The glucose-dependent insulinotropic polypeptide and glucose-stimulated insulin response to exercise training and diet in obesity

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1Department of Pathobiology, Lerner Research Institute; 2Department of Endocrinology, Diabetes and Metabolism, Cleveland Clinic; 3Department of Nutrition, School of Medicine, Case Western Reserve University; and 4Department of Gastroenterology/Hepatology, Cleveland Clinic, Cleveland, Ohio

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Kelly KR, Brooks LM, Solomon TP, Kashyap SR, O’Leary VB, Kirwan JP. The glucose-dependent insulinotropic polypeptide and glucose-stimulated insulin response to exercise training and diet in obesity. Am J Physiol Endocrinol Metab 296: E1269–E1274, 2009. —Aging and obesity are characterized by decreased β-cell sensitivity and defects in the potentiation of nutrient-stimulated insulin secretion by GIP. Exercise and diet are known to improve glucose metabolism and the pancreatic insulin response to glucose, and this effect may be mediated through the incretin effect of GIP. The purpose of this study was to assess the effects of a 12-wk exercise training intervention (5 days/wk, 60 min/day, 75% VO2max) combined with a eucaloric (EX, n = 10) or hypocaloric (EX-HYPO, pre: 1,945 ± 190, post: 1,269 ± 70, kcal/day; n = 9) diet on the GIP response to glucose in older (66.8 ± 1.5 yr), obese (34.4 ± 1.7 kg/m2) adults with impaired glucose tolerance. In addition to GIP, plasma PYY3–36, insulin, and glucose responses were measured during a 3-h, 75-g oral glucose tolerance test. Both interventions led to a significant improvement in VO2max (P < 0.05). Weight loss (kg) was significant in both groups but was greater after EX-HYPO (−8.3 ± 1.1 vs. −2.8 ± 0.5, P = 0.002). The glucose-stimulated insulin response was reduced after EX-HYPO (P = 0.02), as was the glucose-stimulated GIP response (P < 0.05). Furthermore, after the intervention, changes in insulin (ΔI0–30/ΔG0–30) and GIP (ΔG0–30) secretion were correlated (r = 0.69, P = 0.05). The PYY3–36 (ΔΔ0–30) response to glucose was increased after both interventions (P < 0.05). We conclude that 1) a combination of caloric restriction and exercise reduces the GIP response to ingested glucose, 2) GIP may mediate the attenuated glucose-stimulated insulin response after exercise/diet interventions, and 3) the increased PYY3–36 response represents an improved capacity to regulate satiety and potentially body weight in older, obese, insulin-resistant adults.

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GLUCOSE-DEPENDENT INSULINOTROPIC POLYPEPTIDE (GIP) and glucagon-like peptide (GLP-1) represent the primary incretin hormones and have been shown to stimulate insulin secretion in response to nutrient or glucose ingestion (4, 11, 14). Although GIP and GLP-1 share similar metabolic functions, they act through independent pathways, and their effects are mediated through unique G protein-coupled receptors (9, 33). GIP is secreted from K cells in the small intestine, and increased secretion has been reported in obesity (15, 31, 39), impaired glucose tolerance (IGT) (39), and type 2 diabetes mellitus (15). It has been suggested that this overactivation of the enterino-
mine the effect of an exercise training intervention on GIP and its incretin effect on glucose-stimulated insulin secretion in older obese adults. Since weight loss may be an important factor in determining GIP release, we also assessed the effects of exercise training combined with a calorie-reduced diet on basal and glucose-stimulated gastrointestinal peptides (GIP and PYY3–36). We hypothesized that exercise training plus a hypocaloric diet would reduce glucose-stimulated insulin and GIP release and increase PYY3–36 secretion, and because of the greater weight loss this improvement would be greater than exercise training alone.

METHODS

Participants. Nineteen older obese men and women with IGT were recruited to participate in the study. Participants were classified as IGT if they had a fasting glucose that was ≥100 mg/dl but <126 mg/dl and a 2-h oral glucose tolerance test (OGTT) value between 140 and 199 mg/dl in accordance with the criteria of the American Diabetes Association (3). Medical screening included a complete history and physical examination, an electrocardiogram, an exercise stress test, and a complete metabolic blood profile. Individuals with diabetes, significant hypertension, and heart, liver, gastrointestinal, kidney, or pulmonary disease were excluded from the study. In addition, individuals with physical ailments that would have prevented them from exercising were also excluded. All participants were sedentary and weight stable for 6 mo prior to the study. All metabolic and physiological measurements were performed during a 3-day in-patient hospital stay in the General Clinical Research Center. The study was approved by the Institutional Review Board of the Cleveland Clinic, and all subjects provided written, informed consent in accordance with the guidelines for the protection of human subjects.

Intervention. Subjects were randomized to one of two groups, an exercise training group that maintained its usual caloric intake [eucaloric diet (EX): n = 10, 8 males and 2 females; age: 65.7 ± 1.6 yr; BMI: 34.2 ± 1.8 kg/m²] or an exercise training group that was counseled to reduce its caloric intake by ~500 kcal/day [hypocaloric diet (EX-HYPO): n = 9, 4 males and 5 females; age: 68.0 ± 1.4 yr; BMI: 34.5 ± 1.7 kg/m²]. Three-day diet records were collected prior to the beginning of the study, and individual nutritional counseling was provided weekly to reinforce and support the dietary restrictions and to monitor caloric intake. Exercise training included 50–60 min/day of supervised moderate-intensity aerobic exercise (treadmill/cycle ergometer) at ~75% of maximal oxygen uptake capacity performed 5 days/wk (excluding weekends) for 12 wk.

Body composition. Height without shoes was measured to the nearest 1.0 cm. Body weight was measured to the nearest 0.1 kg, with the subjects wearing only undergarments and a hospital gown. Waist circumference was measured at the level of the umbilicus. Body density was determined by hydrostatic weighing, and body fat mass was calculated using Siri equations, as described previously (35).

Aerobic fitness. Physical fitness was measured using an incremental treadmill exercise test to determine subjects’ maximal oxygen consumption (VO2max), as described previously (40).

OGTT. A 75-g OGTT was performed after a 12- to 14-h overnight fast pre- and postintervention. Postintervention, the OGTT was conducted within 18 h following the last exercise bout. Fasting baseline samples were drawn to determine initial glucose, insulin, and gut peptide measurements. Following baseline draws, a 75-g glucose drink was ingested within a 10-min period. Blood samples were drawn for glucose, insulin, and gut peptides at 30, 60, 90, 120, and 180 min after the glucose was ingested. Plasma glucose concentrations were measured immediately using the glucose oxidase method (Beckman Instruments, Fullerton, CA). For GIP and PYY3–36 analysis, blood was collected in vacutainers containing EDTA and the protease inhibitor aprotonin and was centrifuged at 1,000 rpm for 10 min at 4°C. The samples were stored at ~80°C for subsequent analysis. An index of glucose-stimulated-insulin secretion was calculated as insulin Δ0–30 min/glucose Δ0–30 min (1).

Blood analysis. Insulin was assayed via commercial RIA (Linco, St. Charles, MO). Plasma GIP (total) and PYY3–36 were analyzed via commercial ELISA kits (Linco). To correct for interassay variability, all pre- and postmeasurements for each individual subject were run on the same plate.

Statistical analysis. All values are expressed as means ± SE. Total hormonal responses (area under the curve) were calculated using the trapezoidal approach. In addition, immediate hormone secretory responses to glucose ingestion were estimated as Δ0–30 min. Repeated-measures ANOVA was used for between-group comparisons (Statview; SAS Institute, Cary, NC). Tukey post hoc tests were applied to identify relationships created as a result of the intervention. Statistical significance was accepted at P < 0.05.

RESULTS

Dietary intake. Based on weekly diet counseling with a research dietitian and 3-day diet records collected every 4 wk during the 12-wk intervention, we determined that those in the EX group consumed a diet similar (both calorific and macronutrient composition) to their prior usual diet (Table 1). The EX-HYPO group consumed ~700 fewer kcal daily (Table 1), reduced fat intake by ~5% [%kcal; preintervention (pre): 30 ± 1.3%, postintervention (post): 25 ± 1.1%], and increased carbohydrate intake by ~6% (pre: 50.6 ± 2.6%, post: 56.9 ± 1.5%). The macronutrient composition was similar between the

Table 1. Participant characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>EX Group</th>
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<th>EX-HYPO Group</th>
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<tbody>
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<td></td>
<td>Preintervention</td>
<td>Postintervention</td>
<td>Preintervention</td>
<td>Postintervention</td>
</tr>
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<td>Body weight, kg</td>
<td>93.1±5.2</td>
<td>90.3±5.0*</td>
<td>98.4±5.7</td>
<td>90.1±4.9†</td>
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<td>BMI, kg/m²</td>
<td>34.2±2.1</td>
<td>33.8±1.6*</td>
<td>34.5±1.6</td>
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<td>Fat mass, kg</td>
<td>40.9±3.1</td>
<td>39.4±3.4*</td>
<td>38.8±3.8</td>
<td>33.5±3.5†</td>
</tr>
<tr>
<td>FFM, kg</td>
<td>52.2±2.8</td>
<td>50.9±2.2</td>
<td>61.2±4.6</td>
<td>59.9±4.1</td>
</tr>
<tr>
<td>V02max, ml·kg·FFM⁻¹·min⁻¹</td>
<td>34.1±1.1</td>
<td>38.2±1.6*</td>
<td>36.1±1.7</td>
<td>39.9±1.5*</td>
</tr>
<tr>
<td>Caloric intake, kcal/day</td>
<td>1,633±78</td>
<td>1,637±175</td>
<td>1,945±190</td>
<td>1,269±70*</td>
</tr>
<tr>
<td>Macronutrients, %carbohydrate/fat/protein</td>
<td>48/34/18</td>
<td>46/34/20</td>
<td>51/30/19</td>
<td>57/25/18</td>
</tr>
</tbody>
</table>

Data represent means ± SE. Participant characteristics prior to and following 12 wk of aerobic exercise training and either a eucaloric (EX) or hypocaloric (EX-HYPO) diet. BMI, body mass index; FFM, fat-free mass. V02max, maximal volume of oxygen consumption. EX, n = 10; EX-HYPO, n = 9.*Significantly lower than preintervention value, P < 0.05. †Significantly lower than the EX group, P < 0.05.
EX and EX-HYPO subjects both pre- and postintervention (Table 1).

**Body composition.** At the end of the 12-wk intervention, body weight (P < 0.0001), BMI (P = 0.0012), and fat mass (P < 0.0001) were significantly reduced in both groups (Table 1). As expected, subjects in the EX-HYPO group showed greater changes in these parameters (body weight, P = 0.002; BMI, P = 0.01; fat mass, P = 0.0008). Notably, there was no significant change in fat-free mass (FFM; P = 0.09) for either group.

**Aerobic fitness.** VO$_{2\text{max}}$ (ml·kg FFM$^{-1}$·min$^{-1}$) was used as an index of aerobic fitness. Both groups achieved a significant improvement (P = 0.02) in VO$_{2\text{max}}$ over the course of the intervention (Table 1).

**Glucose and insulin.** Fasting plasma glucose was significantly reduced after EX-HYPO (P = 0.04) but not after EX alone. Furthermore, the glucose response (area under the curve) was significantly reduced (P = 0.04) in the EX-HYPO group but not the EX group (Table 2). The EX-HYPO intervention induced a decrease in both fasting insulin (P = 0.04) and area under the insulin response curve (P = 0.01) (Table 2). EX alone was not sufficient to significantly reduce fasting insulin or the insulin response to glucose. Insulin secretion was significantly reduced in the EX-HYPO group following the intervention (pre: 0.86 ± 0.15, post: 0.55 ± 0.09; P = 0.03) and was decreased, but not significantly, in the EX group (pre: 0.85 ± 0.16; post: 0.78 ± 0.15) (Fig. 1, A and B). Insulin sensitivity was calculated using the Matsuda index, which is a function of fasting glucose and insulin, and the mean glucose and insulin responses during the OGGT (26). Insulin sensitivity was increased for EX-HYPO (pre: 2.69 ± 0.5; post: 4.16 ± 0.55; P = 0.01); however, there was no correlation between the change in insulin sensitivity and GIP (r = −0.335, P = 0.16).

**GIP and PYY$_{3-36}$.** After the intervention, fasting GIP concentrations were unchanged in both groups (Table 2 and Fig. 2, A and B). However, the GIP response to glucose was significantly reduced in the EX-HYPO group compared with baseline and with the EX group (P = 0.04 and P = 0.007, respectively; Fig. 2B). Furthermore, the immediate GIP secretory response as determined by the change from time 0 to 30 min was reduced in EX-HYPO compared with prestudy (P = 0.04; Fig. 2, inset) and EX poststudy (P = 0.06). There was no change in the GIP response to glucose or secretion following EX (Fig. 2, A and A, inset, and Table 2). In addition, there was a significant correlation between the percent change in insulin and GIP after EX-HYPO (r = 0.85, P = 0.002; Fig. 3).

Table 2. Fasting glucose, insulin, and gut peptides and responses following glucose ingestion

<table>
<thead>
<tr>
<th>Variable</th>
<th>Preintervention</th>
<th>Postintervention</th>
<th>Preintervention</th>
<th>Postintervention</th>
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<tbody>
<tr>
<td></td>
<td>EX Group</td>
<td>EX-HYPO Group</td>
<td>EX Group</td>
<td>EX-HYPO Group</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>112.3 ± 5.8</td>
<td>110.2 ± 4.5</td>
<td>112.0 ± 4.7</td>
<td>100.5 ± 4.2*</td>
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<tr>
<td>AUC, mg·dl$^{-1}$·h</td>
<td>30.903 ± 1.670</td>
<td>29.183 ± 1.220*</td>
<td>28.629 ± 1.478</td>
<td>25.281 ± 1.477*</td>
</tr>
<tr>
<td>Insulin, μU/ml</td>
<td>20.1 ± 3.8</td>
<td>16.9 ± 2.8</td>
<td>16.9 ± 2.2</td>
<td>13.4 ± 1.6†</td>
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<tr>
<td>AUC, μU·ml$^{-1}$·h</td>
<td>11.724 ± 2.242</td>
<td>10.421 ± 1.951</td>
<td>10.310 ± 1.511</td>
<td>6.585 ± 1.259*</td>
</tr>
<tr>
<td>GIP, pg/ml</td>
<td>83.9 ± 16</td>
<td>77.7 ± 22</td>
<td>61.7 ± 12</td>
<td>59.3 ± 18</td>
</tr>
<tr>
<td>AUC, pg·ml$^{-1}$·h</td>
<td>32.107 ± 6976</td>
<td>37.314 ± 6314</td>
<td>33.498 ± 5.658</td>
<td>26.688 ± 5.134†</td>
</tr>
<tr>
<td>PYY$_{3-36}$ pg·ml</td>
<td>43.3 ± 6.9</td>
<td>39.7 ± 2.7</td>
<td>43.1 ± 5.0</td>
<td>40.1 ± 6.9</td>
</tr>
<tr>
<td>AUC, pg·ml$^{-1}$·h</td>
<td>7.843 ± 895</td>
<td>7.542 ± 1431</td>
<td>6.495 ± 1400</td>
<td>6.994 ± 2100</td>
</tr>
</tbody>
</table>

Data represent means ± SE. AUC, area under the curve; GIP, glucose-dependent insulinootropic polypeptide; PYY$_{3-36}$, peptide YY$_{3-36}$. Fasting plasma glucose, insulin, and gut peptide levels and plasma responses following oral glucose before and after the interventions. EX, n = 10; EX-HYPO; n = 9. *Significantly lower than preintervention. †Significantly lower than the EX group, P < 0.05.
DISCUSSION

The effect of exercise on gut peptides, the gut peptide response to nutrient stimulation, and insulin secretion, especially in insulin-resistant conditions, has not been studied extensively. Our results show, for the first time, that an exercise/diet lifestyle intervention reduces plasma GIP and increases the PYY3–36 response following glucose stimulation in older, obese, insulin-resistant adults. This study also shows that the change in glucose-stimulated insulin secretion was related to the change in glucose-stimulated GIP response after the respective interventions. These data suggest that physical activity and moderate caloric restriction reduce insulin secretion via attenuation of a GIP-related incretin effect following nutrient stimulation, and this leads to improved glucose tolerance in a population that is at risk for the development of type 2 diabetes.

Previous studies in humans and animals have shown that exercise training by itself generally improves β-cell sensitivity and reduces the insulin response to glucose (16, 22, 30, 36). However, these findings are not universal, and a recent study in young subjects with type 2 diabetes reported no change in glucose-stimulated insulin secretion following exercise training (10). Insulin secretion has also been reported to increase after exercise training in subjects with type 2 diabetes (13). These different responses may be related to the effect of the exercise intervention on weight loss. The cited studies that did not find a reduced insulin response following exercise training also reported that subjects lost little if any body weight. In the present study, weight loss in the EX-HYPO group was substantially greater than EX alone, and greater changes in the glucose-stimulated insulin response and insulin secretion were clearly evident.

In the present study, the GIP response to glucose was reduced after the EX-HYPO intervention but was unchanged after EX alone. We had previously observed an attenuation of the GIP and insulin response to glucose ingestion after an acute bout of exercise in young trained athletes (34), and Blom et al. (6) reported a similar response in young untrained men. When combined with data from Krotkiewski et al. (23), who reported a reduced GIP response to exercise training in severely obese women, we were expecting a decrease in glucose-stimulated GIP after exercise training. However, all of the data supporting a decrease in the GIP response to glucose have been generated in younger subjects; it is likely that older, impaired, glucose-tolerant subjects may need a bigger stimulus to modify the K cell response and alter GIP release. Based on results from the EX-HYPO trial, it would seem that the greater weight loss that was accomplished by exercise and diet together may have provided enough of a stimulus to elicit this response. The observation that changes in the glucose-stimulated GIP response and changes in insulin secretion were correlated after...
the intervention lends support to the known incretin effect of GIP on insulin secretion (4, 15). Thus, it may be that the EX-HYPO intervention induces cellular changes in the β-cell and the K cell, which in turn helps to restore normal incretin function. The identification of the factors that mediate this effect and its related mechanism could not be accomplished in the current study but represent an area of emerging interest. Collectively, these observations lead us to suggest that weight loss, which can be more effectively achieved by a combination of exercise and diet, is an important determinant of improved β-cell function, improved K cell function, and possibly the cross-talk between these cells that allows GIP to help control insulin release in response to nutrient stimulation.

To date, little is known about the effects of exercise training alone, or calorie restriction in conjunction with exercise, on fasting or nutrient-stimulated PYY3–36 responses (17, 32, 37). Some studies have reported a short-lived increase in PYY3–36 levels during an acute exercise bout (25, 41), but it was shown recently that the PYY3–36 response to meal ingestion is elevated for up to 8 h after an acute bout of aerobic exercise (8). The present study is the first to address the effects of exercise/diet interventions on PYY3–36 levels in humans. In both EX and EX-HYPO there was an increase in the immediate PYY3–36 (Δ0–30 min) response to glucose, suggesting an improvement in PYY3–36 sensitivity following exercise training. Exercise has been reported to have an anorexic effect (21), and PYY3–36 is an anorectic hormone that inhibits food intake via hypothalamic signaling (5). Our results demonstrating an increase in glucose-stimulated PYY3–36 release, taken together with previous reports of an increase in PYY3–36 levels during exercise, suggest a possible mechanism whereby exercise can reduce food intake via increased sensitivity to satiety cues, ultimately leading to reductions in body weight.

We recognize that there are aspects of the current study design that limit the conclusiveness of our findings. The absence of a diet-only group limits our ability to distinguish between the effects of diet-induced responses vs. exercise/diet or exercise alone. Now that we have uncovered an exercise/diet design that limit the conclusiveness of our findings. The absence of a diet-only group limits our ability to distinguish between the effects of diet-induced responses vs. exercise/diet or exercise alone. Now that we have uncovered an exercise/diet response in 65-year olds. J Gerontol B: M122–M127, 1993.


REFERENCES

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GRANTS


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GIP RESPONSE TO EXERCISE AND DIET


