Hyperinsulinemia in the Physiologic Range Is Not Superior to Short-Term Fasting in Suppressing Insulin Secretion in Obese Men

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The negative-feedback control exerted by plasma insulin on β-cell insulin release in normal-weight and obese subjects is still a matter of debate. Subjects submitted to a euglycemic insulin clamp undergo a suppression of insulin secretion that is due to both the infused insulin and the 2- to 3-hour fast during the procedure. We elected to elucidate the role of physiologic hyperinsulinemia per se in the insulin negative autofeedback in obese men. Ten men with massive uncomplicated obesity (age, 18 to 37 years; body mass index [BMI], 41 ± 1.15 kg/m^2) and 6 normal-weight healthy men (age, 22 to 30 years; BMI, 22 ± 0.28 kg/m^2) underwent 2 studies in random order: (1) a euglycemic-hyperinsulinemic glucose clamp with an insulin infusion rate of 1 mU/kg/min and (2) a control study with saline infusion. Serum C-peptide concentrations were significantly higher in obese versus control subjects at baseline (2.54 ± 0.178 *v* 1.63 ± 0.256 ng/mL, *P* < .05). Exogenous insulin infusion significantly suppressed serum C-peptide at steady state ([SS] last 30 minutes of insulin or saline infusion) in controls (mean of the last 4 measurements from 120 minutes to 150 minutes, 0.86 ± 0.306 ng/mL, *P* < .05 *v* baseline) but not in obese patients (2.03 ± 0.26 ng/mL, nonsignificant [NS] *v* baseline). During the saline infusion studies, C-peptide levels slightly and similarly declined over time in both groups (2.71 ± 0.350 at baseline *v* 2.31 ± 0.300 ng/mL at SS in obese patients, NS, and 1.96 ± 0.189 *v* 1.62 ± 0.150 ng/mL in controls, NS). This study shows that in obese men hyperinsulinemia within the postprandial range is not superior to a 2.5-hour fast for the suppression of β-cell activity, suggesting an impairment of the insulin negative autofeedback in this clinical condition.

THE NEGATIVE-FEEDBACK interaction between plasma insulin and β-cell insulin release in normal-weight and obese patients has been the subject of a still unsettled debate. In vitro studies both support[1] and refute[2] the existence of an insulin-negative feedback interaction. Studies in normal humans using the euglycemic-hyperinsulinemic glucose clamp method at a variable insulin infusion rate and plasma C-peptide level as indicators of insulin secretion have also provided conflicting results.[3-5] In obese individuals compared with lean subjects, Elahi et al,[6] Cavallo-Perin et al,[7] and our group[8] reported a subnormal reduction in plasma C-peptide levels during euglycemic hyperinsulinemia, while others[9,10] reported a similar suppression of the peptide. The existence of an insulin autofeedback mechanism is of particular importance, since its impairment might play a role in the hyperinsulinemia of obesity.[11] Subjects submitted to a euglycemic insulin clamp undergo a suppression of β-cell secretion due to both the administered insulin[12] and the 2- to 3-hour fast during the procedure, since starvation per se reportedly induces a decline in insulin secretion both in animals[13] and in lean or obese humans.[14,15]

None of the previous studies investigating insulin autofeedback in obesity have tried to establish the role of physiologic hyperinsulinemia independently of fasting in the suppression of insulin secretion. To shed further light on this issue in obese patients and normal-weight healthy subjects, we evaluated insulin secretion, as indicated by serum C-peptide levels, during a euglycemic glucose clamp allowing postprandial circulating insulin levels and during a saline-controlled study.

SUBJECTS AND METHODS

Ten men with massive uncomplicated obesity (age, 18 to 37 years; mean ± SEM, 27.8 ± 2.25; body mass index [BMI], 41 ± 1.15 kg/m^2) and 6 normal-weight healthy men (age, 22 to 30 years; mean ± SEM, 25.0 ± 1.41; BMI, 22 ± 0.28 kg/m^2) were studied. All obese patients had a waist to hip ratio greater than 0.90. None of the subjects were hypertensive, had a family history of diabetes, or used medications known to affect carbohydrate metabolism. Tests for liver, kidney, and thyroid function were normal, as well as carbohydrate tolerance following standard 75-g oral glucose load. All subjects were fed a weight-maintaining balanced diet and consumed at least 300 g carbohydrate daily over the 3 days preceding the study. The experimental nature and potential risks of the study were explained to the subjects, who provided informed consent to participate. The protocol was approved by the Ethical Committee of our Institution.

All patients and controls were admitted to our department 14 hours before initiation of each study. The previous evening, they consumed a standard dinner (55% carbohydrate, 30% fat, and 15% protein) between 7:00 and 8:00 PM. After an overnight fast and beginning at 8:00 to 8:30 AM, each subject underwent 2 studies in random order and at least 1 week apart: (1) a euglycemic-hyperinsulinemic glucose clamp as described by DeFronzo et al[16] and (2) a control study with saline infusion.

Study 1

A 19-gauge, 24-inch intravenous catheter was inserted into an antecubital vein for insulin, 20% glucose, and potassium infusion. Insulin (Actrapid HM U-40; Novo Nordisk, Bagsvaerd, Denmark) diluted to 1 U/mL in 2 mL of the subject's whole blood and 0.9% NaCl to a final volume of 100 mL was infused for 150 minutes at the rate of 1 mU · kg^{-1} · min^{-1} (1 nmol = 162 mU) to achieve high physiologic circu-

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lating insulin levels. During insulin infusion, glucose was infused at a variable rate to maintain blood glucose at fasting baseline levels according to the principle of the glucose clamp technique. Hypokalemia was prevented by administration of KCl infused at a rate of 10 mmol/h. To obtain arterialized venous blood samples, a contralateral hand vein was cannulated in a retrograde fashion with an indwelling catheter, and the hand was maintained at 60° to 65°C in a thermoregulated Plexiglass (Rohm & Haas, Philadelphia, PA) box.

Study 2

All patients and controls underwent identical procedures except that insulin, glucose, and potassium were substituted with matched quantities of 0.9% saline. When saline studies preceded insulin clamp experiments, the volume of saline for infusion was calculated by inferring a glucose infusion rate during the steady-state (SS) period (M value) of approximately 2.5 mg/kg/min for the obese and 7.5 mg/kg/min for normal-weight subjects. This assumption was based on our previous studies in obese or normal-weight subjects of comparable BMI[8] and was in fact confirmed by the data of the subsequent glucose clamp studies.

Serial blood samples were drawn at baseline after a minimum of 30 minutes of rest following cannulation and at regular intervals for the following 150 minutes for the estimation of blood glucose (BG), immunoreactive insulin (IRI), C-peptide, and free fatty acid (FFA) concentrations.

Analytical Procedures

BG was determined immediately after sampling by the glucose oxidase method on a glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). The intraassay coefficient of variation for this method is less than 3%. Blood samples were collected on ice, centrifuged under refrigeration within 20 minutes, and separated. They were stored at −20°C until analysis. Serum insulin and C-peptide were determined by radioimmunoassay using commercial kits (Incstar, Stillwater, MN, for insulin and Technogenetics, Milan, Italy, for C-peptide). The sensitivity of the assay is 2 μU/mL for insulin and 0.08 ng/mL for C-peptide; the interassay and intraassay coefficient of variation is 8.2% and 4.2% for insulin and 7.3% and 4.5% for C-peptide. Serum FFAs were determined using an acyl-coenzyme A oxidase-based colorimetric kit (Wako, Osaka, Japan). The interassay and intraassay coefficient of variation is 4.1% and 1.6%, respectively, and the normal range for our laboratory is 100 to 600 μEq/L.

Data Analysis

The last 30 minutes of insulin or saline infusion was considered as the SS period, and the SS values were calculated as the average of the last 4 measurements (120, 130, 140, and 150 minutes). The glucose infusion rate during the SS period (M value) was taken as an index of whole-body insulin sensitivity.[16] The metabolic clearance rate of insulin (MCR_i) during the euglycemic-hyperinsulinemic glucose clamp was calculated using the formula,[17] MCR_i = insulin infusion rate $\times 10^3$/[$insulin_{ss}$-($insulin_{basal} \times$ C-$peptide_{ss}$/C-$peptide_{basal}$)], where $insulin_{ss}$ represents the mean serum insulin at SS and the ratio in the denominator represents the correction for changes in endogenous insulin secretion induced by exogenous insulin infusion.

Statistical Methods

The data are presented as the mean ± SEM. Statistical analysis was performed by ANOVA for repeated measurements (StatView II; Abacus Concepts, Calabasas, CA). A *P* value less than .05 was considered statistically significant.

RESULTS

Study 1: Euglycemic-Hyperinsulinemic Glucose Clamp

Fasting BG levels were similar in obese and lean subjects and did not change during the SS period of the clamp (baseline *v* SS, 87 ± 1.9 *v* 87 ± 2.7 mg/dL and 87 ± 2.1 *v* 84 ± 2.6 mg/dL in obese and control subjects, respectively; Table 1). The coefficient of variation of BG during the insulin clamp was 5.1% ± 0.63% in the obese and 6.0% ± 0.65% in lean subjects (nonsignificant [NS]).

Baseline serum insulin was slightly higher in obese versus control subjects, whereas the level at SS of the clamp was similar in both groups (100 ± 4.4 *v* 104 ± 6.6 μU/mL, respectively, NS; Table 1). The MCR_i was 11.8 ± 0.60 versus

Table 1. BG, Serum IRI, Rate of Infusion of Exogenous Glucose (M value), Serum C-Pep, and Serum FFAs at Baseline and During a Euglycemic-Hyperinsulinemic Clamp in 10 Obese Patients and Six Normal-Weight Healthy Subjects

	Time (min)							
Parameter	0	30	60	90	120	130	140	150
BG (mg/dL)								
Patients	87 ± 1.9	82 ± 2.7	84 ± 3.4	89 ± 2.7	88 ± 2.5	87 ± 3.2	86 ± 2.9	87 ± 2.7
Controls	87 ± 2.1	82 ± 3.2	82 ± 4.3	85 ± 1.8	83 ± 2.2	84 ± 2.6	84 ± 3.2	84 ± 3.0
IRI (μU/mL)								
Patients	15 ± 1.7	102 ± 4.6*	101 ± 5.6*	100 ± 3.9*	100 ± 3.5*	98 ± 5.5*	101 ± 4.9*	102 ± 4.9*
Controls	11 ± 2.9	91 ± 16.3*	92 ± 5.3*	108 ± 6.6*	104 ± 8.2*	106 ± 6.8*	103 ± 6.9*	104 ± 6.5*
M value ($mg \cdot kg^{-1} \cdot min^{-1}$)								
Patients	0	0.8 ± 0.12*	2.2 ± 0.26*†	2.4 ± 0.34*†	2.3 ± 0.28*†	2.4 ± 0.28*†	2.5 ± 0.33*†	2.5 ± 0.33*†
Controls	0	1.4 ± 0.36*	5.9 ± 0.76*	7.1 ± 0.43*	7.4 ± 0.45*	7.5 ± 0.44*	7.5 ± 0.36*	7.6 ± 0.33*
C-peptide (ng/mL)								
Patients	2.54 ± 0.178†	2.19 ± 0.206†	2.12 ± 0.328†	2.23 ± 0.245†	2.23 ± 0.268†	2.21 ± 0.304†	1.84 ± 0.234*†	1.86 ± 0.26*†
Controls	1.63 ± 0.256	1.24 ± 0.293*	1.07 ± 0.316*	1.07 ± 0.294*	0.96 ± 0.331*	0.94 ± 0.327*	0.84 ± 0.287*	0.71 ± 0.282*
FFA (μEq/L)								
Patients	703.7 ± 59.18†	625.7 ± 73.67†	451.0 ± 60.55*†	309.5 ± 62.33*†	253.6 ± 49.99*†	214.5 ± 47.67*	201.1 ± 40.63*	172.82 ± 46.13*
Controls	433.6 ± 67.31	294.6 ± 49.30*	153.9 ± 22.69*	98.96 ± 28.33*	86.7 ± 24.99*	100.7 ± 24.31*	96.44 ± 30.86*	87.8 ± 20.02*

NOTE. Values are the mean ± SEM.

*$P < .05$ *v* baseline.

†$P < .05$ *v* controls.

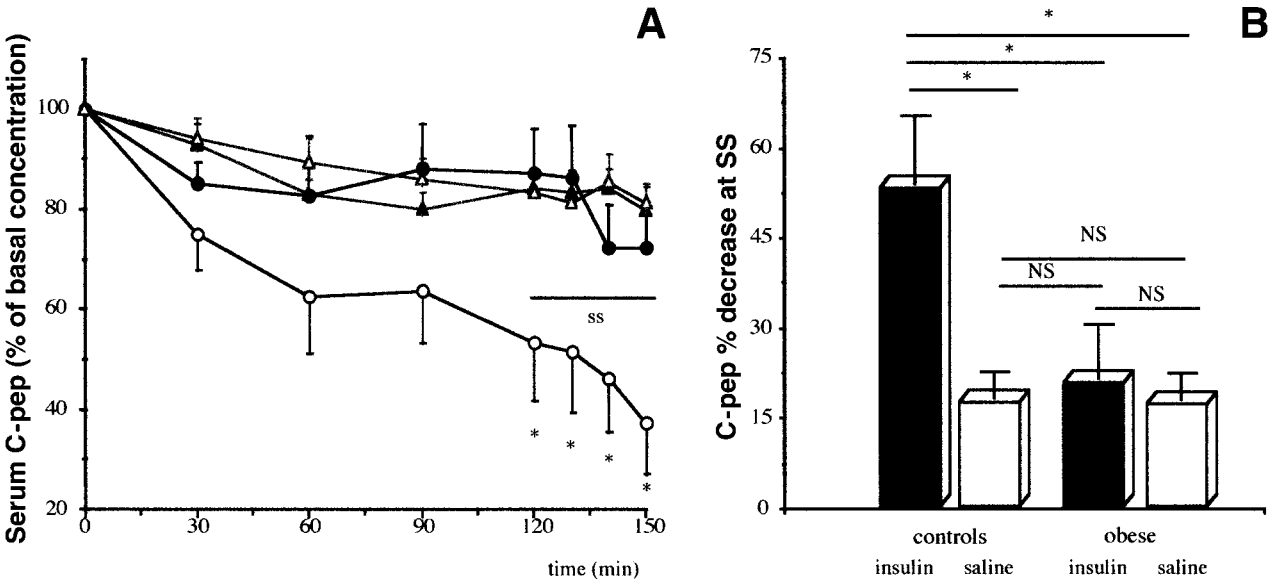

Fig 1. (A) Serum C-peptide (C-pep) expressed as percent of basal concentration during insulin clamp or saline infusion in obese patients (● and ▲, respectively) and control subjects (○ and △, respectively). *$P < .05$, controls undergoing insulin clamp studies *v* all other groups. (B) C-pep percent decrease at SS in obese patients and control subjects undergoing insulin clamp (■) or saline infusion (□). *$P < .05$.

12.5 ± 0.89 mL · kg^{-1} · min^{-1} (NS) in obese and normal-weight subjects, respectively.

The rate of exogenous glucose infusion (M value) needed to maintain euglycemia at SS was significantly higher in controls than in obese patients (7.5 ± 0.39 *v* 2.4 ± 0.30 mg · kg^{-1} · min^{-1}, respectively, $P < .05$; Table 1).

Serum C-peptide concentrations were significantly higher in obese versus control subjects at baseline, with a molar ratio between C-peptide and insulin of 8.6 ± 0.77 versus 9.5 ± 1.81, respectively (NS). Exogenous insulin infusion significantly suppressed serum C-peptide at SS in controls (0.86 ± 0.306 ng/mL, $P < .05$ *v* baseline) but not in obese patients (2.03 ± 0.26 ng/mL, NS *v* baseline) (Table 1). C-peptide at SS decreased at $46.9\% \pm 11.03\%$ of baseline levels in controls, but only at $79.5\% \pm 8.93\%$ in obese patients ($P < .05$; Fig 1).

Serum FFA concentrations were significantly higher in obese versus control subjects at baseline and were suppressed to a comparable extent ($70.4\% \pm 4.69\%$ and $74.6\% \pm 7.36\%$ of baseline, respectively) by insulin infusion at SS (216.7 ± 45.73 and 92.9 ± 23.7 μEq/L, respectively, $P < .05$ for one *v* the other and *v* baseline; Table 1).

Study 2: Saline Infusion

BG levels did not change during saline infusion in obese and control subjects (baseline *v* SS, 83 ± 3.6 *v* 80 ± 2.4 mg/dL, NS, and 78 ± 1.5 *v* 76 ± 1.6 mg/dL, NS, respectively; Table 2).

Serum insulin slightly decreased over time in both groups (baseline *v* SS, 12.9 ± 3.16 *v* 8.8 ± 2.07 μU/mL, NS, in obese and 9.6 ± 0.51 *v* 6.5 ± 0.61 μU/mL, NS, in controls; Table 2).

Table 2. BG, Serum IRI, Serum C-Peptide, and Serum FFAs at Baseline and During Saline Infusion in 10 Obese Patients and Six Normal-Weight Healthy Subjects

	Time (min)							
Parameter	0	30	60	90	120	130	140	150
BG (mg/dL)								
Patients	83 ± 3.6	82 ± 3.9	81 ± 3.3	82 ± 2.7	81 ± 2.9	80 ± 2.4	80 ± 2.5	80 ± 2.5
Controls	80 ± 1.5	77 ± 1.8	75 ± 2.0	76 ± 1.6	75 ± 2.0	76 ± 1.7	77 ± 1.7	78 ± 2.0
IRI (μU/mL)								
Patients	12.9 ± 3.16	10.0 ± 2.02	10.9 ± 3.6	6.9 ± 0.84	8.3 ± 1.37	9.0 ± 2.29	9.3 ± 2.29	8.6 ± 2.47
Controls	9.6 ± 0.51	7.1 ± 0.75	6.9 ± 1.15	7.7 ± 0.74	6.5 ± 0.65	6.7 ± 0.56	6.7 ± 0.65	6.1 ± 1.24
C-peptide (ng/mL)								
Patients	2.71 ± 0.350†	2.55 ± 0.300	2.33 ± 0.321	2.24 ± 0.303	2.34 ± 0.307	2.33 ± 0.308	2.36 ± 0.327	2.19 ± 0.285*
Controls	1.96 ± 0.189	1.85 ± 0.195	1.77 ± 0.220	1.70 ± 0.203	1.63 ± 0.158	1.60 ± 0.181	1.65 ± 0.134	1.58 ± 0.140
FFA (μEq/L)								
Patients	783.6 ± 60.14†	853.8 ± 48.36†	792.3 ± 56.38†	828.4 ± 39.65†	943.3 ± 70.61†	937.4 ± 51.39†	946.4 ± 60.09†	918.3 ± 203.12†
Controls	483.3 ± 75.35	460.0 ± 36.78	481.6 ± 49.76	545.0 ± 59.14	541.6 ± 58.10	595.0 ± 63.81	593.3 ± 65.86	593.3 ± 50.37

NOTE. Values are the mean ± SEM. *$P < .05$ *v* baseline.
†$P < .05$ *v* controls.

C-peptide concentrations were higher in obese versus control subjects at baseline (Table 2) and displayed a similar modest decline in both groups at SS of saline infusion (2.31 ± 0.300 ng/mL, 17.2% ± 4.02% decrease from baseline, and 1.62 ± 0.150 ng/mL, 17.3% ± 4.12% decrease from baseline, respectively, NS).

Serum FFA concentrations were higher in obese versus control subjects at baseline (783.6 ± 60.14 *v* 483.3 ± 75.35 μEq/L, $P < .05$) and showed a comparable slight increase in both groups at SS (936.3 ± 54.14 μEq/L in obese and 580.8 ± 57.88 μEq/L in controls, NS; Table 2).

DISCUSSION

To the best of our knowledge, this is the first study showing that in obese men a hyperinsulinemia in the physiologic range is not superior to short-term fasting per se in suppressing β-cell secretion, whereas in normal-weight healthy subjects, hyperinsulinemia of the same degree inhibits insulin secretion 2-fold more than fasting. The finding in obese subjects of a superimposable C-peptide suppression at a serum insulin concentration of 100 or 10 μU/mL provides direct evidence of a strong β-cell refractoriness to usual ambient inhibitory signals, ie, physiologic postprandial hyperinsulinemia, and points to an altered negative short-loop insulin β-cell feedback in obesity. This alteration may arise from a primary lesion of the pancreatic islet. The resistant β cell would continue to secrete insulin even when the hormone in the extracellular fluid reaches a level at which normal β cells are inhibited.[1] The observation that pancreatic islets of Zucker fatty rats (*fa*/*fa*) in vitro secrete substantially more insulin than islets from lean control rats[18] also points to an intrinsic alteration in β-cell function in obesity.

In normal-weight subjects, supraphysiologic elevations in plasma insulin do not result in a further decrease in endogenous insulin secretion above that achieved with mild hyperinsulinemia.[19] In obese patients, pharmacologic doses of exogenous insulin are needed to suppress insulin secretion in vitro.[1] In cirrhotic patients, in whom hyperinsulinemia is a frequent feature, feedback inhibition of insulin secretion is also altered and suppression of basal insulin secretion is never achieved, not even by insulin concentrations of about 10,000 μU/mL.[7] Thus, it would be interesting to study in vivo in obese patients the effect of higher-dose insulin than that used in our study, which aimed only at reproducing high physiologic insulin concentrations. On the other hand, it cannot be excluded that a more prolonged hyperinsulinemia than that used in our study is able to inhibit insulin secretion significantly. Studies including appropriate saline control experiments are needed to verify this hypothesis. Only two studies[3,20] evaluating insulin autofeedback in healthy subjects have performed sham tests and reported results consistent with ours, ie, an effective insulin suppression following exogenous insulin infusion but not saline infusion. Similar findings have been reported in vivo in lean rats[21]: a hyperinsulinemic clamp, independent of the ambient glucose concentration, caused a marked reduction in pancreatic proinsulin mRNA, while saline infusion failed to do so.

Insulin removal was not altered in our obese patients, as indicated by the normal C-peptide to insulin ratio at fasting, a finding already reported in obese children and adults,[22,23] and by a similar MCR_i in our obese and control subjects. This latter finding is in agreement with previous studies[24] showing that it is not obesity per se but the accompanying basal hyperinsulinemia (absent in our patients) which is the major contributing factor to the reduced insulin clearance in obesity. Since there is no evidence for decreased C-peptide clearance in obese patients[24] and care was exercised in this study to avoid methodologic sources of error (plasma glucose was strictly maintained during the clamp at the basal level coefficient of variation < 7% in both groups), a primary dysregulation of the β cell, not responding to the physiologic inhibitory signals, is likely in play in obesity.

How obesity might induce β-cell dysfunction remains to be established. Insulin autosuppression in humans might occur either directly via a neurally mediated mechanism or indirectly via suppression of insulinotropic substrates such as FFAs or ketone bodies or hormones such as glucagon. In any case, the in vivo environment with intact nerve connections and blood supply appears to be essential for a normal β-cell responsiveness to modulatory signals.[2] Along this line, an impaired insulin negative autofeedback has been documented in insulin-dependent diabetic patients after combined pancreas and kidney transplantation but not after kidney transplantation alone,[25] a finding supporting the view that the process of insulin autoinhibition is neurally mediated. In obesity, the high circulating levels of FFAs also may play a role in the impairment of the insulin autofeedback mechanism. In normal-weight subjects, and much more so in obese subjects, a reduction of circulating FFA levels is associated with decreased basal and glucose-stimulated insulin secretion.[26,27] In our study, insulin was equally effective for inhibiting FFA release in obese and control subjects; however, serum FFA levels at SS remained higher in the obese versus the controls. Since leptin has been shown to suppress insulin secretion in human pancreatic islets,[28] the possibility that the diminished sensitivity to leptin in obese patients might cause a dysregulation of β-cell activity deserves exploration.

In our saline experiments performed in the postabsorptive state, serum FFA levels markedly increased while BG levels remained relatively stable. The increased secretion of counterregulatory hormones (epinephrine, norepinephrine, glucagon, growth hormone, and cortisol)[29] together with the diminished insulin concentrations fully account for these homeostatic adjustments during short-term fasting, when glucose production equals glucose utilization.[30]

In conclusion, this study has shown that in obese men, a hyperinsulinemia within the postprandial range is not superior to short-term fasting per se in the suppression of β-cell activity, suggesting an impairment of the insulin negative feedback in this clinical condition and providing an additional piece of information regarding the pathogenesis of hyperinsulinemia in obesity.

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