obese hyperinsulinaemic IGT and Type 2 diabetes groups was evaluated. After reducing the blood glucose to euglycaemia with the AEP, euglycaemia was maintained for at least 1 h before the arginine infusion was started. In this experiment, the insulin was infused for 30 min to increase plasma IRI levels by 3 to 4-fold of those observed in non-obese healthy subjects, by changing the parameters of insulin infusion algorithm of the AEP [1, 2, 7, 8].

Table 2. Blood glucose, plasma IRI, and plasma IRG responses during and after 30-min arginine infusion in non-obese healthy subjects, obese normoinsulinaemic patients and obese hyperinsulinaemic patients

Sampling time (min)	0	5	10	15	30	60	120
Blood glucose (mmol/l)							
Non-obese healthy subjects	4.5 ± 0.2	4.6 ± 0.3	4.6 ± 0.2	4.8 ± 0.5	5.1 ± 0.3	4.6 ± 0.5	4.4 ± 0.4
Obese normoinsulinaemic patients	4.7 ± 0.2	4.9 ± 0.6	5.0 ± 0.5	5.2 ± 0.5	5.4 ± 0.5	5.0 ± 0.5	4.8 ± 0.5
Obese hyperinsulinaemic patients NGT	4.6 ± 0.3	4.7 ± 0.5	4.8 ± 0.3	4.9 ± 0.4	5.2 ± 0.4	4.7 ± 0.4	4.5 ± 0.4
IGT	5.1 ± 0.3	5.2 ± 0.4	5.3 ± 0.3	5.9 ± 0.4	6.5 ± 0.2[a]	6.2 ± 0.3[a]	5.1 ± 0.4
Type 2 diabetes	6.7 ± 0.2[a]	6.8 ± 0.2[a]	6.8 ± 0.3[a]	6.9 ± 0.3[a]	7.0 ± 0.3[a]	6.8 ± 0.3[a]	6.4 ± 0.3[a]
Plasma IRI (mU/l)							
Non-obese healthy subjects	14.6 ± 1.6	45.4 ± 5.8	38.7 ± 4.2	43.4 ± 3.8	51.6 ± 6.2	20.8 ± 3.6	16.3 ± 2.7
Obese normoinsulinaemic patients	16.6 ± 2.2	51.3 ± 9.7	43.8 ± 7.1	48.9 ± 6.7	57.4 ± 9.2	23.9 ± 4.2	18.6 ± 3.8
Obese hyperinsulinaemic patients NGT	24.1 ± 1.5[a]	78.4 ± 8.3[a]	69.6 ± 4.7[a]	70.6 ± 5.5[a]	102.8 ± 8.4[a]	69.3 ± 5.7[a]	18.6 ± 1.6
IGT	25.4 ± 2.2[a]	101.4 ± 6.9[a]	92.2 ± 7.6[a]	105.8 ± 7.2[a]	118.6 ± 8.7[a]	91.2 ± 6.9[a]	40.5 ± 2.1[a]
Type 2 diabetes	28.5 ± 1.9[a]	87.9 ± 5.8[a]	78.1 ± 5.6[a]	90.8 ± 7.9[a]	108.6 ± 9.2[a]	82.7 ± 7.1[a]	36.6 ± 2.1[a]
Plasma IRG (ng/l)							
Non-obese healthy subjects	59.7 ± 6.6	174.2 ± 23.8	231.9 ± 31.2	275.8 ± 32.8	284.1 ± 24.6	193.3 ± 27.4	81.8 ± 7.3
Obese normoinsulinaemic patients	65.0 ± 10.2	182.4 ± 19.4	251.1 ± 17.4	285.5 ± 27.6	309.5 ± 24.4	178.4 ± 21.4	84.6 ± 11.9
Obese hyperinsulinaemic patients NGT	60.5 ± 7.3	165.7 ± 31.1	219.5 ± 33.4	288.3 ± 34.4	272.9 ± 36.6	183.6 ± 19.9	78.3 ± 5.2
IGT	110.0 ± 22.4[a]	348.3 ± 38.8[a]	408.3 ± 48.3[a]	438.1 ± 51.8[a]	510.6 ± 50.2[a]	289.4 ± 37.7[a]	183.5 ± 21.1[a]
Type 2 diabetes	99.6 ± 19.8[a]	315.6 ± 31.0[a]	396.1 ± 43.3[a]	468.7 ± 48.2[a]	473.5 ± 29.8[a]	266.1 ± 35.6[a]	175.6 ± 16.7[a]

NGT, normal glucose tolerance; IGT, impaired glucose tolerance; IRI, immunoreactive insulin; IRG, immunoreactive glucagon.
Results are expressed as mean ± SEM. [a] $p < 0.05$ as compared with non-obese healthy subjects

Experiment (2): Infusion of high dose insulin during intravenous arginine infusion while blood glucose levels were clamped at values simulating those of the arginine infusion test with no insulin

Each patient from the obese hyperinsulinaemic IGT and Type 2 diabetic groups was evaluated. In this experiment, during arginine infusion, the same rates of insulin infusion required to overcome insulin resistance were performed by AEP. Blood glucose was continuously monitored and clamped at the levels observed in the arginine infusion test with no insulin infusion by infusing glucose with the aid of the AEP [2].

Method of blood sampling and hormone assays

Blood samples for hormone determinations were obtained from the antecubital vein, at 0, 5, 10, 15, 30, 45, 60, 90 and 120 min after initiation of the arginine infusion. Plasma IRI was measured by radioimmunoassay using the double-antibody technique [9]. For glucagon determination, blood was immediately placed into heparinized tubes containing 0.6 ml EDTA-Trasylol solution (Trasylol, Bayer, Leverkusen, FRG, 5000 U/ml; Na_2EDTA: 1.2 g/l). The blood samples were centrifuged for 15 min at 3000 rev/min, and the plasma samples were stored at −40°C until assay. Plasma immunoreactive glucagon (IRG) was measured by radioimmunoassay using OAL 123 Daiichi Radioisotope Institute, Tokyo, Japan [10], the antibody specific for pancreatic glucagon. Blood glucose was measured by the glucose oxidase method (AutoAnalyzer, Technicon, N.Y., USA).

Statistical analysis

Results were shown as mean ± SEM. Student's *t*-tests were used for statistical analysis.

Results

Insulin resistance in obese hyperinsulinaemic patients

As shown in Figure 1, the obese hyperinsulinaemic IGT and Type 2 diabetic groups showed the delayed hyper-response patterns of plasma IRI after an oral glucose load. IRG responses after oral glucose load were suppressed in the obese hyperinsulinaemic NGT, obese normoinsulinaemic patients and non-obese healthy subjects, but tended to rise in both obese hyperinsulinaemic IGT and Type 2 diabetic groups.

The mean values of glucose metabolized (M) in the obese hyperinsulinaemic NGT, IGT and Type 2 diabetic groups were significantly lower than those of the obese normoinsulinaemic patients and non-obese healthy subjects (5.4 ± 0.6, 2.3 ± 0.6 and 2.8 ± 0.6 vs 7.8 ± 1.1 and 9.9 ± 0.7 mg·kg^{-1}·min^{-1}, respectively, $p < 0.01$, Table 1) and there was no significant difference between the obese hyperinsulinaemic IGT and Type 2 diabetic groups.

Glucagon response to arginine in obese hyperinsulinaemic patients

As shown in Table 2, blood glucose, plasma IRI and plasma IRG responses in obese normoinsulinaemic patients were not statistically different from those in non-obese healthy subjects. In the obese hyperinsulinaemic NGT group, blood glucose concentrations and IRG responses

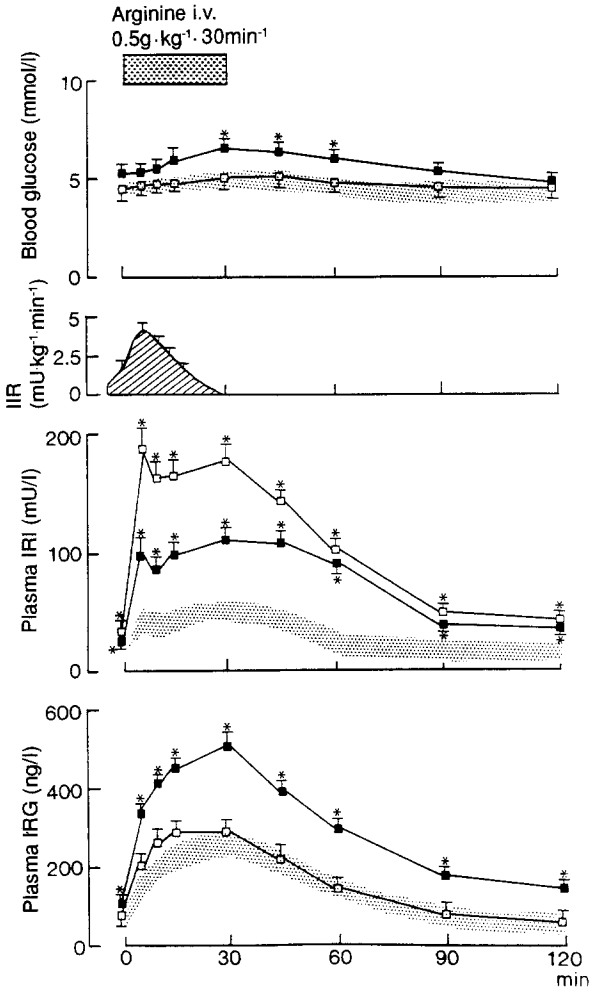

Fig. 2. Blood glucose, plasma immunoreactive insulin (IRI) and glucagon (IRG) responses during and after 30-min arginine infusion in six obese hyperinsulinaemic patients with impaired glucose tolerance under high dose insulin infusion using the artificial endocrine pancreas (□—□) and without insulin infusion (■—■). The mean values in 10 non-obese healthy subjects are also depicted (shaded area). IIR, insulin infusion rate. Mean ± SEM, * $p < 0.05$ vs non-obese healthy subjects

to arginine stimulation were not statistically different from those in non-obese healthy subjects and obese normoinsulinaemic patients.

In the obese hyperinsulinaemic IGT group, the mean blood glucose concentrations at 30, 45 and 60 min were statistically higher than the corresponding values of non-obese healthy subjects. Plasma IRG values rose rapidly from 110.0 ± 22.4 ng/l at 0 min to 510.6 ± 50.2 ng/l at 30 min, and were significantly higher than those of non-obese healthy subjects.

In the obese hyperinsulinaemic Type 2 diabetic group, the mean blood glucose concentrations at all sampling points were significantly higher than those of non-obese healthy subjects. Fasting plasma IRG levels were significantly higher than those of non-obese healthy subjects, but not different from those of the obese hyperinsulinaemic IGT group. Plasma IRG rose rapidly from 99.6 ± 19.8 ng/l at 0 min to 473.5 ± 29.8 ng/l at 30 min (Table 2).

Normalization of exaggerated glucagon response to arginine in obese hyperinsulinaemic patients

In the obese hyperinsulinaemic IGT group, after infusing high dose insulin, blood glucose responses were normalized as shown in Figure 2. The mean plasma IRI levels were increased to 193.3 ± 22.7 mU/l at 5 min and 184.0 ± 25.1 mU/l at 30 min. The amount of insulin infused during 30 min of arginine infusion was 4.2 ± 0.7 IU. By infusing high dose insulin, the exaggerated IRG response was normalized.

In the obese hyperinsulinaemic Type 2 diabetic group, after infusing high dose insulin, blood glucose responses were also normalized. The mean plasma IRI levels were increased to 185.8 ± 34.8 mU/l at 5 min and 174.2 ± 26.7 mU/l at 30 min. During 30 min of arginine infusion, 3.8 ± 0.5 IU of insulin was infused. The insulin infusion also normalized the IRG response (Fig. 3).

In the obese hyperinsulinaemic IGT and Type 2 diabetic groups, blood glucose concentrations were clamped while high dose insulin was infused at levels similar to those observed in the arginine infusion test with no insulin infusion. Even under these conditions, the exaggerated IRG responses in both groups were again normalized.

Discussion

It is widely accepted that the excessive glucagon secretion in response to arginine stimulation occurs in hypoinsulinaemic diabetic patients. Similar abnormal glucagon secretion to arginine has been reported in obese hyperinsulinaemic patients [3], but the opposite results have also been reported [11, 12]. In these reports, the degrees of glucose intolerance and obesity of the patients were not taken into consideration. We examined 120 obese patients (body mass index > 27 kg/m^2) and found that 60, 30 and 10% were hyperinsulinaemic, normoinsulinaemic and hypoinsulinaemic, respectively. In the hyperinsulinaemic obese patients, 60, 32 and 8% showed NGT, IGT and Type 2 diabetes, respectively. An excessive glucagon secretion during arginine infusion was found only in the obese hyperinsulinaemic patients with IGT and Type 2 diabetes, but not in patients with NGT. These results suggested that exaggerated glucagon responses to arginine were relevant to the degree of glucose intolerance in obese hyperinsulinaemic patients.

Kawamori et al. [1, 2] demonstrated that in hypoinsulinaemic Type 1 and Type 2 diabetic patients, the normalization of exaggerated glucagon response to arginine was achieved when plasma insulin concentrations simulated those of healthy subjects. Recently, Paolisso et al. [13, 14] reported that the arginine-induced hyperglucagon response in Type 1 diabetic patients was reduced greatly when insulin was administered in a pulsatile manner in an attempt to reproduce the pulsatile physiological release of insulin. These results support the concept that the exaggerated glucagon secretion to arginine in hypoinsulinaemic patients is secondary to their insulin deficiency.

However, it is not yet clear whether the exaggerated glucagon response observed in obese hyperinsulinaemic

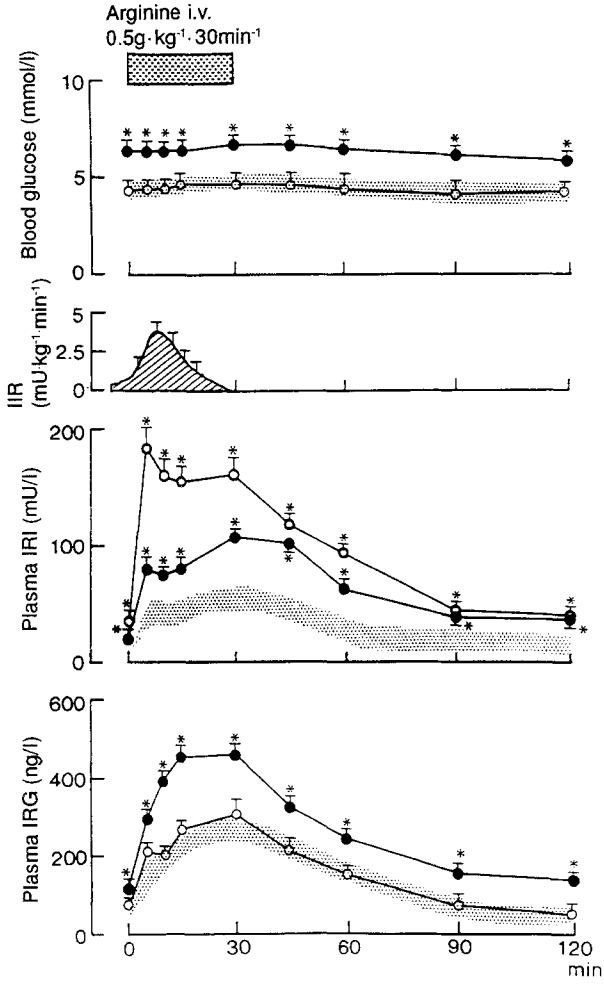

Fig. 3. Blood glucose, plasma immunoreactive insulin (IRI) and glucagon (IRG) responses during and after 30-min arginine infusion in six obese hyperinsulinaemic patients with Type 2 (non-insulin-dependent) diabetes mellitus under the high dose insulin infusion using the artificial endocrine pancreas (○—○) and without insulin infusion (●—●). Symbols and abbreviations used are the same as those used in Figure 2

patients can be normalized or not. Elahi et al. [15] have shown that hyperglucagonaemia in insulin resistant obese subjects declined during euglycaemic clamps, whereas Starke et al. [16] demonstrated in dogs the independency of direct insulin action on Alpha cells to the ambient glucose concentration. There is also evidence suggesting that the exaggerated glucagon response to arginine in obese patients may be independent of the plasma insulin concentrations [4]. This study was, therefore, designed to determine if the glucagon hyper-responsiveness to arginine in obese hyperinsulinaemic patients would be improved by a dosage of infused insulin high enough to overcome insulin resistance.

It was found that in obese hyperinsulinaemic patients with IGT and Type 2 diabetes, a previously exaggerated glucagon response to arginine could be normalized by infusing insulin at a high enough dose to overcome insulin resistance. The abnormal plasma glucose response was also normalized. To normalize the abnormal glucagon response, insulin doses of 4.2 ± 0.7 and 3.8 ± 0.5 IU were required in obese hyperinsulinaemic IGT and Type 2 diabetic patients, respectively (compared to 1.2 ± 0.3 IU in hypoinsulinaemic Type 1 diabetic patients [1, 2]), reaching peak IRI levels of three to four-fold higher than those observed in non-obese healthy subjects. Supplemented insulin doses were inversely correlated with the values of glucose metabolized (M), as an index of insulin resistance measured by the euglycaemic clamp method.

To clarify whether or not normalization of glycaemic excursions contributes to the normalization of the exaggerated glucagon response to arginine in these patients, blood glucose was clamped while high dose insulin was infused at the same levels as those observed in the arginine infusion test with no insulin infusion. As a result, normalization of the exaggerated plasma glucagon response was achieved, whether hyperglycaemia existed or not. These results clearly demonstrate that the exaggerated Alpha-cell function in obese hyperinsulinaemic patients with glucose intolerance is also secondary to the reduced insulin action on the pancreatic Alpha cell. We also found that in some of the obese hyperinsulinaemic IGT and Type 2 diabetic patients studied, paradoxic glucagon rises during OGTT could be normalized by the insulin infused at a high enough dose to overcome insulin resistance (data not shown). These results were also supported by the fact that in obese hyperinsulinaemic patients who succeeded in weight reduction, the previously elevated plasma glucagon responses to arginine and paradoxic rises in glucagon during OGTT were also reduced to the levels of those in non-obese healthy subjects, accompanied by significant improvements in blood glucose and IRI responses (data not shown).

The exact mechanism of normalization of an abnormal glucagon response to arginine infusion has not been elucidated. Samols et al. [17–19] suggested the involvement of a paracrine mechanism by which insulin could suppress glucagon in vivo and in vitro. Since then, the concept of normalizing an abnormal glucagon response to arginine using insulin has been discussed [16, 20–24]. The results of Asplin et al. [25] also support this concept. Asplin's study stated that, in non-obese non-diabetic patients, the overall effect on the pancreatic Alpha cell was a progressively greater acute glucagon response to arginine during the insulin infusions, probably due to a lesser Alpha-cell suppression by paracrine Beta-cell activity. However, in our present studies with obese hyperinsulinaemic IGT and Type 2 diabetic groups, high dose insulin infusion normalized the exaggerated glucagon response to arginine. Plasma C-peptide levels, however, during high dose insulin were suppressed, accompanied with the decrease in blood glucose (fasting and peak levels of plasma C-peptide levels in obese hyperinsulinaemic IGT patients: 1.1 ± 0.1 and 4.0 ± 0.3 nmol/l in arginine infusion test without insulin infusion, 0.9 ± 0.1 nmol/l and 3.0 ± 0.6 nmol/l in arginine and high dose insulin infusions, respectively). However, in the experiment with blood glucose concentrations clamped at levels similar to those of the arginine infusion test without insulin infusion, fasting and peak levels of plasma C-peptide were 1.0 ± 0.1 nmol/l and 3.8 ± 0.4 nmol/l. The same trend was observed in the obese hyperinsulinaemic Type 2 diabetic group. These present findings do not exclude the possibility of a para-

crine mechanism between islet cells. With the aid of the euglycaemic clamp technique, further studies on the glucagon responses to arginine with a high dose of insulin infusion in control groups, whether obese normoinsulinaemic or obese hyperinsulinaemic NGT groups are necessary to clarify the possibility of the paracrine mechanism for normalization of abnormal glucagon responses to arginine.

The regulatory mechanism of insulin on the Alpha cell is believed to be mediated by specific cell surface receptors, but this has not been investigated thoroughly. Our present results indirectly favour such a concept. Firstly, a dosage of infused insulin high enough to overcome insulin resistance could normalize the glucose response as well as the glucagon response to arginine infusion. Secondly, insulin doses high enough to overcome insulin resistance were found to be inversely correlated with the peripheral insulin resistance measured by the euglycaemic clamp technique. Patel et al. [26] demonstrated that the specific autoradiographic grains associated with radioactively labelled insulin were found on Alpha cells. However, by using receptor assay techniques, Van Schravendijk et al. [27] reported that no specific binding of insulin was detected on purified pancreatic Alpha cells, because of low receptor assay sensitivity and possible cellular damage inflicted by the cell isolation technique. Quantification and characterization of insulin receptors on the Alpha cell remain to be fully understood, especially concerning the possible physiological role.

In conclusion, our study shows that the exaggerated Alpha-cell response to arginine infusion in obese hyperinsulinaemic patients with glucose intolerance is secondary to the reduced insulin action on the pancreatic Alpha cell, and that expression of insulin action is very important to normalize these abnormalities. These findings are consistent with our earlier reports on the normalization of the exaggerated glucagon response to arginine [1, 2] and of the paradoxic rise of glucagon to an oral glucose load [28] in hypoinsulinaemic Type 1 and Type 2 diabetic patients.

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