Coadministration of Glucagon-Like Peptide-1 During Glucagon Infusion in Humans Results in Increased Energy Expenditure and Amelioration of Hyperglycemia

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Glucagon and glucagon-like peptide (GLP)-1 are the primary products of proglucagon processing from the pancreas and gut, respectively. Giving dual agonists with glucagon and GLP-1 activity to diabetic, obese mice causes enhanced weight loss and improves glucose tolerance by reduction of food intake and by increase in energy expenditure (EE). We aimed to observe the effect of a combination of glucagon and GLP-1 on resting EE and glycemia in healthy human volunteers. In a randomized, double-blind crossover study, 10 overweight or obese volunteers without diabetes received placebo infusion, GLP-1 alone, glucagon alone, and GLP-1 plus glucagon simultaneously. Resting EE—measured using indirect calorimetry—was not affected by GLP-1 infusion but rose significantly with glucagon alone and to a similar degree with glucagon and GLP-1 together. Glucagon infusion was accompanied by a rise in plasma glucose levels, but addition of GLP-1 to glucagon rapidly reduced this excursion, due to a synergistic insulinotropic effect. The data indicate that drugs with glucagon and GLP-1 agonist activity may represent a useful treatment for type 2 diabetes and obesity. Long-term studies are required to demonstrate that this combination will reduce weight and improve glycemia in patients. Diabetes 62:1131–1138, 2013

RESEARCH DESIGN AND METHODS

The study was approved by the Hammersmith and Queen Charlotte’s Research Ethics Committee (09/H0707/76) and carried out according to the principles of the Declaration of Helsinki. Ten overweight/obese volunteers were recruited by advertisement (Table 1). Potential participants were screened and determined to be healthy by medical history, physical examination, hematological and biochemical testing, and 12-lead electrocardiogram. All female participants were premenopausal and had regular menstrual cycles. None were taking the combined oral contraceptive pill, but one volunteer was taking a progestogen contraceptive pill that was deemed acceptable, as progestogen-based contraception has previously been found not to alter EE (17).
Volunteers were placed briefly under a calorimeter canopy to confirm that they would be comfortable in it during study visits.

Volunteers attended for five consecutive study visits spaced ≥2 days apart. The initial visit was an unblinded “dummy” visit where gelofusine (B. Braun, Crawley, U.K.) was infused to acclimatize volunteers to study procedures. For avoidance of liver glycogen depletion, volunteers were asked to avoid alcohol and strenuous exercise for 24 h prior to each study visit and were given a high-energy snack (each containing 3.8 g fat, 20.4 g carbohydrate, and 1.9 g protein; United Biscuits) at 0700 h. Upon arrival, volunteers arrived at the clinical research facility at 0830 h having eaten a small, low-fat energy snack (each containing 3.8 g fat, 20.4 g carbohydrate, and 1.9 g protein; United Biscuits) at 0700 h. Upon arrival, volunteers were asked to empty their bladder; urine was collected at the end of each study visit to provide an estimate of urinary nitrogen excretion.

After dual cannulation to provide a blood sampling point and to allow intravenous infusion of hormones, volunteers were asked to swallow a core temperature measurement capsule (Equivital; Hidalgo, Swavesey, U.K.). They were placed under the indirect calorimeter canopy (Gas Exchange Monitor; GEM Nutrition, Daressbury, U.K.) at 0 min (0900 h). Before each measurement, the calorimeter was calibrated with “zero” (0.00% O2 and 0.00% CO2) and “span” (20.00% O2, 1.00% CO2) gases (BOC Gases, Surrey, U.K.). Volunteers lay semirecumbent on a bed and were allowed to watch television or listen to music. Calorimeter measurements were allowed to stabilize during the first 30 min. Resting EE, respiratory quotient (RQ), and carbohydrate and fat oxidation rates were estimated from measurements of VO2 and VCO2 recorded each minute, with adjustment for urinary nitrogen excretion. Protein oxidation rate over the entire study visit was estimated from urinary nitrogen excretion (18,19). Baseline resting EE was defined as the mean of measurements taken during the final 15 min of this 45-min baseline phase.

At 45 min, a hormone infusion was started, which lasted for 45 min. Volunteers received intravenous infusions of placebo (gelofusine), glucagon (50 ng/kg/min; Novo Nordisk, Crawley, U.K.), GLP-1 (7–36 amide) (0.8 pmol/kg/min; Clinafé Basic, Bachem, Switzerland), or combined GLP-1 and glucagon at the above-mentioned doses, with infusion allocation determined in a four-way, randomized design. Gelofusine was used as the vehicle for hormone infusions in order to minimize absorption of peptides to infusion lines and syringes (20). The infusion was “ramped” to establish stable plasma hormone levels rapidly, with an infusion rate four times the nominal rate for the initial 5 min reduced to twice the nominal rate for the next 5 min and then to the nominal rate for the remaining 35 min. The dose of glucagon was selected after a dose-finding study to determine a well-tolerated dose of glucagon with consistent effects on EE.

Calorimetry continued during the 45-min infusion. As with the baseline phase, calorimetry measurements were allowed to stabilize for 30 min before measurements from the final 15 min were used to calculate the infusion-phase resting EE, RQ, and substrate oxidation rates. At 90 min, the infusion and calorimetry were stopped. Each study visit was terminated at 105 min.

Blood samples were collected at 30, 45, 60, 75, 90, and 105 min and assayed for insulin, glucose, nonesterified fatty acids (NEFAs), thyroid hormones, cortisol, GLP-1, glucagon, and total/acetyl ghrelin levels. Samples for analysis of plasma GLP-1 and glucagon were collected in lithium heparin tubes with 100 μL aprotinin (1,000 kallikrein inhibitor units) and were measured using established radioimmunoassays (21,22). NEFAs were measured by the Department of Chemical Pathology, Great Ormond Street Hospital National Health Service Trust. Ghrelin was measured using total ghrelin and active (acyl) ghrelin ELISA assays (Millipore, Watford, U.K.). Other analytes were measured by the Department of Chemical Pathology, Imperial College Healthcare National Health Service Trust.

Statistical analysis was carried out, as noted in the text and Figure captions, using GraphPad Prism 5.0d (GraphPad Software, San Diego, CA) and STATA 12 (StataCorp, College Station, TX). The change from baseline in the calorigraphic data was modeled using a linear mixed model with random intercepts to model patient heterogeneity. This model allowed the assessment of the presence of a period effect or a period-by-treatment interaction. The within-subject comparison was based on the treatment effect as long as there was not a significant period-by-treatment interaction. In addition, the baseline calorigraphic data and patient weight were included in the model to estimate the adjusted treatment effect. Two-way repeated-measures ANOVA with Bonferroni post hoc test was used to compare differences in glucose, insulin, and ghrelin levels. Area under the curve was calculated using the trapezoidal rule, and differences were compared using one-way repeated-measures ANOVA with Tukey post hoc test. Plasma NEFA concentrations were analyzed with one-way repeated-measures ANOVA and Bonferroni post hoc test. Substrate oxidation rates were analyzed using one-way repeated-measures ANOVA. Unless otherwise noted, results are presented as means ± SEM. Statistical significance was defined as P < 0.05.

## RESULTS

Mean plasma levels of GLP-1 at 30 min before the start of the infusion were 15–23 pmol/L. In those arms receiving the infusion of GLP-1 at 0.8 pmol/kg/min, GLP-1 levels rose to 90–103 pmol/L 60 min after initiation (Fig. 1A). Similarly, glucagon levels at 30 min before starting were 8–11 pmol/L. In those arms receiving the infusion of glucagon at 50 ng/kg/min, glucagon levels rose to 239–260 pmol/L (Fig. 1B). After the infusion was stopped at 90 min, glucagon and GLP-1 levels also rapidly fell to baseline. No adverse events were recorded with the hormone infusions.

GLP-1 infusion was accompanied by a slight decline in plasma glucose levels from 5.3 ± 0.1 to 4.1 ± 0.1 mmol/L as expected (Fig. 2A). Glucagon infusion, conversely, induced a rise in plasma glucose to a peak of 8.3 ± 0.4 mmol/L at 75 min. This rise was blunted when GLP-1 and glucagon were combined, with a peak of 7.1 ± 0.5 mmol/L at 75 min, declining to 6.2 ± 0.7 mmol/L at the end of the infusion at 90 min (Fig. 2A).

Consistent with its glucose-dependent insulinotropic effect, GLP-1 induced an early, small rise in insulin levels from 8.1 ± 2.2 to 22.0 ± 4.1 μU/mL, which fell back to baseline at later time points (Fig. 2C). Glucagon infusion caused a more marked rise in insulin to a peak of 56.2 ± 10.2 μU/mL at 90 min. The combination of GLP-1 and glucagon caused a synergistic rise in insulin levels to a peak of 135.6 ± 35.7 μU/mL—a sixfold rise compared with GLP-1 alone and a 2.4-fold rise compared with glucagon alone (Fig. 2C).

Infusion of glucagon caused a sharp drop in NEFA levels from 0.46 ± 0.07 to 0.18 ± 0.03 mmol/L (Fig. 3). Similarly, the GLP-1 plus glucagon combination caused a fall in NEFA levels from 0.38 ± 0.06 to 0.13 ± 0.18 mmol/L. No significant change in NEFA levels was seen in the placebo or GLP-1 arms.

Baseline resting EE was not significantly different between infusion arms. A linear mixed model analysis of repeated measurements, taking into account subject weight, baseline measurements, and the order of infusion (period by treatment), was used to analyze the differences in resting EE induced by the different infusions: no significant interactions were found with these three possible confounding factors (Fig. 4A and Table 2). There was no significant change in resting EE during GLP-1 infusion. However, resting EE increased significantly during glucagon infusion by a mean of 146.99 kcal/day. This effect was retained when GLP-1 and glucagon were infused in

| TABLE 1 | Demographic details of volunteers in the study |
|-------|------------------|
| Volunteer | Height (m) | Weight (kg) | BMI (kg/m²) | Age (years) | Sex |
| G02     | 1.66         | 99.1        | 36.0        | 26          | F   |
| G03     | 1.63         | 80.0        | 30.1        | 28          | F   |
| G04     | 1.78         | 101.5       | 32.0        | 31          | M   |
| G05     | 1.94         | 99.4        | 25.6        | 23          | M   |
| G06     | 1.81         | 99.0        | 30.2        | 49          | M   |
| G07     | 1.84         | 89.1        | 26.3        | 48          | M   |
| G08     | 1.87         | 100.3       | 28.7        | 32          | M   |
| G09     | 1.82         | 91.1        | 27.5        | 32          | M   |
| G10     | 1.71         | 77.2        | 26.4        | 45          | M   |
| G11     | 1.61         | 79.1        | 29.8        | 45          | M   |
| Mean    | 1.77         | 91.3        | 29.3        | 25.8        | M/3 F |

F, female; M, male.
combination, with a mean increase in resting EE of 146.26 kcal/day.

Baseline RQ values were again not significantly different between infusion arms. The changes in RQ upon infusion were analyzed using the linear mixed model (Fig. 4B and Table 2). There was no significant change in RQ for the GLP-1 infusion arm. There was a significant increase in RQ in the glucagon arm as well as in the GLP-1 plus glucagon arm.

Substrate oxidation rates are summarized in Table 3, which shows that there were no significant differences comparing all arms of the study at baseline. Carbohydrate and fat oxidation rates were analyzed using the linear mixed model (Fig. 4C and D and Table 2). There was no significant change in carbohydrate oxidation rates with GLP-1 infusion. There was a significantly increased carbohydrate oxidation rate with glucagon infusion and a similar increase with GLP-1 plus glucagon infusion. There was no significant change in fat oxidation rate either with GLP-1 infusion or with glucagon infusion, whereas the combined GLP-1 plus glucagon infusion significantly decreased fat oxidation rates.

There was no significant variation in thyroid hormone levels between the baseline phase and the infusion phase apart from a small, statistically significant reduction in thyroid levels when volunteers were given glucagon, which was not seen in the GLP-1 plus glucagon arm and which is therefore of uncertain biological significance (data not shown). There was no significant difference in core temperature (data not shown). There were no significant changes in systolic and diastolic blood pressure, although there was a trend toward a higher pulse rate in the glucagon arm.
and GLP-1 plus glucagon infusion arms compared with the placebo and GLP-1 infusion arms (data not shown).

Ghrelin is a key appetite-regulatory hormone that increases food intake (23). Acyl ghrelin, incorporating an octanoyl group linked to the Ser3 residue, is the active form of ghrelin that activates its growth hormone secretagogue receptor 1a (24). As glucagon and GLP-1 have been known to suppress appetite (6,25) and ghrelin secretion (26,27), the plasma levels of ghrelin during infusion were examined (Fig. 5). Infusion of GLP-1 alone did not influence circulating total and acyl ghrelin levels. Glucagon infusion alone reduced total and acyl ghrelin but not to a statistically significant degree. By contrast, the combination of GLP-1 and glucagon significantly reduced total and acyl ghrelin.

**DISCUSSION**

In this clinical study, we have verified for the first time that combined infusion of GLP-1 and glucagon increases resting EE acutely, by the equivalent of ~150 kcal/day, in a manner similar to that of glucagon infusion alone. We speculate that this phenomenon could be mediated by increased thermogenesis in brown adipose tissue (28) and by futile substrate cycling (29). These effects may be triggered directly by glucagon acting on tissue GcgR (e.g., in brown adipose tissue) or indirectly via an increase in catecholamines (2). No consistent effects of glucagon or GLP-1 plus glucagon were seen on the thyroid hormone axis or on cortisol secretion (data not shown). One caveat is that our observations on EE relate to the acute effects of the GLP-1 plus glucagon combination; a longer-term study will be required to confirm that chronic GLP-1R/GcgR agonism in humans leads to a sustained elevation of EE, as was seen in diet-induced obesity rodent models (15). If such an elevation of EE were to be sustained, it would represent ~7.5% of the typical 2,000 kcal/day resting EE of a 40-year-old male of 100 kg in weight and 1.75 m in height.
At the dose used, glucagon provokes an acute rise in plasma glucose levels, probably via glycogen breakdown. Hyperglycemia is an expected effect of glucagon. Indeed, glucagon antagonists have been mooted as treatments for diabetes on the basis that sustained hyperglucagonemia contributes to the pathogenesis of diabetes (1), although there are major concerns that this strategy may promote hepatic steatosis and liver injury (30). We found that coinfusion of GLP-1 blunted the hyperglycemic effect of glucagon via a synergistic insulinotropic effect, although it did not completely neutralize the acute rise in glucose. It remains possible that the acute hyperglycemic effect would resolve with continued combination treatment, due to increased insulin release and exhaustion of liver glycogen stores. Indeed, the initial hyperglycemia did resolve toward the end of the 45-min infusion of GLP-1 plus glucagon—unlike with glucagon alone (Fig. 2A). It is also noteworthy that oxyntomodulin, administered for 4 weeks to human volunteers, did not result in hyperglycemia (13).

Our study used overweight-obese healthy volunteers without diabetes. Would the synergistic insulinotropic effect of the GLP-1 and glucagon combination be seen in patients with type 2 diabetes and decreased β-cell reserve? Despite the fact that such patients exhibit decreased sensitivity to incretins at physiological levels, it is notable that GLP-1 at pharmacological levels is still able to stimulate a significant insulin response (31). Early indications from clinical trials also suggest that long-term GLP-1R agonist therapy carries positive benefits for insulin secretion and resistance.

**FIG. 3.** Effects of glucagon, GLP-1, and GLP-1 plus glucagon infusion on NEFA levels. Means ± SEM plotted for NEFA levels during the baseline phase (30 min) and at the end of the infusion phase (90 min). ***P < 0.001 for difference between baseline and infusion phase values.

**FIG. 4.** Effects of infusions on EE. The mean changes in each parameter (±95% CI) between baseline and infusion phases are plotted. Baseline resting EEs were as follows: 1,487 ± 70.9 kcal/day (placebo), 1,487 ± 65.9 kcal/day (GLP-1), 1,469 ± 66.0 kcal/day (glucagon), and 1,485 ± 67.4 kcal/day (GLP-1 plus glucagon); they were not significantly different (P = 0.7253, one-way repeated-measures ANOVA). Baseline RQ values for all arms were 0.822 ± 0.018 (placebo), 0.857 ± 0.018 (GLP-1), 0.851 ± 0.020 (glucagon), and 0.854 ± 0.022 (GLP-1 plus glucagon), and these were not significantly different (P = 0.5138, one-way repeated-measures ANOVA). A linear mixed-model analysis of repeated observations within subjects was used to compare these differences for each arm. P values for the observed differences in each parameter are indicated at the top of each graph. A: ∆Resting EE (expressed as kilocalories per day). B: ∆RQ (VCO2/V02). C: ∆Carbohydrate (CHO) oxidation (ox) rates (grams per minute). D: ∆Fat oxidation rates (grams per minute).
GLUCAGON PLUS GLP-1 INCREASES RESTING EE

TABLE 2
Statistical analysis of calorimetry data

| Parameter               | ΔResting EE (kcal/day) | ΔRQ | ΔCarbohydrate oxidation (g/min) | ΔFat oxidation (g/min) |
|-------------------------|------------------------|-----|---------------------------------|------------------------|
| Treatment               |                        |     |                                 |                        |
| P                       | 0.0001                 | 0.001 | 0.0001                           | 0.005                  |
| GLP-1 vs. placebo       | 2.47 (−63.49 to 68.43), | 0.02 (−0.01 to 0.06), | 0.02 (−0.01 to 0.05), | −0.01 (−0.02 to 0.004), |
|                         | P = 0.94               | P = 0.17 | P = 0.24                         | P = 0.21               |
| Glucagon vs. placebo    | 146.99 (83.75–210.24), | 0.03 (0.003–0.07), | 0.05 (0.02–0.08), | −0.004 (−0.02 to 0.01), |
|                         | P = 0.0001             | P = 0.03 | P = 0.003                        | P = 0.47               |
| GLP-1 plus glucagon vs. | 146.26 (82.58–209.95), | 0.07 (0.03–0.10), | 0.08 (0.04–0.11), | −0.02 (−0.03 to −0.01), |
| placebo                 | P = 0.0001             | P = 0.000 | P = 0.0001                        | P = 0.001               |
| SD (95% CI)             |                        |     |                                 |                        |
| Between subjects        | 46.2 (17.0–125.4),     | 0.02 (0.01–0.05), | 0.01 (0.004–0.05), | 0.008 (0.004–0.02)     |
|                         | P = 0.20               | P = 0.20 | P = 0.20                         | P = 0.28               |
| Within subjects         | 71.9 (54.2–95.6)       | 0.04 (0.03–0.05) | 0.03 (0.03–0.05) | 0.01 (0.01–0.02)       |

Data are means (95% CI) unless otherwise indicated. A linear mixed-model analysis of repeated observations within subjects was used, adjusting for baseline measurements and subject weight. For each calorimetry parameter, the P value for the difference, the mean difference (comparing each treatment to placebo), and between- and within-subjects SDs are tabulated. The order-by-treatment interaction was tested to ensure that this did not affect subject response (37–39). VPC, variance partition coefficient.

β-cell function (32). Moreover, if chronic treatment induces enhanced weight loss, this may improve glycemia in the long run by enhancing insulin sensitivity. Further studies are therefore required to understand the effect of the GLP-1 and glucagon combination when given for extended time periods and to verify that there is an overall beneficial effect on glycemia in patients with type 2 diabetes.

We observed that plasma NEFA concentrations fell with glucagon and with combined GLP-1 plus glucagon infusions, triggered by the insulinoefective property of these infusions. We did not observe an increase in fat oxidation rates but, rather, observed a drop with the GLP-1 plus glucagon combination. Although it is traditionally taught that glucagon stimulates lipolysis (33), at the doses used combined glucagon and GLP-1 infusions did not appear to have any direct effect on lipolysis by adipose tissue. Indeed, it has been shown that glucagon’s insulinoefective property causes a fall in NEFA levels, probably mediated by inhibition of hormone-sensitive lipase (34,35). This finding contrasts with results of studies by Day et al. (15), where chronic treatment with a GLP-1R/GcgR coagonist in diet-induced obese mice was shown to cause phosphorylation and activation of hormone-sensitive lipase. It remains to be seen, therefore, whether chronic GLP-1R/GcgR stimulation may induce a lipolytic state in humans, and this should be addressed in long-term studies of the combination.

Lastly, we show that an intravenous infusion of GLP-1 and glucagon is able to suppress the secretion of ghrelin and that there was a trend toward suppression of ghrelin levels with intravenous glucagon alone. The suppression of ghrelin secretion by glucagon appears to be mediated by central mechanisms involving the hypothalamic and pituitary and not via insulin-mediated suppression of ghrelin secretion (26,36). We did not observe an effect of GLP-1 alone on ghrelin secretion in our study, unlike Hagemann et al. (27), who found that GLP-1 suppressed ghrelin secretion. The differences may be explained by the fact that their study used a 50% higher dose of GLP-1, and it is notable that the suppression of ghrelin levels was observed after a longer infusion (210–360 min), whereas our study was curtailed after a 45-min infusion. Our data suggest that the combination of GLP-1 and glucagon may indirectly influence appetite by reducing the levels of the orexigenic hormone ghrelin in addition to their respective direct effects on appetite.

In conclusion, our observations provide initial evidence that GLP-1R/GcgR coagonists, such as oxyntomodulin and related hormone analogs, may be suitable to treat type 2 diabetes and obesity. However, further studies are required to investigate whether sustained weight loss and beneficial effects on glycemia persist during chronic treatment.

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TABLE 3
Substrate oxidation rates

| Oxidation rates (g/min) | Placebo       | GLP-1        | Glucagon      | GLP-1 plus glucagon | P       |
|-------------------------|---------------|--------------|---------------|---------------------|---------|
| Carbohydrate            | 0.098 ± 0.019 | 0.128 ± 0.021 | 0.124 ± 0.021 | 0.105 ± 0.022      | 0.6209  |
| Fat                     | 0.062 ± 0.007 | 0.034 ± 0.009 | 0.041 ± 0.007 | 0.046 ± 0.008      | 0.3798  |
| Protein                 | 0.039 ± 0.005 | 0.044 ± 0.006 | 0.034 ± 0.005 | 0.044 ± 0.006      | 0.1207  |

Data are means ± SEM. Baseline values presented for carbohydrate and fat. Oxidation rate over entire calorimetry period presented for protein.
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T.M.T. designed the study, conducted the study, wrote the manuscript, and reviewed and commented on the manuscript. B.C.T.F. designed the study, conducted the study, and reviewed and commented on the manuscript. K.A.M., R.C.T., E.S.C., and V.S. conducted the study and reviewed and commented on the manuscript. J.G.M. performed the statistical analysis and reviewed and commented on the manuscript. K.C.R.B., A.D.S., A.V., and A.A. conducted the study and reviewed and commented on the manuscript. G.S.F. advised on the caloric measurements and data analysis and reviewed and commented on the manuscript. M.A.G. advised on the hormone immunoassays and reviewed and commented on the manuscript. S.R.B. designed the study and reviewed and commented on the manuscript. S.R.B. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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![FIG. 5. Effects of infusions on total (A) and acylated (B) ghrelin levels (picograms per milliliter). Means ± SEM plotted for the baseline phase and at the end of the infusion phase. *P < 0.05, **P < 0.01.](diabetes.diabetesjournals.org)
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