Loss of Early Phase of Insulin Release in Humans Impairs Glucose Tolerance and Blunts Thermic Effect of Glucose

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SUMMARY

Loss of the early phase of insulin release has been documented in both type I (insulin-dependent) and type II (non-insulin-dependent) diabetes; however, the physiological importance of this loss is unsettled. We created a model of loss of the early phase of insulin release in normal volunteers. Somatostatin (SRIF) was briefly infused (from −5 to 15 min) during intravenous (IVGTT) and oral (OGTT) glucose tolerance tests. The thermic response to oral glucose was determined under these conditions by indirect calorimetry. Early insulin release was totally blocked during IVGTT and OGTT by SRIF infusion. During the IVGTT, glucose tolerance was deteriorated in association with loss of the early phase of insulin release as indicated by a decrease in the *K* value (control 1.9 ± 0.36 vs. SRIF 1.1 ± 0.27, $P < .001$). Higher plasma glucose concentrations were observed during SRIF tests in the OGTT at 60, 90, 120, 150, and 180 min; total glycemic excursion was larger during the SRIF test (9473 ± 3089 mg · dl^{-1} · 5 h^{-1}) when compared with the control condition (6583 ± 2329 mg · dl^{-1} · 5 h^{-1}). During the OGTT the total amount of glucose oxidized (control 56 ± 4.2 vs. SRIF 55 ± 3.4 g/5 h) was similar in both conditions, suggesting that nonoxidative pathways of glucose disposal were responsible for the deterioration in glucose tolerance. Surprisingly, we found that glucose-induced thermogenesis was reduced in association with loss of the early phase of insulin release (control 102 ± 21.3 vs. SRIF 72 ± 27.8 J/5 h, $P < .001$). Thus, loss of the early phase of insulin release is associated with deterioration of glucose tolerance and blunted glucose-induced thermogenesis. These data underscore the key role of the early phase of insulin release in glucose homeostasis and provide a possible mechanism that could lead eventually to obesity and diabetes. *Diabetes* 36:1167–72, 1987

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Two abnormalities common to insulin-dependent (type I) and non-insulin-dependent (type II) diabetes are loss of the early phase of insulin secretion (1–5) and insulin resistance (6–11). The physiological importance of the loss of the early phase of insulin release is unsettled; however, qualitative defects in insulin secretion and peripheral insulin resistance might be linked, because insulin deficiency may lead to insulin resistance (12,13). It has been shown that glucose- and insulin-induced thermogenesis is blunted in insulin-resistant states (14). The magnitude of this defect correlates with the degree of insulin resistance (15) and could presumably be a contributory factor in obesity (16). We present data to support the hypothesis that deletion of the early phase of insulin release causes impaired glucose tolerance, diminishes the thermic response to glucose, and thereby provides a mechanism that could be linked to the promotion of obesity and insulin resistance.

MATERIALS AND METHODS

Seven healthy volunteers without family history of diabetes and free of medication were studied. Routine laboratory evaluations, thyroid function, and oral glucose tolerance tests (OGTT) were normal (17). Informed consent was obtained before the study. Tests were done in the Clinical Research Center of the University of Vermont.

The volunteers consumed a weight-maintaining diet with at least 250 g of carbohydrate daily and refrained from strenuous physical activity. None were involved in physical training programs. Each volunteer underwent four tests in random order: two intravenous glucose tolerance tests (IVGTT) and two OGTT.

Volunteers were admitted at 0700 h for the IVGTT, and an intravenous catheter was placed. To inhibit the early phase

of insulin release, somatostatin (SRIF, Bachem, Torrance, CA), freshly dissolved in saline, was briefly infused (200 μg/h) from −5 to 15 min during the IVGTT. After baseline samples were taken, glucose (50% solution) was given acutely at a dose of 500 mg/kg body wt (maximum dose 25 g). Samples for glucose and insulin determination were taken at 2, 3, 5, 10, 15, 20, 30, 40, 50, and 60 min. Saline replaced SRIF for control IVGTT studies.

For the OGTT, volunteers were admitted the night before the test. Supper was served between 1700 and 1800 h to standardize fasting period (~14 h). Urine was collected overnight and during the test to measure urinary urea nitrogen excretion. An intravenous line was placed at ~0700 h. Resting metabolic rate was measured with indirect calorimetry with the hood method for 30–45 min. To inhibit early insulin release, SRIF or saline infusion (control), as described for the IVGTT, was begun at −5 min and continued until 15 min. After baseline blood samples were taken, the volunteer ingested 75 g of glucose dissolved in water with a straw in <10 min (mean, 4.6 ± 1.1 min). Samples for glucose, insulin, and C-peptide were taken at 2.5, 5, 7.5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, and 300 min.

The thermic response to the ingestion of glucose was measured by the hood method (18). Briefly, the head of the volunteer was enclosed in a plastic, transparent, and ventilated (35–40 L/min) hood. Oxygen concentration was measured in expired air with a zirconium fuel cell analyzer (Applied Electrochemistry, Sunnyvale, CA) and carbon dioxide with an infrared analyzer (Applied Electrochemistry). Flow was measured with a pneumotachograph (Vertek, Burlington, VT). The voltage outputs of the analyzers were converted to digital signals and read continuously in a desktop computer (Hewlett-Packard HP-85). Total rate of CO_2 production (V_{CO_2}) was calculated by the formula

$$V_{CO_2} = \Delta\%CO_2 \cdot \text{flow rate} \cdot U$$

where $\Delta\%CO_2$ is the difference between the $\%CO_2$ mea-

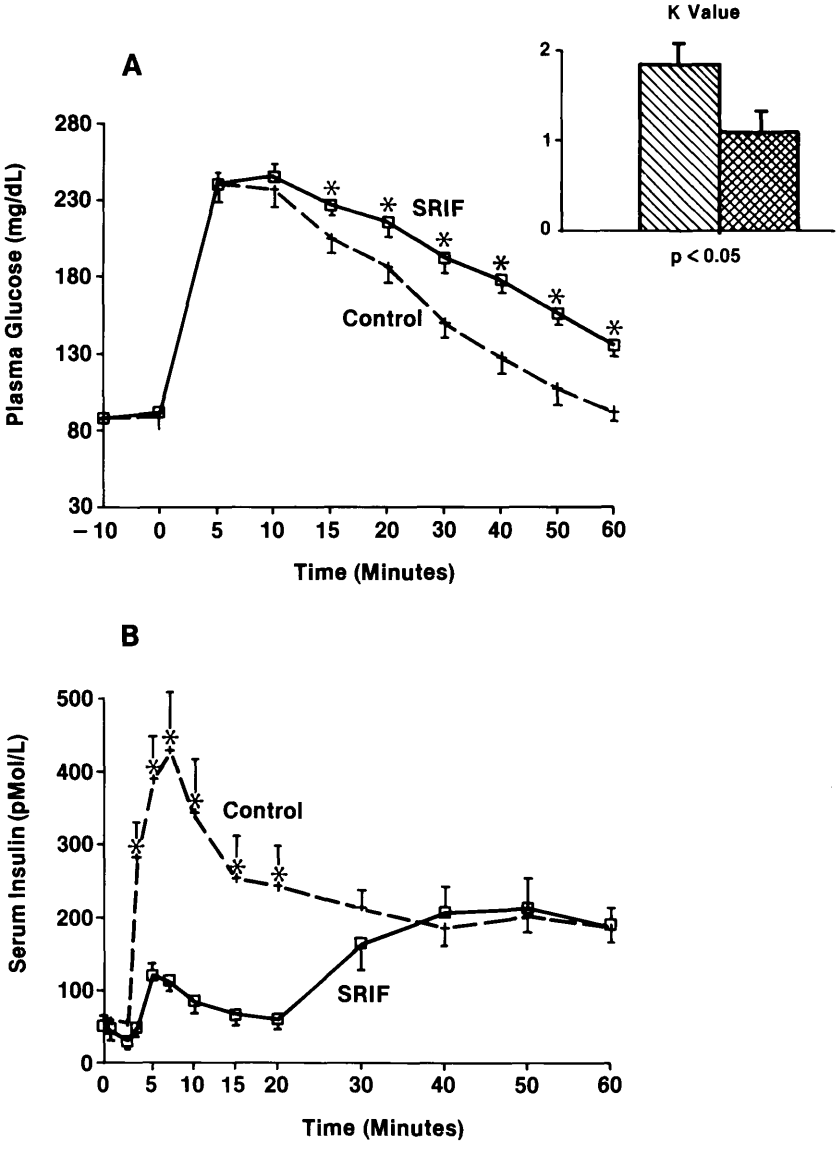

FIG. 1. Intravenous glucose tolerance test (IVGTT) showing plasma glucose response (*A*) and insulin response (*B*). Infusion of somatostatin (SRIF) from −5 to 15 min totally blocked early insulin release. *Insert* depicts *K* values for control (*hatched*) and SRIF (*cross-hatched*). *K* value (% decrease/min) calculated from 10 to 60 min, assuming that decrease in plasma glucose concentration in first 10 min of IVGTT is merely a reflection of physical distribution of glucose in blood and extracellular space. *R* value calculated (log glucose vs. time) from 10 to 60 min was always >.98 for all subjects. *$P < .05$ vs. control.

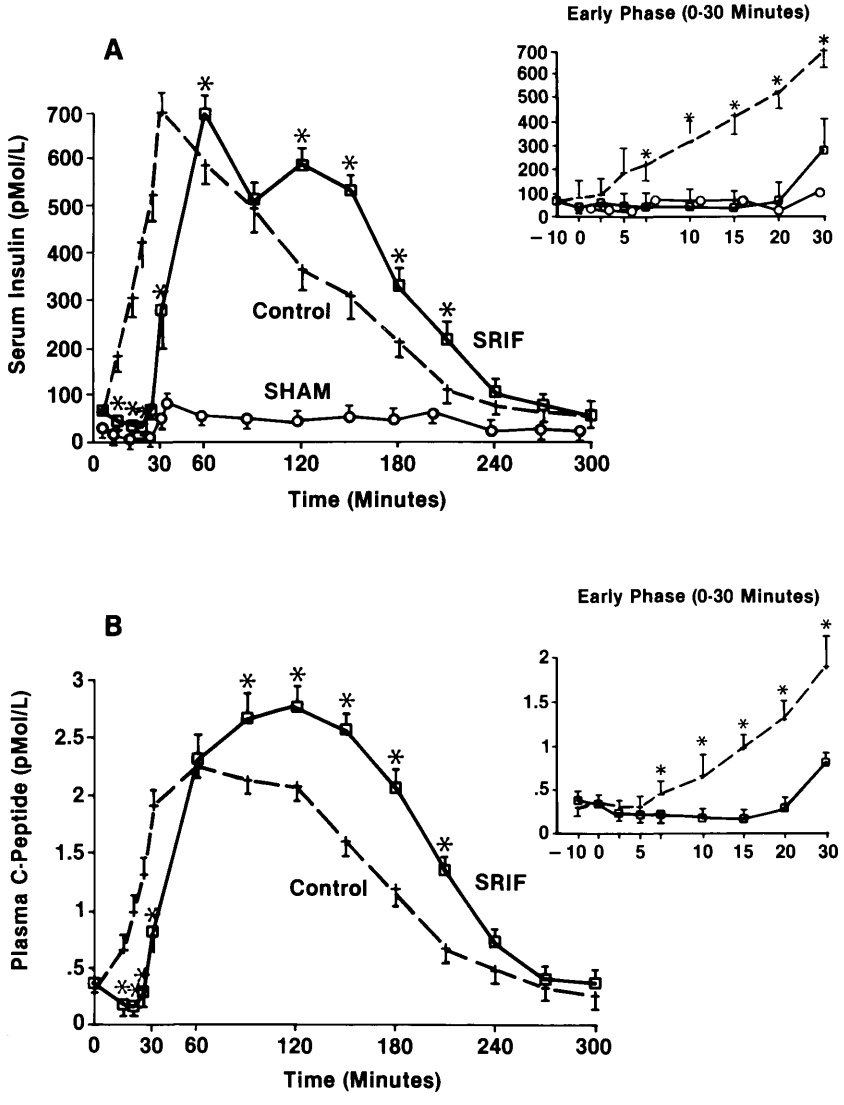

FIG. 2. Oral glucose tolerance test (OGTT) showing β-cell response. *A*, insulin response; *B*, plasma C-peptide secretion during OGTT. *Inserts* show early phase of insulin release (0–30 min) and demonstrate that SRIF infusion totally blocked β-cell response to ingestion of glucose. Rebound response by β-cell is graphically more evident by examining plasma C-peptide response. Areas under curve not distinguished from each other by statistical analysis. In sham studies, other than very modest decrease in first 15 min, serum insulin was maintained at baseline concentrations. *$P < .05$ vs. control.

sured at the end of the hood system and atmospheric $\%CO_2$ concentration (0.04). Flow rate in the hood is standardized to STPD conditions by U, calculated by the formula

$$U = \frac{P - [(5.1265 \cdot \exp^{0.068 \cdot T} \cdot H)/100]}{760(1 + 0.00367 \cdot T)}$$

where P is barometric pressure, H is humidity, and T is temperature. Respiratory gaseous exchange ratio (RQ) was calculated by

$$RQ = 0.7905/\{[(20.94 - \%O_2)/(\%CO_2 - 0.04)] - 0.2095\}$$

which takes into account the Haldane correction for inequality of mixing gases. Total oxygen consumption (V_{O_2}) is then calculated by

$$V_{O_2} = V_{CO_2}/RQ$$

To investigate the effects of SRIF per se on thermogenesis, sham studies were done in four additional subjects. These volunteers were studied exactly as described for the OGTT; however, no glucose was ingested. Glucose, insulin, and thermogenesis were measured as described.

Analytical methods. Plasma glucose was measured by the glucose oxidase method in a glucose analyzer (glucose analyzer 2, Beckman, Fullerton, CA). Serum insulin and plasma C-peptide were measured by radioimmunoassay (19,20).

Calculations and statistical analysis. The *K* value during the IVGTT was calculated between 10 and 60 min with log concentration of glucose versus time. Areas under the curve were calculated by the trapezoidal approach. Unless stated otherwise, all data are expressed as means ± SE.

Energy expenditure was estimated by the caloric equivalent of the oxygen consumption adjusted for total respiratory exchange ratio. For substrate utilization we used the tables of Lusk (21) after calculation of the nonprotein respiratory exchange ratio (NPRQ) assuming that 1 g of urinary urea excretion = 6.25 g of protein oxidized. Briefly, the amount of V_{O_2} and V_{CO_2} due to protein oxidation are subtracted from the initially measured V_{O_2} and V_{CO_2}. A new NPRQ is calculated. Relative contributions of carbohydrate and fat oxida-

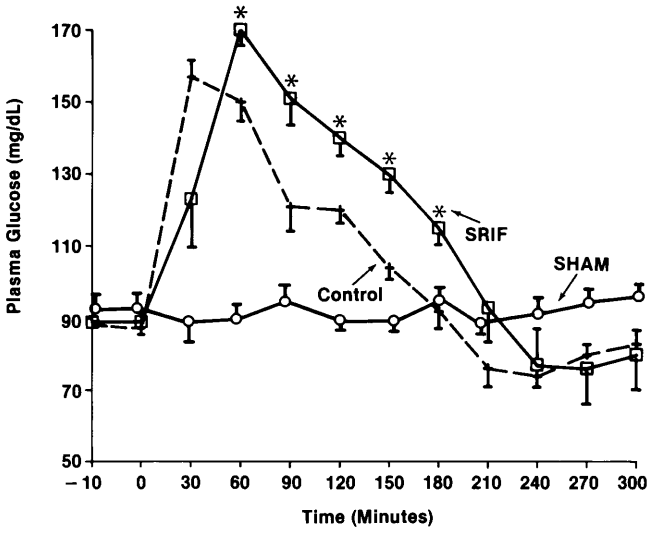

FIG. 3. Oral glucose tolerance test (OGTT) showing plasma glucose response. There was deterioration of glucose tolerance associated with loss of early phase of insulin release; no episode of reactive hypoglycemia was observed. There was no difference in total amount of carbohydrate oxidized, suggesting that nonoxidative disposal of glucose was responsible for this deterioration. *$P < .05$ vs. control.

tion to nonprotein V_{O_2} are calculated taking into consideration that at a NPRQ of 0.7, 100% of the nonprotein V_{O_2} is due to fat oxidation, whereas at a NPRQ of 1.00, carbohydrate oxidation accounts for 100% of V_{O_2}. Intermediate values are interpolated.

Two-way ANOVA (time and treatment) with repeated measurements was used to compare SRIF versus control studies. Post hoc separation of mean values was employed when the F ratio was indicative of statistical significance, predefined as $P < .05$. Student's paired t test was used for comparison of areas under the OGTT curve and for the K values (22).

RESULTS

Intravenous glucose tolerance test. The SRIF infusion totally blocked the early phase of insulin secretion in response to acute hyperglycemia (Fig. 1). After stopping the SRIF infusion, β-cell secretion returned and concentrations of serum insulin were indistinguishable from control studies from 30 to 60 min. The K value during control IVGTT was 1.9 ± 0.36, and by comparison, there was a significant decrease during the SRIF test (1.1 ± 0.27, $P < .001$).

Oral glucose tolerance test. As expected, β-cell activity increased during the first minutes of the OGTT in control studies; a difference was observed versus baseline as early as 10 min (205 ± 36 vs. 62 ± 24 pM, $P < .05$) and reached a peak value of 708 ± 33.2 pM at 30 min (Fig. 2). Thereafter, there was a steady decline to a nadir of 63 ± 19 pM at 240 min. In marked contrast, SRIF totally blocked insulin secretion during the first 20 min of the OGTT. Later, serum concentrations of insulin rebounded and were similar to control at 60 min. Afterward, insulin concentrations were greater than control concentrations, with differences demonstrable at 120, 150, and 180 min. Glucose tolerance deteriorated in association with the changes in β-cell activity. Plasma glucose concentrations were higher after SRIF at 60, 90, 120, 150, and 180 min (Fig. 3). Total glycemic excursion was greater during the SRIF test (9473 ± 3089 vs. 6583 ± 2329 mg · dl^{-1} · 5 h^{-1}, $P < .05$). The total amount of glucose oxidation, calculated by indirect calorimetry, was similar in both conditions (55 ± 3.4 vs. 56 ± 4.2 g/5 h).

Preingestion energy expenditure was similar in both conditions (control 4.6 ± 0.1 vs. SRIF 4.4 ± 0.2 kJ/min). In control studies, oral glucose produced an increase in energy expenditure; a peak value of 5.5 ± 0.25 kJ/min at 90 min slowly declined to resting values at 240 min (Fig. 4). In association with the loss of the early phase of insulin release, the thermic response to glucose was reduced in magnitude. During the SRIF test energy expenditure rose to 4.9 ± 0.15 kJ/min at 120 min ($P < .05$ vs. control) and then declined to baseline values. Total energy expenditure (above resting metabolic rate) was 102 ± 21.3 J for control studies versus 72 ± 27.8 J for SRIF tests ($P < .001$).

As expected, serum insulin decreased in the sham studies in the first 15 min of the test in association with SRIF infusion from a baseline of 75 ± 35 to 45 ± 15 pM; thereafter, concentrations were indistinguishable from baseline values. Neither plasma glucose nor energy expenditure was affected by the SRIF infusion.

DISCUSSION

The pattern of insulin secretion during both the IVGTT and the OGTT during SRIF tests resembled that in the diabetic state (23). During the OGTT an initial sluggish response (in association with loss of the early phase) followed by late rebound has been described as an early abnormality of diabetes (1) and has been thought to explain the reactive hypoglycemia observed in diabetics in early stages of the

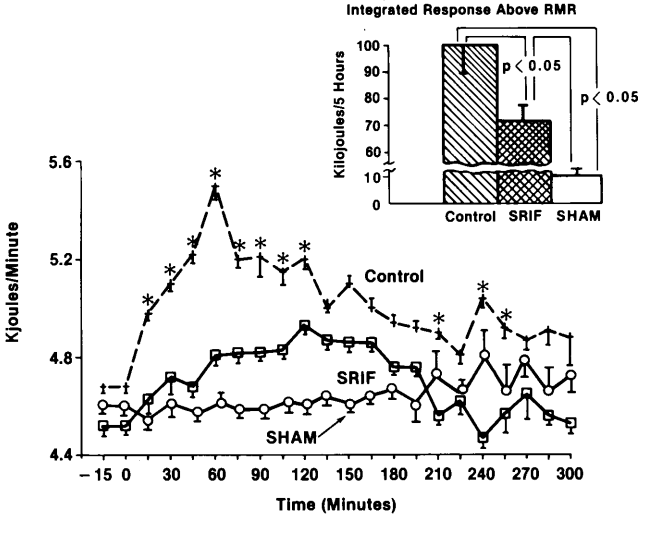

FIG. 4. Oral glucose tolerance test showing thermic response. Energy expenditure measured by indirect calorimetry with hood system. Values expressed as kJ/min (mean ± SE); to convert to kcal/min divide by 4.184. In all subjects, caloric expenditure and respiratory gaseous exchange ratio (RQ) came to values that were statistically indistinguishable from baseline after 4 h. Between 240 and 300 min, there were large fluctuations in readings in most subjects, attributable to restlessness of volunteers. Total thermic response (*inset*) calculated from 0 to 5 h as area under curve minus baseline, assuming that resting metabolic rate (RMR) would remain at same level throughout the test. In sham studies, RMR did not change compared with baseline; fluctuations in readings were also observed between 4 and 5 h, caused mainly by mild anxiety in subjects. *$P < .05$ vs. control.

disease (24). Loss of the early phase of insulin release could initiate diabetes (5,6); however, it could also be a consequence of a reduced β-cell mass and thus not a primary event. In type I diabetes, loss of the early phase of insulin release correlates with the appearance of islet cell antibodies (2). It was recently found that hyperglycemia per se can inhibit insulin release (25,26). Thus, loss of the early phase of insulin release could be a consequence rather than the cause of an abnormal metabolic milieu.

In these studies, the loss of the early phase of insulin release or its delay was associated with decreased tolerance to intravenous and oral glucose. Concentrations of glucose did not reach the levels seen in diabetes; however, note that this deterioration occurred acutely in normal volunteers. That SRIF per se is not responsible for this deterioration is supported by the unchanged plasma glucose response during the sham studies. Thus, our model for inhibiting the early phase of insulin release reproduces key metabolic changes seen in early diabetes (1–4), i.e., glucose intolerance to both intravenous and oral glucose and late hyperinsulinemia during the OGTT.

We also found that the thermic effect of glucose was clearly diminished in association with loss of the early phase of insulin secretion. This could be related to loss of the early phase of insulin release or to an effect of SRIF on thermogenesis. In humans, short-term administration of SRIF has not been shown to decrease caloric expenditure (27), and the very short-lived biologic activity attributable to the acute administration of SRIF (28) could not reasonably account for the prolonged inhibition of thermic response to glucose observed in this study. The SRIF inhibits gastrointestinal function (28); a delay in absorption could create a shift in time in metabolic responses and this could account for the blunted thermogenesis during the OGTT. The fact that all metabolic parameters (energy expenditure, RQ, glucose, insulin, and C-peptide) had returned to baseline values after 4 h in all the studies suggests that glucose absorption was complete during the control and SRIF conditions. Furthermore, thermogenesis was not affected by the infusion of SRIF per se as demonstrated in the sham studies. Thus, we can tentatively conclude that SRIF was not directly responsible for the inhibition of the thermic effect of glucose.

Insulin increases energy expenditure by enhancing rates of glucose disposal (29) and possibly by activating the membrane-bound Na^+-K^+-ATPase (30) and/or by stimulating the sympathetic nervous system (31). As reported in other studies, diabetic and obese patients have a blunted thermogenic response to glucose and insulin infusions that correlates with the magnitude of glucose intolerance (14–16). This defect is overcome by large amounts of insulin, suggesting that it is related to insulin resistance (32). In athletes with diminished early-phase insulin secretion, LeBlanc et al. (33) observed reduced thermic effect of food. In contrast, recent observations suggest that insulin per se plays a minor role, if any, as a thermogenic hormone, because intracellular metabolism correlates better with the thermic response than changes in plasma insulin (34,35). Note that insulin is the primary signal for internalization and intracellular metabolism of glucose, especially in muscle (36). Plasma levels of catecholamines increase after ingestion of glucose (37) catecholamines increase oxygen consumption (38). The interaction of insulin, catecholamines, and glucose is apparently responsible for most of the postprandial thermogenesis in rodents and humans (39–42). In light of our results, an early intravenous rapid increase in serum insulin could activate the sympathetic nervous system and increase energy expenditure. Thus, the apparent conflict in published data could be reconciled if insulin is thought of not as a direct thermogenic effector but as a signal for other thermogenic systems.

We have shown that acute loss of the early phase of insulin release creates impaired glucose tolerance and decreased thermic response to glucose. The long-term consequences of a lower thermic effect of food is an open question; however, it is conceivable that it could promote or at least be a contributing factor on a chronic basis to obesity (43) and insulin resistance. Our data underscore the pivotal role of the early phase of insulin release in glucose homeostasis and thermogenesis in response to glucose.

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