Increased Nonoxidative Glucose Metabolism in Idiopathic Reactive Hypoglycemia

Frida Leonetti, Monica Foniciello, Patricia Iozzo, Oliviero Riggio, Manuela Merli, Paola Giovannetti, Paolo Sbraccia, Andrea Giaccari, and Guido Tamburrano

Idiopathic reactive hypoglycemia (IRH) is responsible for postprandial hypoglycemia. Normal insulin secretion and reduced response of glucagon to acute hypoglycemia, but mostly increased insulin sensitivity, represent the metabolic features of this syndrome. The present study has two aims: first, to investigate the fate of glucose utilization inside the cells to assess whether increased glucose disposal in IRH is due to the oxidative and/or nonoxidative pathway; and second, to evaluate glucagon response to prolonged insulin-induced hypoglycemia. In eight patients with IRH and eight normal (N) subjects, we performed two studies on different days: (1) 120-minute euglycemic-hyperinsulinemic (1.0 mU · kg^{-1} · min^{-1} regular human insulin) clamp associated with indirect calorimetry; and (2) 180-minute hypoglycemic (2.22 to 2.49 mmo/L achieved through 0.85 mU · kg^{-1} · min^{-1} intravenous [IV] regular human insulin) clamp. The results showed an increased insulin-mediated glucose uptake in IRH (9.10 ± 0.19 *v* 6.78 ± 0.18 mg · kg^{-1} · min^{-1}, $P < .005$). Glucose oxidation was similar in IRH subjects and controls both in basal conditions (1.39 ± 0.16 *v* 1.42 ± 0.15 mg · kg^{-1} · min^{-1}) and during the clamp studies (2.57 ± 0.21 *v* 2.78 ± 0.26 mg · kg^{-1} · min^{-1}). In contrast, nonoxidative glucose disposal was significantly higher in IRH than in N subjects (6.53 ± 0.30 *v* 4.00 ± 0.21 mg · kg^{-1} · min^{-1}, $P < .001$). During insulinization, fat oxidation was reduced slightly more in IRH than in control subjects. During the hypoglycemic clamp, a significant ($P < .01$) increase in plasma glucagon concentrations was observed in normal subjects as compared with baseline, whereas no change occurred in IRH patients. In conclusion, in IRH: (1) increased insulin-mediated glucose disposal is due to the increase of nonoxidative glucose metabolism; and (2) glucagon secretion has been confirmed to be inadequate. The increase of insulin sensitivity associated with a deficiency in glucagon secretion can widely explain the occurrence of hypoglycemia in the late postprandial phase.

IN THE POSTPRANDIAL state, the transitory phase from the utilization of exogenous to endogenous substrates is especially related to hepatic glucose production as the main energy source. Dissipation of insulin and activation of counterregulatory hormones, mainly glucagon, are necessary to ensure euglycemia through hepatic glycogenolysis.[1]

Postprandial hypoglycemia is due to the presence of idiopathic reactive hypoglycemia (IRH), which, as established in 1987,[2,3] is a syndrome characterized by biochemical blood glucose less than 2.5 mmol/L and symptomatic hypoglycemia occurring after a meal, increased insulin sensitivity,[4-6] and inadequate glucagon secretion.[4,7] Our previous study[6] showed that replacement of basal normal plasma glucagon concentrations allowed a normalization of the glucose requirement during the euglycemic-hyperinsulinemic clamp in IRH patients. Whether this effect of glucagon occurred in the liver and/or in peripheral tissues is still unclear, because the ability of glucagon to affect insulin-mediated glucose uptake on muscle is still controversial.[8-11]

Once inside the cell, glucose disposal has two major fates: (1) oxidation to carbon dioxide and water; and (2) storage as glycogen. The latter process, together with a minor contribution of anaerobic glycolysis and pentose shunts, represents nonoxidative glucose metabolism.[12]

This study was planned to assess the role of oxidative and nonoxidative glucose disposal in the increased insulin-mediated glucose uptake of IRH subjects. Furthermore, a new contribution to the knowledge of glucagon secretion has been investigated by studying α-cell activity during prolonged hypoglycemia in these patients.

SUBJECTS AND METHODS

The subjects were selected from patients referred to our Institute for suspected postprandial hypoglycemia on the basis of accurate case histories and blood glucose measurements performed at least twice in their daily life at the onset of symptoms, using a portable device for home glucose monitoring (Glucometer II; Ames, Elkart, IN). The cutoff point for low plasma glucose (≤2.49 mmol/L) was chosen in accordance with the statement on IRH.[2,3]

Eight patients (IRH) with postprandial hypoglycemia (age, 37.8 ± 2.3 years; body mass index (BMI), 21.5 ± 0.5 kg/m^2) and eight normal (N) volunteers as the control group (age, 31.6 ± 3.2 years; BMI, 20.8 ± 0.6 kg/m^2) were included in the study. Patients and controls were matched either for gender or for physical activity. Athletes were excluded from the protocol, since they usually have an elevated insulin-mediated glucose utilization. All individuals were on a weight-maintaining diet containing approximately 250 g carbohydrate/d for 3 days before each test. Female participants were in the follicular phase of the menstrual cycle.

None of the subjects suffered from diabetes, impaired glucose tolerance, hypertension, malignancy, or liver or endocrine disease, or had a family history of diabetes mellitus. None were taking any medication; informed consent was obtained in accordance with the Helsinki Declaration.[13]

Experimental Protocol

All studies were performed after a 12-hour overnight fast.

Study 1. All subjects underwent a 5-hour oral glucose tolerance test (OGTT) with 75 g glucose (Curvosio 50%; Sclavo Diagnostic, Siena, Italy). Plasma glucose levels were evaluated with a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA) on

From the Servizio Speciale Emergenze Metaboliche, Cattedra di Gastroenterologia 2, Universita "La Sapienza," Rome, Italy.

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Address reprint requests to Frida Leonetti, MD, II Clinica Medica, Policlinico Umberto I, Viale del Policlinico, 00161 Rome, Italy.

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arterialized venous samples obtained while keeping the hand bearing the sampling line in a heated box (65°C). Plasma insulin (IRI), glucagon (IRG), and cortisol were determined as previously described,[14-16] in basal conditions and every 30 minutes after the exose intake.

Study 2. After 1 week, each subject underwent euglycemic-hyperinsulinemic clamp study (1.0 mU · kg^{-1} · min^{-1} human regular insulin) as previously described.[17] Arterialized blood samples obtained via a teflon catheter (Abbocath) inserted retrogradely into a wrist vein and kept patent using an infusion of isotonic saline were drawn every 20 minutes to measure IRI, IRG, free fatty acid (FFA), and glycerol concentrations. FFAs and glycerol were assessed by spectrophotometric methods.[18,19]

In brief, during 120 minutes of insulin infusion, euglycemia was maintained by a variable infusion of 20% glucose, determined by measurements of plasma glucose at 5-minute intervals. In these experimental conditions, glucose requirement equals glucose uptake by all tissues.

Whole-body glucose uptake (GU) was calculated during the last part (80 to 120 minutes) of the clamp. Twenty-five minutes before the glucose/insulin infusion and during the steady-state period of the clamp (80 to 120 minutes), a calorimeter (MMC Horizon System; Sensor Medics, Anaheim, CA) with a canopy system was used to measure respiratory gas exchange to calculate glucose (Gox), lipid (Fox), and protein (Pox) oxidation rates as previously described.[21] Continuous measurements of O_2 consumption ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) were computed, and a printout was obtained at 2-minute intervals. The average $\dot{V}O_2$ and $\dot{V}CO_2$ was used to calculate the energy production rate (EPR). Timed urine samples were collected twice between 6 AM and noon (before and after insulin infusion), and urinary nitrogen excretion was determined using the NA Automatic Analyzer (Carlo Erba Instruments). Then this value was used to calculate the nonprotein respiratory quotient (npRQ). All calculations were performed using a built-in computer.

Resting energy expenditure was also predicted in each subject, using the Harris-Benedict equation[20] and the regression equation developed by Owen et al.[21]

Nonoxidative glucose disposal (G nonox) was calculated as the difference between whole-body glucose uptake (GU) and glucose oxidation (G nonox = GU − Gox).

Study 3. On a different day, five subjects of each group underwent a hypoglycemic clamp with a constant infusion of 0.85 mU · kg^{-1} · min^{-1} human regular insulin for 180 minutes.

Arterialized blood glucose was clamped at 2.22 ± 0.2 mmol/L and so preserved with a variable infusion of 20% glucose, determining plasma glucose at 5-minute intervals. During the study, blood samples were collected every 30 minutes to evaluate glucagon concentrations. The test was planned to be interrupted if severe neuroglycopenia occurred.

Statistical Methods

Results are expressed as the mean ± SE. Statistical analysis was performed with Student's *t* test for paired or unpaired data: *P* values less than .05 were considered statistically significant.

RESULTS

Study 1

Mean basal plasma glucose concentrations (4.86 ± 0.13 and 4.69 ± 0.13 mmol/L in N and IRH, respectively) were similar in both groups. All patients with IRH had plasma glucose values during the OGTT of not greater than 2.49 mmol/L associated with symptoms attributable to neuroglycopenia (ie, headache, confusion, blurred vision, paresthesia, and dizziness) and, to a minor extent, to sympathetic activity (palpitations, sweating, anxiety, tremor, and hunger). N subjects did not complain of any symptom during the test. Mean glycemic nadirs of 2.47 ± 0.01 mmol/L at 211 ± 10 minutes and of 3.60 ± 0.15 mmol/L at 250 ± 20 minutes occurred in IRH and control subjects, respectively.

Plasma insulin levels both basally (57.9 ± 8.8 and 54.6 ± 8.1 pmol/L in N and IRH, respectively) and during the OGTT (Fig 1) were similar in the two groups. Basal plasma glucagon (98.9 ± 12.5 and 91.3 ± 14.0 ng/L in N and IRH) and cortisol (342 ± 54 and 376 ± 12.5 nmol/L in N and IRH) concentrations were normal in the IRH group. However, during the OGTT (Fig 1), a different response to the glycemic nadir occurred between patients and control

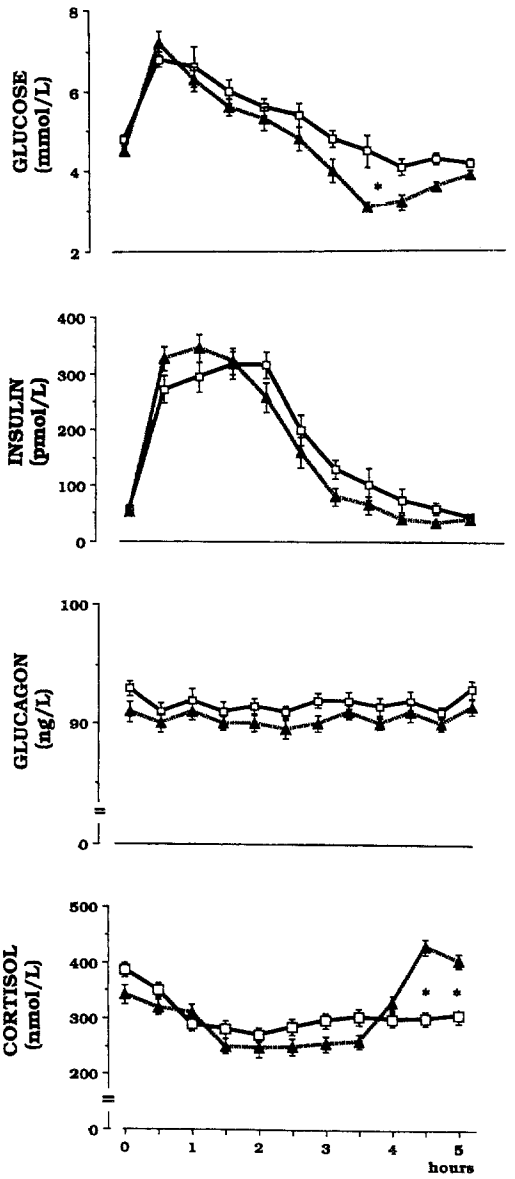

Fig 1. Plasma glucose, insulin, glucagon, and cortisol in IRH patients (▲) and N subjects (□) during a 5-hour OGTT. *P < .01 v normals.

subjects. In fact, a significant ($P < .005$) increase of cortisol levels in the fifth hour, without any increment of glucagon concentrations throughout the test, was observed only in IRH patients.

Study 2

Steady-state plasma glucose and insulin concentrations were comparable in both groups, and the coefficients of variation for glycemia were less than 6% in each clamp study. In the same interval, plasma glucagon levels (Table 1) slightly decreased in both groups.

During the clamp studies, concentrations of FFA and glycerol were suppressed by 68% and 35%, respectively, in N subjects, whereas in IRH patients this suppression was 77% for FFA and 31% for glycerol (Table 1) as compared with baseline.

Insulin-mediated glucose uptake (Fig 2) was significantly higher ($P < .001$) in patients with IRH than in the control group (9.10 ± 0.19 *v* 6.78 ± 0.18 mg · kg^{-1} · min^{-1}, respectively). Glucose oxidation (Fig 2) was similar in both groups in basal conditions (1.39 ± 0.16 and 1.42 ± 0.15 mg · kg^{-1} · min^{-1} in N and IRH, respectively). In contrast, nonoxidative glucose disposal (Fig 2) was significantly higher ($P < .005$) in IRH than in N subjects (6.53 ± 0.30 *v* 4.0 ± 0.21 mg · kg^{-1} · min^{-1}, respectively).

In the steady-state period of the study, the npRQ (Table 1) increased similarly in both groups from 0.85 ± 0.01 to 0.98 ± 0.04 in IRH and from 0.85 ± 0.01 to 0.98 ± 0.03 in controls.

Protein and fat oxidation (Table 1) were normal in IRH subjects in the basal state, as well as during the clamp, although fat oxidation tended to be slightly lower in IRH in the last phase of the study.

Study 3

After the insulin infusion was started, hypoglycemia was observed at 18 ± 5 minutes in patients and at 29 ± 8 minutes in N subjects, and glycemia was clamped at 2.42 ± 0.02 mmol/L throughout the test in all subjects. No one showed severe neuroglycopenia, and therefore, the test was entirely run in each individual.

Plasma glucagon concentrations after the same degree of hypoglycemia significantly ($P < .01$) increased to 189% of baseline only in N. In contrast, no increment of plasma glucagon levels was observed in IRH patients during the hypoglycemic phase of the clamp (Fig 3).

DISCUSSION

Hypoglycemia in IRH patients throughout their daily life is to be ascribed to an imbalance between glucose utilization and production during the late postprandial phase.[1] Hypoglycemic symptoms in the fasting state are not reported by these patients, and the clinical picture is generally ameliorated by a carbohydrate-restricted diet.[22] Therefore, stimulation of insulin secretion by sugar-rich meals is required to provoke symptoms, which explains why the syndrome is defined as "reactive."[2]

The pathogenesis of IRH was initially attributed to a higher and delayed peak in plasma insulin concentrations during the OGTT, which was adduced to be present in these patients with regard to normal subjects.[23] Subsequently, more accurate studies showed the presence of a normal insulin secretion in this syndrome.[4,23]

Results emerging from our previous studies[4,6] confirm this assumption while pointing out that patients with IRH display increased insulin-mediated peripheral glucose uptake, as evaluated through the glucose clamp technique. This alteration itself could explain, at least in part, the occurrence of hypoglycemia in the late postprandial phase, when the decrease of both insulin blood levels and action are required to promote hepatic glucose production.

Also, the role of counterregulatory hormones in enhancing hepatic glycogenolysis should be emphasized, insofar as an impaired glucagon response to decreasing glycemia could explain the onset of postprandial hypoglycemia in the presence of increased insulin sensitivity.

The present study confirms the existence of a reduced glucagon response to both prolonged and OGTT-induced

Table 1. Euglycemic-Hyperinsulinemic (1.0 mU/kg · min) Clamp (mean ± SE)

Variable	Basal State		Steady State	
	N (n = 8)	IRH (n = 8)	N (n = 8)	IRH (n = 8)
Glucose level (mmol/L)	4.91 ± 0.07	4.85 ± 0.19	4.85 ± 0.04	4.95 ± 0.09
Insulin (pmol/L)	51.4 ± 4.2	55.6 ± 3.6	498 ± 15.6	516 ± 18.4
Glucagon (ng/L)	103 ± 17.8	84.3 ± 10.7	83.6 ± 9.6	63.6 ± 7.5
FFA (mmol/L)	396 ± 38	400 ± 52	126 ± 13	93 ± 18
Glycerol (mmol/L)	112 ± 12	122 ± 10	72 ± 16	83 ± 17
Glucose parameters (mg · kg^{-1} · min^{-1})				
Uptake	—	—	6.78 ± 0.18	9.10 ± 0.19*
Oxidation	1.42 ± 0.15	1.39 ± 0.16	2.78 ± 0.26	2.57 ± 0.21
Nonoxidation	—	—	4.00 ± 0.21	6.53 ± 0.30*
Fat oxidation (mg · kg^{-1} · min^{-1})	0.84 ± 0.09	0.87 ± 0.1	0.32 ± 0.1†	0.21 ± 0.1†
Protein oxidation (mg · kg^{-1} · min^{-1})	0.68 ± 0.1	0.96 ± 0.2	0.69 ± 0.1	0.96 ± 0.1
RQ ($\dot{V}co_2/\dot{V}o_2$)	0.85 ± 0.01	0.84 ± 0.01	0.98 ± 0.03	0.98 ± 0.04

*$P = .001$ *v* N.

†$P < .01$ *v* basal state.

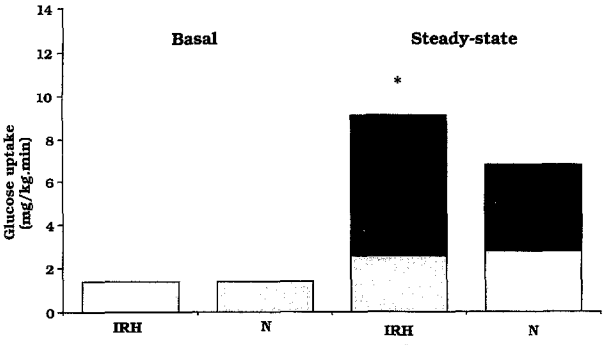

Fig 2. Basal glucose oxidation and total glucose uptake, divided into oxidative (□) and nonoxidative (▥) glucose disposal during a hyperinsulinemic-euglycemic clamp in IRH and N groups. *P < .001 v N.

hypoglycemia in patients with IRH.[4,6] In fact, a partial glucagon secretion deficiency has already been reported[4,6] in IRH patients, raising the question of whether increased insulin sensitivity is the cause and/or the effect of the glucagon lack.

In support of the first hypothesis, the pancreatic α-cell surface has been demonstrated to express inhibitory insulin binding sites.[24] Therefore, increased insulin sensitivity would reduce the glucagon response to hypoglycemia, as well as to specific stimuli, ie, arginine.[7]

In favor of the second hypothesis, high plasma glucagon concentrations have been documented in non–insulin-dependent diabetes mellitus and are considered to be at least in part responsible for the insulin resistance of this metabolic disease.[25] Similar conclusions have been reached by comparing totally pancreatectomized and type I diabetic patients.[26] Moreover, somatostatin therapy, by inhibiting glucagon secretion, improves insulin sensitivity in diabetes.[26] Furthermore, Del Prato et al[11] showed that a 48-hour continuous glucagon infusion induces a 20% decrease in peripheral insulin-mediated glucose uptake. However, when direct effects of glucagon on muscle have been investigated through the forearm-balance technique,[9,10] only a small variation, if any, has been found.

According to the literature, no physiologic or pathologic condition reproduces the same metabolic picture found in IRH, but physical exercise and anorexia nervosa show an increased insulin sensitivity.[27-29]

In a previous study,[4] we concluded that an increased insulin sensitivity in the presence of a normal insulin response to a glucose load should contribute to the pathogenesis of IRH. Subsequently, we asserted that an increased glucose requirement during the hyperinsulinemic clamp appears to be completely normalized through glucagon replacement in these patients.[6]

Our present study clearly points out that the increased insulin-mediated glucose uptake observed in IRH is entirely due to the nonoxidative glucose pathway, proving that insulin sensitivity changes are to be ascribed to nonoxidative glucose metabolism. The opposite occurs in the presence of insulin-resistant syndromes, which are mainly linked to a deficit in the nonoxidative glucose compartment.[30] Actually, glucose oxidation is normal in IRH patients both in the basal state and during experimental insulinization.

Since it is well known that peripheral insulin-mediated glucose uptake is mainly linked to muscle tissue, we have to emphasize the fact that glycogen synthase activity, as the expression of glycogen synthesis, is the crucial step regulating nonoxidative glucose disposal, particularly in the insulinization state. Furthermore, if we consider the opposite effects of insulin and glucagon on this enzyme, we can speculate that increased insulin sensitivity associated with a

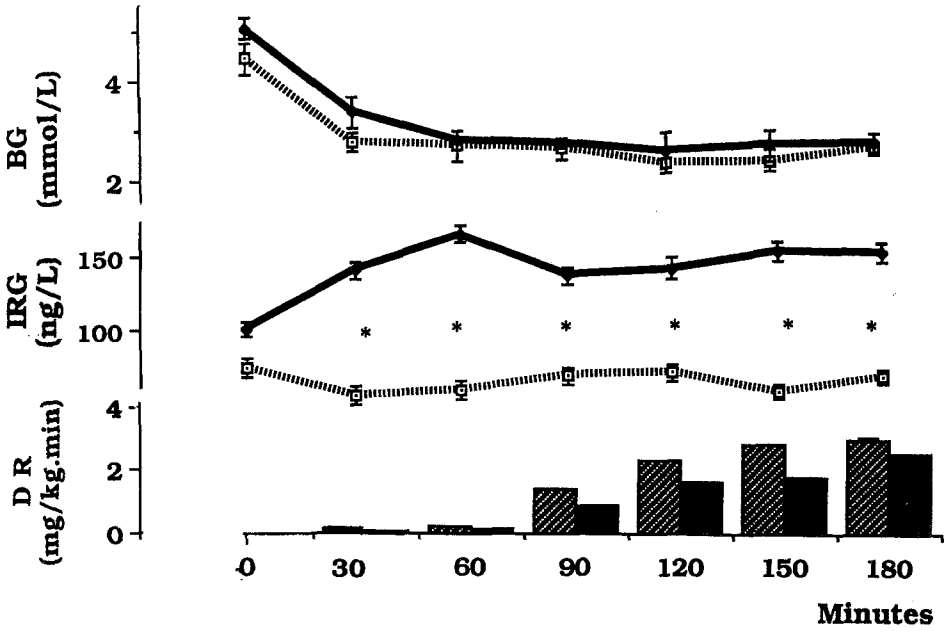

Fig 3. Plasma glucose (BG) and glucagon (IRG) associated with glucose requirement (DR) during a 3-hour hypoglycemic clamp in IRH (---, ▨) and N subjects (=, ■). *P < .05 v N.

partial glucagon deficiency leads to an increment of glycogen synthase activity. The effect of this enzyme enhances glycogen synthesis, thus increasing nonoxidative glucose disposal in the muscle, while at the same time reducing hepatic glucose production through an inhibition of glycogenolysis in the liver. Whether the augmented activity of this enzyme could positively influence peripheral glucose uptake remains unknown.

In conclusion, the present study demonstrates that in patients with IRH, increased insulin-mediated glucose uptake is entirely due to an enhanced nonoxidative glucose disposal, while oxidative glucose metabolism remains unaltered; partial glucagon secretion deficiency occurs also during prolonged hypoglycemia. These data suggest the hypothesis that an augmented insulin sensitivity and a partial glucagon deficit would contribute to the stimulation of glycogen synthase activity leading to the increase of nonoxidative glucose metabolism.

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