Insulin

Its Role in the Thermic Effect of Glucose

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Abstract

To investigate the possible role of insulin per se in the thermic response to glucose/insulin infusions, respiratory exchange measurements were performed on eight healthy young men for 45 min before and 210 min after somatostatin infusion. Two tests were performed on separate days and each had two consecutive phases of 90 min each. *Test 1.* Two different rates of glucose uptake were imposed, one at euglycemia (phase 1) and the other at hyperglycemia (phase 2) while insulinemia was maintained constant throughout.

***Test 2.* Glucose uptake was maintained constant throughout while insulin was infused at two different rates: 1 mU/kg per min with hyperglycemia (phase 1) and 6.45 mU/kg per min with "euglycemia" (phase 2).**

The thermic effect of glucose and insulin, obtained from phase 1 in both tests, was 5.9±1.2 and 5.8±0.5% (NS) of the energy infused, respectively. A step increase in glucose uptake alone, test 1, phase 2, (0.469±0.039 to 1.069±0.094 g/min) caused an increase in energy expenditure of 0.14±0.03 kcal/min (thermic effect 5.9±1.1%). When insulin was increased by 752±115 μU/ml, with no change in glucose uptake, energy expenditure rose by 0.05±0.02 kcal/min, which correlated with the increase in plasma catecholamines.

It is concluded that a large proportion of the thermic response to glucose/insulin infusions is due to glucose metabolism alone. The thermic effect of insulin is small and appears to be mediated by the sympathetic nervous system; thus at physiological insulin concentrations, the thermic effect of insulin per se is negligible.

Introduction

The thermic effect of glucose in lean individuals either after oral ingestion or during intravenous infusions is greater (1, 2) than can be attributed to the energy cost of storing glucose as glycogen (3). On the other hand, obese subjects often (4–8) but not always (9–11) present with a reduced thermic effect when compared with lean controls, which is aggravated by increasing insulin resistance (5). Although a diminished thermic effect of food has been proposed to contribute to the pathogenesis of obesity (12–14), it is uncertain whether this is a cause or a consequence of the obese state, particularly since it has been demonstrated that when the glucose uptake is the same in lean and obese individuals the thermic effect of glucose/insulin is also the same (15). Rothwell and Stock (16) have reported that the increase in metabolic rate in response to a meal was reduced in streptozotocin-induced diabetic rats but was reestablished in those receiving insulin. This has led to the suggestion that insulin action is involved in the thermic response to food ingestion (16, 17).

Since the effects of glucose and insulin metabolism are so intimately related, it is difficult to conclude if it is a direct effect of insulin independent of glucose metabolism that is responsible for the observed increase in thermogenesis. This study was therefore designed to separately measure the thermic effect of glucose alone and the influence of insulin itself on energy expenditure in man. For this purpose euglycemic and hyperglycemic insulin clamping was performed in healthy young male subjects with or without somatostatin infusion in combination with continuous respiratory exchange measurements. This approach allowed us to determine whether insulin per se plays a role in the thermic response to glucose/insulin infusions.

Methods

Subjects

Eight healthy young male subjects, whose physical characteristics are presented in Table I, were studied. No subject had a family history of diabetes mellitus and none was taking any medication during the experimental protocol. The protocol was submitted, reviewed, and accepted by the ethical committee of the Faculty of Medicine, University of Lausanne. After receiving a detailed explanation of the experimental protocol, written consent was obtained from each volunteer before acceptance into the study.

Experimental protocol

For 3 d before each test the subject was instructed to eat a diet containing at least 250 g of carbohydrate, supplemented with sugared fruit juice providing an additional 60 g of hexose sugars per day. Each subject was requested to keep a log of his diet and physical activity during this period so that he could replicate similar dietary and activity patterns in the days preceding subsequent tests.

Each subject spent the night before the test at the Institute. In the morning the subject was awakened at 6:30 a.m. and after voiding was transferred to the room in which the test was to be performed. Two venous lines were inserted. One was a venous catheter (Venflon, Viggo, AB, Helsingborg, Sweden) placed in an antecubital vein for infusion of glucose and hormones and the other, a 19-gauge Butterfly (Abbott Ireland Ltd, Sligo, Republic of Ireland) was inserted retrogradely into a hand vein for blood sampling and was kept patent with physiological saline. The hand was then placed in a heated box (~60°C) to achieve arterialization of the venous blood (18).

The subject performed four experimental protocols within a period of 2 mo. The first and second (Fig. 1) and the third and fourth experiments were performed within 2 wk.

Test 1a with constant insulin infusion at two successive steady state plasma glucose concentrations. This test was designed to study the effect of an increase in glucose uptake on energy expenditure, without a concomitant change in insulinemia.

After 45 min of baseline measurements, somatostatin (Stilamin, Ser-

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Table I. Physical Characteristics of the Subjects

Name	Age	Weight	Height	Body fat	FFM
	yr	*kg*	*cm*	*%*	*kg*
C.M.	20	68.24	176.5	12.9	59.6
M.L.	20	78.45	185.5	16.2	65.74
N.F-Y.	21	65.05	175.5	0.6	58.2
C.C.	20	85.60	181.0	14.7	73.0
L.J.	19	62.64	181.5	11.6	55.37
G.T.	22	67.75	186.0	12.0	59.62
B.P-Y.	22	81.72	194.5	15.5	69.05
E.K.	22	67.98	180.0	14.4	58.2
Mean±SD	20.8±1.2	72.2±8.5	182.7±6.1	13.5±2.0	62.3±6.2

ono S.A., CH-1170 Aubonne, Switzerland) was infused at a continuous rate (7 μg/min) for the next 2 h of the experiment. This infusion rate was chosen to effectively inhibit endogenous insulin secretion (19, 20).

After 30 min of somatostatin infusion alone a priming dose of biosynthetic human insulin (Huminsulin Normal, Eli Lilly, Indianapolis, IN) was given in a decreasing manner over a period of 10 min as previously described (18) and was then infused at a continuous rate of 1 mg/kg per min for the remainder of the test. Euglycemia was maintained in arterialized venous blood samples obtained at 5-min intervals, during the somatostatin and insulin infusions, by varying the infusion rate of a 20% glucose solution (18). After 90 min of hyperinsulinemic euglycemia, a priming dose of the 20% glucose solution was given in a logarithmically decreasing manner (18) and was then varied in order to raise and maintain the blood glucose level at 170–180 mg/dl for another 90 min. When hyperglycemia was imposed, a condition that stimulates endogenous insulin secretion, the rate of somatostatin infusion was increased to 9 μg/min to ensure that endogenous insulin did not escape somatostatin's inhibitory effects.

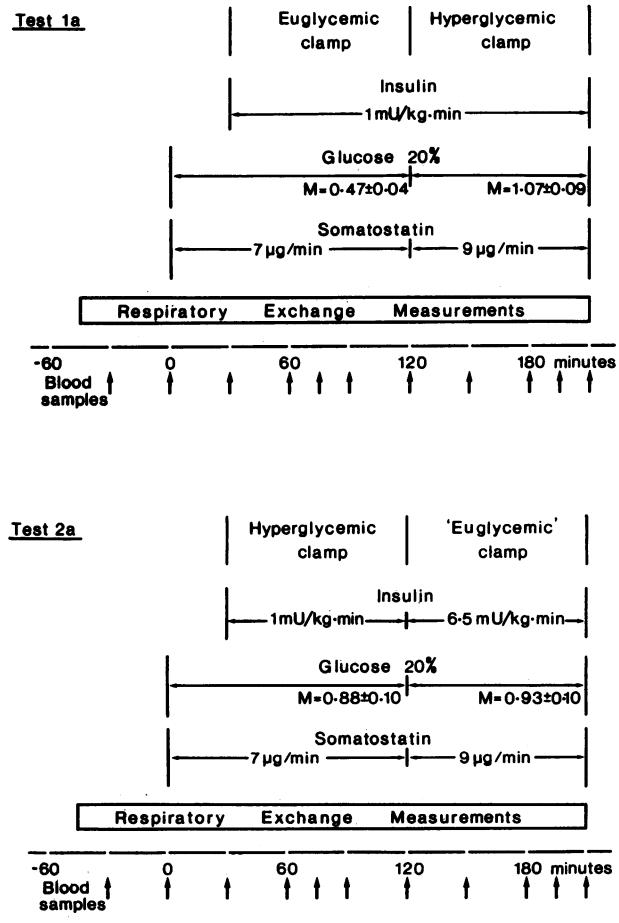

Figure 1. Experimental design. The study was divided into two tests: (*Test 1a*) Plasma insulin concentrations were maintained at ~80 μU/ml throughout the test. Glucose uptake (M) was raised from 0.47±0.04 to 1.07±0.09 g/min (Mean±SEM) at 120 min in order to raise glycemia from 91 to 171 mg/dl and maintain it at that level for another 90 min. (*Test 2a*) M was maintained constant at ~0.9 g/min throughout the test. At 120 min plasma insulin concentrations were raised from 95 to 848 μU/ml. Since M was constant, glycemia decreased from 170 to 101 mg/dl.

Test 1b: Same protocol as 1a but without somatostatin infusion. This test was used as a control for test 1a, in order to check whether somatostatin, which was given in test 1a, had an effect on energy expenditure.

Test 2a with constant glucose infusion at two successive levels of insulinemia. After 45 min of baseline measurements, somatostatin was infused at 7 μg/min for 2 h. When somatostatin had been infused for 30 min a prime continuous infusion of Huminsulin was begun at a rate of 1 mU/kg per min as described above (test 1a). At the same time a priming dose of a 20% glucose solution was infused and then varied in order to raise and maintain glycemia at 170–180 mg/dl for 90 min. After 90 min of hyperinsulinemic hyperglycemia, the somatostatin infusion rate was increased to 9 μg/min and insulinemia was increased by a prime continuous infusion (6.45 mU/kg per min) for a further 90 min, while maintaining the glucose infusion constant.

Test 2b: Same protocol as 2a but without somatostatin infusion. (This test was used as a control for test 2a). Three subjects who participated in the above experiments also volunteered for two additional tests without somatostatin, in which either the 1-mU/kg per min hyperinsulinemic, euglycemic clamp or the hyperinsulinemic, hyperglycemic clamp was continued for the whole duration of the test in order to observe whether the thermic effect of glucose/insulin infusions were changed with time.

During each experiment, continuous respiratory exchange measurements were performed for the duration of the test (baseline and test) using a ventilated hood, open-circuit, indirect calorimeter, the details of which have been described elsewhere (21).

Heart rate was recorded throughout the test by means of a portable, light weight, electronic device (heart rate memory, Difa Benelux BV, Breda, The Netherlands).

Blood samples were taken every 5 min after the baseline for glucose analysis. Samples were also taken at regular intervals (Fig. 1) for free fatty acid, blood urea nitrogen, and hormone analyses.

Urine was collected at the beginning and end of the test and analyzed for glucose, nitrogen, and catecholamines.

Analyses

Blood glucose was analyzed in duplicate on a Beckman II glucose analyzer (Beckman Instruments, Inc., Fullerton, CA). Plasma samples were also analyzed for insulin (22), C-peptide (kit, Byk-Mallinckrodt, Diezenback, Federal Republic of Germany) and glucagon (23) by radioimmunoassay, free fatty acids (24) on the Dole extract (25), and catecholamines by high performance liquid chromatography (26, 27).

Urinary nitrogen was analyzed, after digestion, on a Technicon autoanalyzer (Technicon Instruments, Corp., Tarrytown, NY) (28), and urinary catecholamines were measured by fluorometry (29). Urinary glucose was analyzed with glucose test sticks (Gluketur-test, Boehringer, Mannheim, Federal Republic of Germany). Glucosurea was only obtained in one test and was further analyzed in duplicate on the Beckman II glucose analyzer.

Data analysis

For data presentation, the mean values obtained during the last 30 min of each relevant phase in each protocol were considered. In tests 1a and 1b, phase 1 represents the hyperinsulinemic (~80 μU/ml) euglycemic clamp and phase 2 the hyperinsulinemic hyperglycemic clamp (~171 mg/dl). In tests 2a and 2b, phase 1 represents the hyperinsulinemic,

hyperglycemic (~170 mg/dl) clamp, and phase 2 the "euglycemic" (~101 mg/dl) clamp with a step increase in insulinemia (insulinemia ~ 850 μU/ml).

An index of protein oxidation was calculated from the mean urinary nitrogen excretion during the test, after correction for changes in nitrogen in the urea pool (30).

"Glucose storage" or nonoxidative glucose disposal was calculated by subtracting the rate of glucose oxidation from the rate of the corrected glucose uptake (M), i.e., by taking into account the changes in the glucose pool assuming a distribution volume of 0.21 liter/kg per body wt and subtracting any urinary glucose losses. Urinary glucose was only observed once in 1 subject, during test 2b and amounted to an excretion rate of 0.01 mg/kg per min. Hepatic glucose production was not measured in the present study and was assumed to be totally suppressed during the glucose/insulin infusions (31, 32).

The thermic effect of glucose/insulin (TE_{GI}) was calculated by dividing the increase in energy expenditure (EE) ($EE_{phase\ 1} - EE_{somatostatin}$) by the energy content of the increase in glucose uptake (GU) ($GU_{phase\ 1} - GU_{somatostatin}$).

Tests 1a and 2a

$TE_{GI} = EE_{phase\ 1} - EE_{somatostatin}/(GU_{phase\ 1} - GU_{somatostatin}) \times 3.74 \times 100$. The thermic effect of glucose alone (TE_G) was calculated as follows:

Test 1a

$TE_G = EE_{phase\ 2} - EE_{phase\ 1}/(GU_{phase\ 2} - GU_{phase\ 1}) \times 3.74 \times 100$, where EE is the steady state energy expenditure kcal/min, GU is the glucose uptake g/min, and 3.74 is the energy value 1 g glucose kcal/g.

Statistical analyses were performed using Student's paired t test. Stepwise regression analysis was used to determine which parameter change (glucose uptake, plasma concentration of insulin, norepinephrine, and epinephrine) correlated with each change in energy expenditure. Results are expressed as mean±SEM unless stated otherwise.

Results

The aim of the present protocols were in test 1a, to maintain insulinemia at a constant rate during two different steady state conditions of glucose uptake and in test 2a to maintain a constant glucose uptake during two different steady state conditions of insulinemia. The results of the blood parameters and heart rates measured during the baseline and the different phases of each experiment are summarized in Table II.

Test 1a. It can be seen that with somatostatin infusion there was a slight but significant decrease in both glycemia and insulinemia between the baseline and somatostatin phases after which blood glucose was maintained at 91±1 mg/dl when plasma insulin was 80±4 μU/ml (phase 1). As expected, C-peptide concentrations fell dramatically with somatostatin and decreased further to 0.18±0.01 μg/ml in phase 2 at which time plasma insulin concentrations were 71±3 μU/ml.

Test 1b. Glycemic values were similar to those in test 1a. Without somatostatin infusion, C-peptide concentrations were more elevated than in test 1a, especially during the hyperglycemic

Table II. Changes in the Principal Blood Parameters and Heart Rate in the Tests with and without Somatostatin (n = 8) (Mean±SEM)

Parameter	Baseline (−30–0 min)	Somatostatin (0–30 min)	Phase 1 (90–120 min)	Phase 2 (180–210 min)	Baseline (−30–0 min)	Phase 1 (60–90 min)	Phase 2 (150–180 min)
	Test 1a (with somatostatin)				Test 1b (without somatostatin)		
Euglycemia followed by hyperglycemia							
Glucose (*mg/dl*)	99±1*	95±2 NS	91±1‡	171±2	101±2*	91±1‡	170±3
Insulin (*μU/ml*)	11.2±0.9§	6.7±1.0‡	80±4‡	71±3	12.0±0.9‡	81±7‡	108±9
C-peptide (*ng/ml*)	1.54±0.07‡	0.59±0.02‡	0.23±0.01‡	0.18±0.01	1.74±0.2‡	0.72±0.13‡	3.70±0.30
Glucagon (*pg/ml*)	100±12‡	46±6 NS	44±8 NS	43±5	81±9 NS	69±12‡	53±10
Free fatty acids (*μmol/liter*)	249±25‡	344±47‡	128±9‡	112±11	268±16‡	135±8‡	118±6
Norepinephrine (*pg/ml*)	209±16 NS	204±18 NS	229±19 NS	234±19	205±10§	232±18 NS	242±13
Epinephrine (*pg/ml*)	74±5 NS	72±3 NS	79±5 NS	82±6	71±3 NS	76±4 NS	77±3
Heart rate (*beats/min*)	67±3 NS	65±2 NS	69±3‡	73±3	68±2‡	74±2*	76±2
	Test 2a (with somatostatin)				Test 2b (without somatostatin)		
Hyperglycemia followed by hyperinsulinemia and euglycemia							
Glucose (*mg/dl*)	101±2*	97±2‡	170±2‡	101±2	100±2‡	171±1‡	122±3
Insulin (*μU/ml*)	12.8±1.0‡	6.0±1‡	95±7‡	848±121	10.9±1.6‡	128±8‡	843±43
C-peptide (*ng/ml*)	1.62±0.10‡	0.55±0.04‡	0.25±0.03§	0.02±0.01	1.56±0.13‡	4.40±0.21‡	2.82±0.24
Glucagon (*pg/ml*)	82±11‡	41±6§	31±4 NS	36±7	86±9‡	46±7 NS	44±5
Free fatty acids (*μmol/liter*)	280±18*	439±46‡	119±14 NS	111±12	259±30‡	129±9‡	114±7
Norepinephrine (*pg/ml*)	208±15 NS	204±10 NS	207±15*	244±12	213±21 NS	225±17‡	274±25
Epinephrine (*pg/ml*)	76±4 NS	75±4 NS	75±5*	84±4	76±5 NS	81±6‡	91±6
Heart rate (*beats/min*)	63±2 NS	62±1‡	67±2‡	73±2	65±2‡	74±2‡	80±3

* <0.01. ‡ <0.005. § <0.05.

phase (3.7±0.3 vs. 0.18±0.01 ng/ml, $P < 0.001$). Plasma insulin concentrations were similar during the euglycemic phase (phase 1) in tests 1a and 1b, but was much higher during the hyperglycemic phase (phase 2) in test 1b than in test 1a (108±9 vs. 71±9 μU/ml, respectively, $P < 0.001$). The somatostatin infusion in test 1a thus effectively inhibited endogenous insulin secretion during hyperglycemia.

Test 2a. During phase 1 (hyperinsulinemic hyperglycemic clamp) the plasma glucose concentration reached a similar value to that in phase 2 of test 1a (170±2 vs. 171±2 mg/dl, respectively). At the end of phase 2, the high insulin infusion rate (6.45 mU/kg per min) induced a very high insulinemia (848±121 μU/ml) with a concomitant decrease in plasma glucose concentration towards euglycemia (101±2 mg/dl). Plasma C-peptide concentration was markedly reduced by the somatostatin infusion from 1.62±0.1 to 0.25±0.03 and 0.02±0.01 ng/ml during baseline, phase 1 and phase 2, respectively.

Test 2b. Similar glycemic concentrations were obtained in test 2b as in test 2a, while insulinemia was higher during phase 1 of test 2b (128±8 vs. 95±7 μU/ml, $P < 0.001$ in tests 2b and 2a, respectively). Plasma C-peptide concentrations also increased during hyperglycemia but were significantly inhibited at the high insulin concentrations (843±43 μU/ml) obtained in phase 2.

Plasma concentration of norepinephrine and epinephrine. Plasma norepinephrine concentrations in tests 2 were significantly increased during the hyperinsulinemic phase (phase 2) when compared with values in phase 1 (244±12 vs. 207±15 pg/ml, $P < 0.01$; 274±25 vs. 225±17 pg/ml, $P < 0.005$, in tests 2a and 2b, respectively). Similarly, plasma epinephrine concentrations were slightly increased in tests 2a and 2b during the hyperinsulinemic phase.

Plasma concentration of glucagon. The plasma glucagon concentration was lowered by somatostatin infusions in tests 1a and 2a. In test 2b, plasma glucagon concentrations were decreased from 86±9 (baseline) to 46±7 pg/ml (phase 1; $P < 0.005$) following the step increase in insulinemia.

Glucose uptake, glucose oxidation, and glucose storage. The changes in glycemia, glucose uptake (M), glucose oxidation, and glucose storage are illustrated in Fig. 2 for tests 1a and 2a. During the baseline period glucose oxidation was 0.136±0.020 g/min (test 1a) and 0.112±0.021 g/min (test 2a). Lipid oxidation at this time was 0.061±0.007 g/min in both tests. With somatostatin, glucose oxidation fell to 0.110±0.017 g/min, $P < 0.02$, (test 1a) and 0.098±0.015 g/min NS (test 2a) even though glucose uptake was 0.093±0.018 g/min and 0.094±0.017 g/min, respectively.

During the last 30 min of the hyperinsulinemic (80±4 μU/ml), euglycemic clamp (test 1a) glucose uptake was 0.469±0.039 g/min, of which 0.255±0.021 g/min could be accounted for by oxidation and the remainder, 0.214±0.036 g/min, by glucose storage. At this time lipid oxidation had decreased to almost half the postabsorptive value 0.034±0.006 g/min.

A step change in the rate of glucose uptake from 0.469±0.039 g/min to 1.069±0.086 g/min resulted in an increase in both glucose oxidation to 0.385±0.029 g/min ($P < 0.005$) and storage to 0.684±0.077 g/min ($P < 0.001$), while lipid oxidation decreased to negligible values.

During the last 30 min of test 2a phase 1, where hyperglycemia was maintained at 170±2 mg/dl at an insulinemia of 95±7 μU/ml glucose uptake was 0.884±0.101 g/min, one-third of which was due to oxidation (0.284±0.029 g/min) and 0.600±0.086 g/min to glucose storage. The step change in plasma insulin concentration from 95±7 to 848±121 μU/ml (test 2a, phase 2) caused a slight but significant increase in glucose uptake from 0.884±0.101 to 0.926±0.102 g/min, $P < 0.001$, which was due to a fall in glucose concentration in the glucose space. The disposal of this small increase in glucose uptake could be entirely accounted for by an increase in glucose oxidation since glucose storage remained unchanged (0.570±0.080 g/min, NS).

During both clamp phases lipid oxidation decreased significantly (0.020±0.004 g/min, $P < 0.001$ and 0.006±0.006 g/min, $P < 0.001$, phases 1 and 2, respectively) when compared with the baseline (0.061±0.007 g/min) and somatostatin (0.067±0.004 g/min) phases.

Table III presents the results for glucose uptake, oxidation, and storage obtained in the two tests without somatostatin. In these conditions, although glucose uptake was slightly greater, the contribution of oxidation and storage to glucose uptake was the same (within 4%) in each of the different phases as in the tests with somatostatin. When insulinemia was increased from 128±8 to 843±43 μU/ml glucose uptake increased slightly and was accompanied by a significant increase in glucose oxidation.

The changes in energy expenditure, glucose uptake and insulinemia during the two somatostatin tests are illustrated in Fig. 3. Glucose uptake has been divided into its constituents, oxidation, and storage. It can be seen (test 1a) that an increase in both glucose uptake and insulinemia of 0.377±0.044 g/min and 73±4 μU/ml (phase 1 minus somatostatin), respectively, caused an increase in energy expenditure of 0.08±0.02 kcal/min or a thermic effect of 5.9±1.2%. When the rate of glucose uptake was increased by a further 0.599±0.07 g/min, energy expenditure also increased by 0.14±0.03 kcal/min, which resulted in a thermic effect of glucose alone of 5.9±1.1%.

In test 2a, the increase in both glucose uptake and insulinemia of 0.791±0.110 g/min and 90±7 μU/ml, respectively, caused an increase in energy expenditure of 0.17±0.03 kcal/min. In this

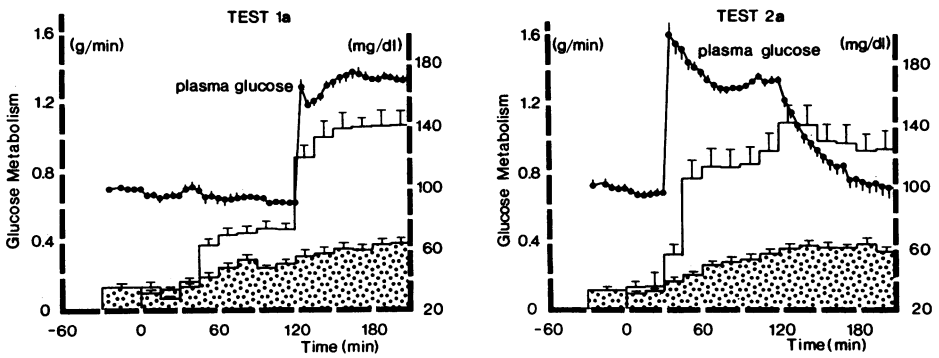

Figure 2. Change in plasma glucose ● —— ●, glucose uptake and its components, oxidation ▩, and storage □ during the two tests with somatostatin ($n = 8$; Mean±SEM).

Table III. Glucose Metabolism and Metabolic Variables during the Two Tests without Somatostatin (n = 8, Mean±SEM)

	Baseline	Phase 1	Phase 2
	−30–0 min	*60–90 min*	*150–180 min*
Test 1b: Euglycemia followed by hyperglycemia			
Glucose uptake (*g/min*)	—	0.524±0.040*	1.168±0.094
Glucose oxidation (*g/min*)	0.116±0.017‡	0.281±0.088‡	0.378±0.024
Glucose storage (*g/min*)	—	0.243±0.026‡	0.790±0.088
Δ Energy expenditure (*kcal/min*)	—	0.14±0.03*	0.27±0.03
Thermic effect (%)	—	7.1±1.4 NS	6.0±0.5
Theoretically derived thermic effect‖ (%)	—	2.5±0.2	3.6±0.1
Lipid oxidation (*g/min*)	0.066±0.006§	0.032±0.008§	0.006±0.004
Test 2b: Hyperglycemia followed by hyperinsulinemia			
Glucose uptake (*g/min*)	—	1.076±0.088 NS	1.097±0.090
Glucose oxidation (*g/min*)	0.137±0.013‡	0.360±0.023‡	0.391±0.020
Glucose storage (*g/min*)	—	0.716±0.075 NS	0.705±0.075
Δ Energy expenditure (*kcal/min*)	—	0.25±0.02*	0.31±0.03
Thermic effect (%)	—	6.5±0.2§	7.6±0.3
Theoretically derived thermic effect (%)	—	3.5±0.1	3.4±0.1
Lipid oxidation (*g/min*)	0.058±0.004‡	0.009±0.006 NS	0.003±0.005

* <0.005. ‡ <0.01. § <0.05. ‖ The theoretically derived thermic effect = glucose storage × 5.3 ÷ glucose uptake, where 5.3% is the thermic effect of glucose storage as glycogen (3).

condition glucose storage was 0.605±0.099 g/min, and the thermic effect was 5.8±0.5%, which was the same as that observed in phase 2 of test 1a. When glucose uptake and storage was maintained constant and insulinemia was increased by 752±115 μU/ml, energy expenditure increased by 0.05±0.02 kcal/min ($P < 0.025$).

Stepwise regression analysis was performed on the changes in glucose uptake, plasma insulin, and catecholamine concentrations, in the somatostatin tests to determine which parameters most influenced the observed increase in energy expenditure (Table IV). It can be seen that in test 1a, a step change in glucose uptake correlated significantly with the increase in energy expenditure ($r = 0.823$, $P < 0.01$) and accounted for 68% (r^2) of this response ($P < 0.025$). The correlation improved slightly and remained significant when the increase in plasma epinephrine concentrations were added to the equation ($r = 0.837$, $P < 0.05$).

In test 2a where glucose uptake was maintained essentially constant and plasma insulin concentrations were increased by 752±115 μU/ml, the change in insulinemia did not correlate with the change in energy expenditure. Although the increases in both plasma norepinephrine and epinephrine concentrations correlated with energy expenditure ($r = 0.745$ and 0.689, respectively, Fig. 4) only norepinephrine correlated significantly when analyzed by stepwise regression analysis ($P < 0.05$).

Table V presents both the metabolic and blood parameters of the tests where either euglycemia or hyperglycemia was maintained continuously for 3 h. These results demonstrate that once steady state conditions were attained, no further changes occurred in any of the measured parameters, except in the euglycemic clamp, where both plasma insulin and free fatty acid concentrations fell slightly but significantly.

Heart rate. Fig. 5 presents the changes in heart rate during the experiments with and without somatostatin. In the postabsorptive state, the resting supine heart rate was 66±1 beats/min in all tests. When somatostatin was infused, there was a transitory fall that was significant (68±3 to 65±2 beats/min, $P < 0.02$ in test 1; 63±2 to 62±1 beats/min, $P < 0.05$ in test 2) and lasted ~10–15 min. During the different clamp phases, the heart rate increased significantly intratest. Although the heart rate tended to be lower with somatostatin in test 1, the differences were not significant. However, in test 2, the differences were more evident and they were significantly different.

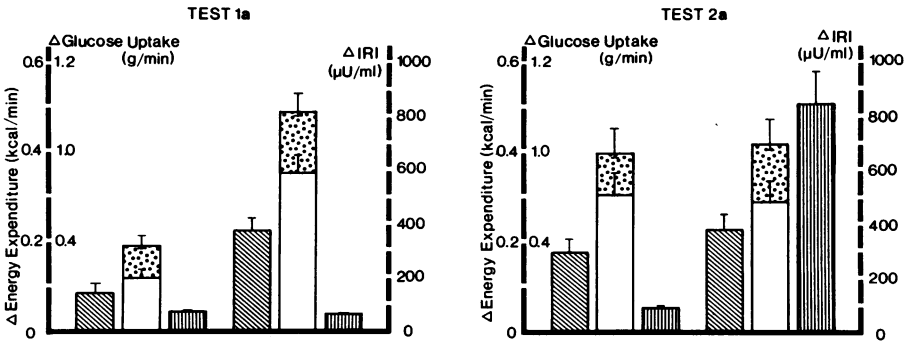

Figure 3. Changes in energy expenditure ■, glucose oxidation ▨, glucose storage □, and plasma insulin concentrations ■, over the somatostatin baseline values during phase 1 on the left and phase 2 on the right of each of the tests in which somatostatin was infused. Note that glucose uptake is the sum of glucose oxidation + glucose storage ($n = 8$; mean±SEM).

Table IV. Simple and Partial Correlation Coefficients by Stepwise Regression Analysis Between the Changes in Energy Expenditure (Dependent Variable) and the Changes in Glucose Uptake, Plasma Insulin, Norepinephrine and Epinephrine Concentrations (Independent Variables) (n = 8)

	Dependent variable Δ energy expenditure kcal/min		
Independent variables	Simple correlation	Partial correlation	*P*
Test 1a: Steady state insulin concentrations with a step increase in glucose uptake (phase 2 − phase 1)			
Δ Glucose uptake (*g/min*)	0.823		<0.025
Δ Plasma epinephrine (*pg/ml*)	0.450	0.837	<0.05
Δ Plasma norepinephrine (*pg/ml*)	−0.583	0.856	NS
Test 2a: Steady state glucose uptake with a step increase in plasma insulin concentration (phase 2 − phase 1)			
Δ Plasma norepinephrine (*pg/ml*)	0.745		<0.05
Δ Plasma epinephrine (*pg/ml*)	0.689	0.754	NS
Δ Plasma insulin (*μU/ml*)	0.333	0.722	NS

Urinary catecholamines. During test 1a urinary norepinephrine and epinephrine excretions were 1.67±0.45 μg/h and 0.35±0.18 μg/h, respectively, and were not significantly different from the excretion rates observed in test 2a (1.49±0.41 μg/h norepinephrine and 0.40±0.28 μg/h epinephrine). In the control tests without somatostatin the rate of norepinephrine excretion was 1.60±0.45 and 1.92±0.37 μg/h (test 1b and 2b, respectively) and that of epinephrine was 0.39±0.23 and 0.41±0.15 μg/h (test 1b and 2b, respectively).

Discussion

This study investigated the influence of glucose metabolism per se and insulin per se upon the thermic effect of glucose/insulin infusions. The results demonstrate that a step change in glucose uptake is highly correlated with a corresponding change in energy expenditure. Although a step change in insulin was accompanied by a slight increase in energy expenditure, the latter was significantly correlated with a concomitant rise in plasma catecholamine concentration rather than with the change in insulinemia.

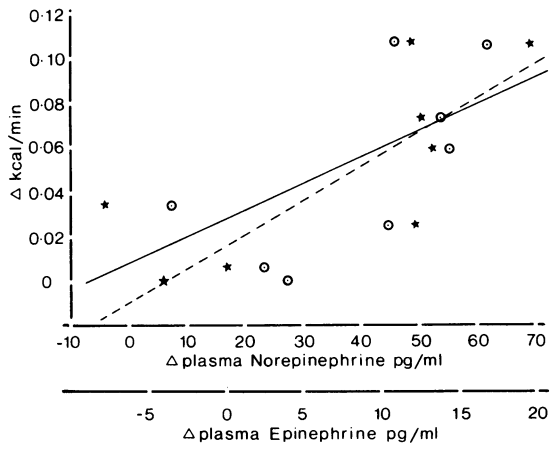

Figure 4. Correlations between the increase in energy expenditure (mean kilocalories per minute) and the changes in plasma catecholamine concentrations with a step increase in insulinemia of 752±115 μU/ml (test 2a, phase 2 minus phase 1). Simple linear correlation analysis showed that both norepinephrine (★ —— ★, $y = 0.001x + 0.008$; $r = 0.745$, $P < 0.05$) and epinephrine (⊙ – – – ⊙, $y = 0.004x + 0.015$; $r = 0.689$, $P < 0.05$) were significantly correlated with mean kilocalories per minute. With stepwise linear correlation analysis only the increase in plasma norepinephrine concentrations was significantly correlated with mean kilocalories per minute and could account for 57% (r^2) of this increase ($n = 8$).

The influence of insulinemia upon plasma norepinephrine concentrations supports the observations of Rowe et al. (33) that hyperinsulinemia, rather than hyperglycemia, stimulates sympathetic nervous activity. The fact that stepwise linear regression analysis shows a significant correlation between the increase in plasma norepinephrine and that of energy expenditure suggests that, when present in large concentrations, insulin can cause a small increase in energy expenditure that is mediated via the sympathetic nervous system. Such a role for insulin has already been suggested (34), possibly acting via receptors that have been found in the ventromedial hypothalamus (35). Insulin concentrations of 840 μU/ml are rarely or never observed, even in pathological conditions. It is thus likely that physiological changes in insulin concentrations can account for only a very small proportion of the observed change in energy expenditure, i.e., 0.05 kcal/min per 752 μU per ml insulin or 0.007 kcal/min for every 100 μU/ml increase in insulin concentration if one assumes a linear relationship between the rise in plasma insulin concentration and the increase in energy expenditure mediated via the sympathetic nervous system.

Flatt has calculated the energy cost of storing glucose as glycogen based on the ratio of the number of moles of ATP used to those produced during glucose oxidation and glucose conversion to glycogen, i.e., 5.3% of the energy content of glucose or 0.2 kcal/g (3). By comparing the thermic effects of glucose/insulin, glucose alone, and insulin alone obtained experimentally with those calculated as above, it is possible to obtain an index of the contribution of the obligatory and facultative thermogenic components (36) during the different phases of the study. The energy cost of glucose storage (obligatory thermogenesis) accounted for ~50–60% of the measured thermic effect (Tables III and V) when no somatostatin was infused and 60% with somatostatin infusion during both hyperglycemic phases (test 1a, phase 2 and test 2a, phase 1). With a step increase in insulinemia, the contribution of the facultative thermogenesis increased from 42 to 51% with somatostatin and from 46 to 55% without somatostatin, demonstrating that insulin and/or insulin-

Table V. Blood Parameters and Metabolic Variables during Either Constant Euglycemia or Hyperglycemia (n = 3, Mean±SEM)

	Euglycemia			Hyperglycemia		
	Baseline (−30–0 min)	Phase 1 (60–90 min)	Phase 2 (150–180 min)	Baseline (−30–0 min)	Phase 1 (60–90 min)	Phase 2 (150–180 min)
Glucose uptake (*g/min*)	—	0.505±0.026 NS	0.536±0.035	—	1.195±0.177 NS	1.178±0.152
Glucose oxidation (*g/min*)	0.146±0.051*	0.286±0.037 NS	0.289±0.026	0.137±0.033*	0.407±0.059 NS	0.403±0.042
Glucose storage (*g/min*)	—	0.220±0.053 NS	0.247±0.061	—	0.788±0.135 NS	0.775±0.121
Δ Energy expenditure (*kcal/min*)	—	0.17±0.05 NS	0.15±0.06	—	0.29±0.05 NS	0.28±0.07
Thermic effect (%)	—	8.9±2.2 NS	6.9±2.4	—	6.7±0.9 NS	6.3±1.3
Theoretically derived thermic effect‡ (%)	—	2.3±0.5	2.7±0.2	—	3.4±0.2	3.4±0.1
Lipid oxidation (*g/min*)	0.048±0.014 NS	0.028±0.009 NS	0.024±0.016	0.061±0.014 NS	−0.002±0.014 NS	0.007±0.001
Glucose (*mg/dl*)	95±1 NS	89±2 NS	93±3	98±0.4§	170±1 NS	171±2
Free fatty acids (*μmol/liter*)	273±76 NS	142±14§	130±13	267±37 NS	135±16 NS	133±19
Insulin (*μU/ml*)	12.6±1.8§	84±6§	78±7	12±2§	128±22 NS	137±18
C-peptide (*ng/ml*)	1.58±0.17§	0.72±0.16 NS	0.58±0.12	1.81±0.21§	5.30±0.68 NS	5.88±0.74
Glucagon (*pg/ml*)	89±47 NS	63±28 NS	61±25	72±21§	35±15 NS	33±13
Norepinephrine (*pg/ml*)	205±7§	252±16 NS	239±7	233±30 NS	253±23 NS	242±30
Epinephrine (*pg/ml*)	64±10§	75±11 NS	72±10	76±8 NS	84±7 NS	77±7
Heart rate (*beats/min*)	60±4§	69±4 NS	69±5	61±3§	70±5 NS	72±5

* <0.05. ‡ The theoretically derived thermic effect = "glucose storage" × 5.3 ÷ glucose uptake; where 5.3 is the thermic effect of glucose storage as glycogen (3); § <0.005.

mediated factors caused a small increase in energy expenditure that was not related to glucose uptake.

It is interesting to note that the increase in energy expenditure over the somatostatin baseline during the hyperglycemic, high plasma insulin clamp (test 2a, phase 2) was 0.23±0.03 kcal/min at a glucose uptake of 0.926±0.102 g/min of which 0.05±0.02 kcal/min or 22% can be accounted for by insulin and/or stimulation of the sympathetic nervous system. If one assumes, as observed elsewhere (37), that a certain sympathetic component to the increase in energy expenditure was already present before the step increase in plasma insulin concentration, then these results confirm our previous observations (36, 37) that stimulation of the sympathetic nervous system can account for ~30% of the increase in energy expenditure during glucose/insulin infusions.

Although it would appear that insulin via stimulation of the sympathetic nervous system can account for 20–25% of the facultative thermogenesis (36) it is possible that other factors may be involved but which cannot be determined from our data.

In this study somatostatin was used as a tool to suppress endogenous insulin secretion. It is unlikely that it influenced our results substantially since tests 1b and 2b carried out without somatostatin gave similar results of glucose metabolism.

Certain aspects of somatostatin actions were observed, however. Somatostatin infusion caused a decrease in blood glucose concentration, which necessitated the infusion of glucose at a rate of 1.3 mg/kg per min in both tests, to maintain euglycemia. It is well documented that this decrease in glycemia is principally due to inhibition of glucagon-stimulated glycogenolysis by somatostatin, which results in a 50–80% reduction in hepatic glucose production (38, 39). Although hepatic glucose production was not measured in the present study, one can assume a basal hepatic glucose production of ~2.2 mg/kg per min (40, 41); somatostatin thus caused a 60% decrease in hepatic glucose

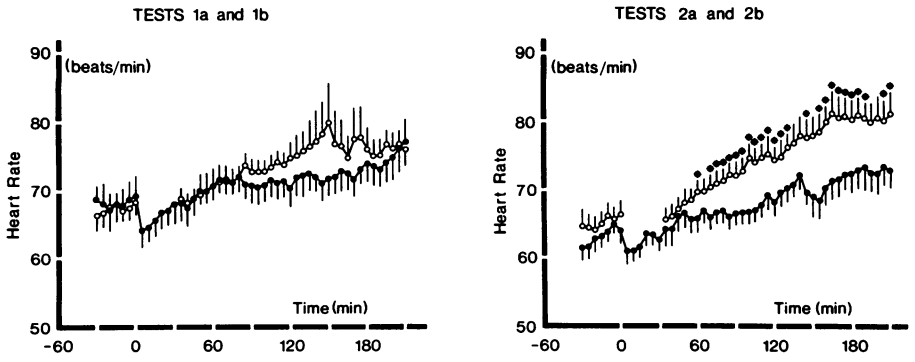

Figure 5. Change in resting heart rate with (● —— ●) and without somatostatin (○ —— ○) infusion during the two clamp protocols (*n* = 8; mean±SEM).

production in our study consistent with the results of others (38, 39).

During the last 30 min of the hyperinsulinemic euglycemic clamp with somatostatin, glucose uptake was somewhat lower (6.5±0.4 mg/kg per min) than during the same phase without somatostatin (7.2±0.5 mg/kg per min), but the difference was not significant. While other studies have reported decreased peripheral glucose utilization after long periods of somatostatin infusion (38, 42), they have been questioned by Bergman et al. (43) who observed an increased glucose clearance with somatostatin infusion and insulin in dogs. They proposed that somatostatin increases insulin receptor sensitivity to insulin but their rate of somatostatin infusion was approximately eight times the rate used in the present study. Such large doses could cause nausea and abdominal pain in human subjects (44).

The initiation of somatostatin infusion not only influenced hepatic glucose production but other metabolic parameters as well. Transient falls were also observed in metabolic rate and glucose oxidation, conversely fat oxidation rose concomitant with an increase in plasma free fatty acids. The latter was probably due to increased lipolysis by the removal of the inhibitory effect of insulin. Whether the fall in glucose oxidation was a result of reduced hepatic glucose production (38, 39), inhibition of glucose oxidation by the glucose fatty-acid cycle (45) or both cannot be determined from our data.

The influence of increasing insulin concentrations on plasma catecholamine concentrations has been mentioned above. Like Rowe et al. (33), we found that these hormonal changes correlated well with our heart rate measurements. An 11% increase in circulating plasma catecholamine concentration was accompanied by an 11% increase in heart rate. It is of interest to note that when the somatostatin infusion was initiated, there was a significant but transient fall in heart rate (see Fig. 5) that lasted 10–15 min. This effect of somatostatin on the heart rate has been observed before (46, 47), but no suggestions were made to explain this phenomenon. Since somatostatin inhibits pancreatic insulin and glucagon secretion, this decrease in heart rate could be due to inhibition of the positive inotropic action of insulin (48) and/or the positive inotropic and chronotropic action of glucagon (49). But this would not explain the transient nature of the response. It is also possible that somatostatin might influence the sympathetic nervous system. Somatostatin or somatostatin-like peptides have been found in the brain (50), and it has been suggested that somatostatin and endorphin neurons interact to modulate sympathetic outflow to the adrenal medulla (51). The same authors have not, however, observed similar effects of somatostatin on peripheral sympathetic nerve endings, nor has it been found to decrease plasma epinephrine or norepinephrine concentrations (52); this is in agreement with the results of this study. Assuming that the slight decrease in heart rate observed with somatostatin infusion represents a change in sympathetic nervous activity, this is of a transitory nature since the resting heart rate was reestablished before the start of the insulin infusion.

In conclusion our results demonstrate that a large proportion of the thermic response to glucose/insulin infusions is due to glucose metabolism alone. The contribution of insulin per se in the stimulation of energy expenditure is small and appears to be mediated via the sympathetic nervous system, thus at physiological insulin concentrations, the thermogenic effect of insulin per se is negligible.

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