Effect of Oxyntomodulin, Glucagon, GLP-1, and Combined Glucagon +GLP-1 Infusion on Food Intake, Appetite, and Resting Energy Expenditure

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Context: The gut hormone, oxyntomodulin, is a proglucagon product with body weight-lowering potential. It binds to both the glucagon-like peptide-1 (GLP-1) receptor and the glucagon receptor; however, the mechanism behind the body weight-lowering effect remains elusive.

Objective: We wanted to delineate the contributions of separate and combined GLP-1 receptor and glucagon receptor activation to the body weight-reducing mechanisms of oxyntomodulin.

Design: This was a double-blinded, randomized, crossover study.

Setting: The study was conducted at a specialized research unit.

Participants: Fifteen young healthy male volunteers (aged 22 [range 18–32] y; body mass index 23 [21–26] kg/m^2; fasting plasma glucose 5.1 [4.4–5.4] mmol/L; and glycated hemoglobin A1c 40 (37–42) mmol/mol).

Interventions: Five 4-hour liquid meal tests during the infusion of saline, GLP-1 (1 pmol $\times$ kg^{-1} $\times$ min^{-1}), glucagon (0.86 pmol $\times$ kg^{-1} $\times$ min^{-1}), oxyntomodulin (3 pmol $\times$ kg^{-1} $\times$ min^{-1}), or glucagon+GLP-1 (same doses).

Main Outcome Measures: We evaluated resting energy expenditure (measured as oxygen uptake, gastric emptying (GE), composite appetite scores (CAS), and food intake.

Results: Oxyntomodulin, GLP-1, and GLP-1+glucagon slowed GE and reduced CAS, whereas glucagon did not affect GE and CAS. All infusions caused a similar decrease in food intake compared with saline (total intake (g [95% confidence interval]), saline 811 [729, 892], GLP-1 669 [586, 750], glucagon 686 [604, 768], oxyntomodulin 689 [608, 771], and glucagon+GLP-1 688 [606, 769]). Oxygen uptake did not change significantly from baseline in response to any peptide infusion compared with saline.

Conclusions: Oxyntomodulin, GLP-1, and glucagon decreased food intake but with no additional effect of combining GLP-1 and glucagon. **(*J Clin Endocrinol Metab* 100: 4541–4552, 2015)**

Acute effects of the gut-derived peptide hormone, oxyntomodulin, include inhibition of gastric emptying (GE), gastric and pancreatic exocrine secretion, and food intake (1, 2), which may translate into body weight loss upon repeated administration in obese subjects (3). Also, oxyntomodulin has been reported to increase resting

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Abbreviations: AUC, area under the curve; CAS, composite appetite score; CI, confidence interval; CK, creatine kinase; FGF21, fibroblast growth factor 21; GE, gastric emptying; GLP-1, glucagon-like peptide-1; ISR, insulin secretion rate; NEFA, nonesterified free fatty acid; PG, plasma glucose; REE, resting energy expenditure; VAS, visual analog scale; VO_2, oxygen uptake.

energy expenditure (REE), potentially contributing to the body weight-lowering effect of the hormone (4). A specific receptor for oxyntomodulin has not been identified, but the peptide shows affinity for both the glucagon receptor and the glucagon-like peptide-1 (GLP-1) receptor (5–7). Like GLP-1, oxyntomodulin is derived from proglucagon and is released from intestinal L cells after meal ingestion, with increases in plasma concentrations being related to the calorie intake (6). The amino acid sequence of oxyntomodulin corresponds to the entire 29-amino acid sequence of the glucagon molecule plus a C-terminal extension of eight amino acids (8, 9). After secretion, the peptide is eliminated with a plasma half-life of approximately 12 minutes (1, 10). Oxyntomodulin activates the GLP-1 receptor with less potency than GLP-1 itself, but the two peptides seem to have similar effects on food intake (11–14). The food intake-lowering effect of intracerebroventricular administration of oxyntomodulin in rats can be blocked with the GLP-1 receptor antagonist exendin(9–39) (2).

Consistent with this, oxyntomodulin has limited effect in preclinical studies with GLP-1 receptor knockout mice (15), suggesting that the interaction with the glucagon receptor has limited importance. However, studies in humans have demonstrated that glucagon may reduce hunger measures, food intake (16–18), and body weight (17). Interestingly, an oxyntomodulin analog with increased affinity for the glucagon receptor demonstrated significant potency with regard to inhibition of food intake and body weight reduction in mice when compared with native oxyntomodulin (19). Studies by Kosinski et al (20) using oxyntomodulin analogs with or without affinity for the glucagon receptor indicated an essential role of glucagon signaling in the body weight-lowering effect of oxyntomodulin in rodents. With the present study, we aimed to evaluate the effects of oxyntomodulin on GE, composite appetite scores (CASs), REE determined by oxygen uptake ($\dot{V}O_2$), and food intake in young healthy men. Furthermore, we aimed to delineate the possible dual-receptor agonistic effects of oxyntomodulin by comparing the effects of oxyntomodulin and saline infusions with separate and combined infusions of GLP-1 and glucagon.

Materials and Methods

The protocol was approved by the Scientific-Ethical Committee of the Capital Region of Denmark (registration number H-4-2010-089) and the Danish Data Protection Agency (registration number 2010-41-5506) and was registered at clinicaltrials.gov (identification NCT01232244). The study was conducted according to the principles of the Helsinki Declaration II. Oral and written informed consent was obtained from all participants before inclusion.

Subjects

Fifteen young males (mean age 22 [range 18–32] years; body mass index 23 [21–26] kg/m^2; fasting plasma glucose 5.1 [4.4–5.4] mmol/L; glycated hemoglobin A1c 5.8% [5.5%–6.0%] [40 (37–42) mmol/mol]) were included. None had hypercholesterolemia, hypertension, or impaired renal or liver function. All subjects were without a family history of diabetes and had normal glucose tolerance according to a 75-g oral glucose tolerance test performed immediately before inclusion in the study. None of the subjects used medication regularly.

Experimental design

All participants were studied in a recumbent position in the morning after an overnight (10 h) fast and tobacco abstinence on five randomized occasions separated by at least 48 hours. A cannula was inserted into a cubital vein, and the forearm was placed in a heating box (55°C) throughout the experiment for collection of arterialized blood samples. Another cannula was inserted into a contralateral cubital vein for hormone infusions through separate infusion lines. The experimental protocol is outlined in Figure 1A. VO_2 was measured 30 minutes before the start of the test infusion and test meal. The continuous infusions of glucagon (0.86 pmol/kg · min), GLP-1 (1 pmol/kg · min), oxyntomodulin (3 pmol/kg · min), glucagon (0.86 pmol/kg · min) + GLP-1 (1 pmol/kg · min), or saline were given in a double-blinded fashion during 4 hours. At time 0 minutes, the infusion was started and a liquid test meal was ingested over 5 minutes. Blood samples were drawn 15 and 0 minutes before and 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, and 240 minutes after meal ingestion. During the 4-hour infusion, hunger scores were collected 10 times using standardized visual analog scales (VAS), recording hunger, satiety, fullness, and prospective food consumption (21), and further inquiry was made regarding general well-being, nausea and thirst. During the final 30 minutes of the experiment (from 210 to 240 minutes), $VO_2$was measured again, after which an ad libitum meal was served with recordings of palatability and impression of the meal using VAS (22). To measure the renal nitrogen excretion for estimation of protein turnover, the urinary bladder was emptied before each experiment, and total urine production during each experiment was collected.

Synthetic GLP-1 and oxyntomodulin (PolyPeptide Laboratories A/S) and glucagon (GlucaGen; Novo Nordisk A/S) were dissolved in sterilized water containing 2% human albumin (Statens Serum Institut, Copenhagen, Denmark), subjected to sterile filtration (followed by testing for sterility and pyrogens), and dispensed into coded frozen vials at the pharmacy of the Capital Region (Herlev, Denmark).

The meal test was ingested within 5 minutes and consisted of 200 mL chocolate-flavored Nutridrink (Nutricia; 300 kcal: 55 g carbohydrates, 17 g fat, and 18 g protein) to which was added 1.5 g of acetaminophen (paracetamol, Panodil; Dungarvan Ltd) dissolved in 50 mL of water.

Arterialized blood was collected in chilled tubes containing EDTA, aprotinin (500 kIU/mL blood; Trasylol; Bayer), and a dipeptidyl peptidase 4 inhibitor (valine pyrrolidide, final concentration 0.01 mmol/L, a gift from Novo Nordisk A/S, Bagsværd, Denmark) for analyses of GLP-1, glucagon, and oxyntomodulin. Blood for analysis of acetaminophen, creatinine, creatine kinase (CK), and triglyceride was collected in lithium heparin tubes. The blood for analysis of insulin, C-peptide,

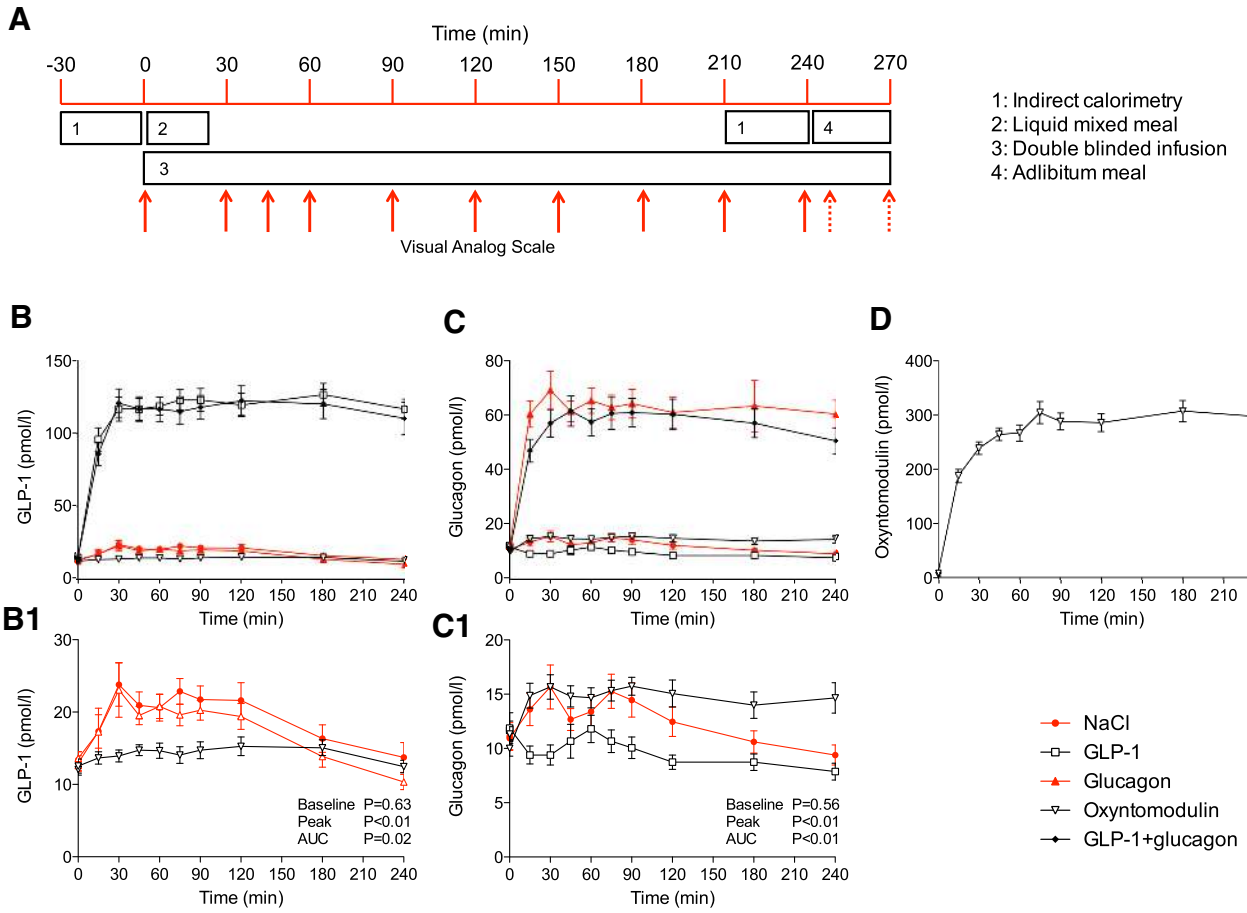

Figure 1. Diagram of experimental procedures. A, Arrows indicate time for appetite scoring (by VAS), and broken arrows indicate time for assessment of palatability (by VAS). Plasma concentrations of GLP-1 (B), glucagon (C), and oxyntomodulin (D) during the infusion of saline (dot), GLP-1 (square), glucagon (upright triangle), oxyntomodulin (downright triangle), and GLP-1+glucagon (diamond), respectively, are shown. Postprandial responses of GLP-1 (B1) and glucagon (C1) are displayed in the lower panels as responses without the levels caused by infusion of the respective peptide along with the significance level of the overall model (*P* value). Red lines indicate rapid gastric emptying and black lines indicate slow gastric emptying as predicted by the acetaminophen levels (see Figure 2A).

nonesterified free fatty acids (NEFAs), and fibroblast growth factor 21 (FGF21) was left to coagulate for 20 minutes at room temperature. All samples were centrifuged for 20 minutes at 1200 × *g* and 4°C. Plasma samples for GLP-1, glucagon, and oxyntomodulin analysis and serum samples for acetaminophen analysis were stored at −20°C and serum samples for insulin, C-peptide, NEFAs, FGF21, and plasma samples for triglyceride, creatinine, and CK at −80°C. For bedside measurement of plasma glucose (PG), blood was added to fluoride tubes and centrifuged immediately at 7400 × *g* for 2 minutes at room temperature. The ad libitum meal served at all five occasions consisted of minced meat, pasta, corn, carrots, and green pepper (37% fat, 13% protein, and 50% carbohydrates, ~1.5 kcal/g).

Analyses

The resting metabolic rate was measured by indirect calorimetry using a tight facemask connected to the calorimeter, which measures the gas exchange breath by breath via an O_2-alkali cell and an infrared CO_2 sensor (CCMexpress; Medgraphics, Medical Graphics Corp). The calorimeter was calibrated immediately before every measurement session. Metabolic rates are represented as averages of measures carried out every 10th second within a 20-minute period. Plasma concentrations of glucose were measured by the glucose oxidase method, using a glucose analyzer (Yellow Springs Instrument model 2300 STAT plus analyzer; YSI Inc). Serum insulin and C-peptide concentrations were measured using a two-sided electrochemiluminescence immunoassay (ADIVA Centaur XP; Siemens). Plasma samples for total GLP-1 and glucagon analysis were extracted with 70% ethanol (final concentration) before RIA measurements. Total GLP-1was analyzed using an antibody (code no 89390) specific for the amidated C terminus (23), and glucagon was analyzed using a C-terminal glucagon-specific antibody (code number 4305) (24). The glucagon analysis showed no cross-reaction with oxyntomodulin. For the measurements of oxyntomodulin, we used a newly developed RIA using an antiserum (code number 9D645) raised in rabbits against a C-terminal fragment of oxyntomodulin. Human oxyntomodulin (Bachem; catalog number H-6058) was used as standard, and the same peptide was ^{125}I-labeled and used as tracer. Analysis of acetaminophen, CK, triglycerides, and creatinine was carried out using enzyme-linked color shift reactions and liquid chromatography (Vitros 5.1 FS; Ortho-Clinical Diagnostics). NEFAs were analyzed using an Acyl-CoA oxidase-linked assay (NEFA-HR;

Wako Chemicals GmbH). FGF21 was measured using an ELISA (www.BioVendor.com).

Statistical analyses and calculations

Baseline, peak, and area under the curve (AUC) values are expressed as mean and 95% confidence intervals (CI). Differences resulting in values of $P < .05$ were considered significant. CAS was calculated from the VAS assessment of appetite measures (hunger + prospective food consumption + [100-satiety] + [100-fullness]/4) (25).

Steady-state levels of the infused peptides were determined graphically for each participant and presented as mean and CI of the mean.

Linear mixed-effect modeling was used for the analysis of longitudinal and repeated measures using statistical software R, with the nlme package. Data were transformed according to distribution pattern. We used a top-down modeling strategy, with subject identity as random variable (26). A homogeneous or heterogeneous residual variance structure was chosen according to likelihood ratios. Results are presented as 95% CIs of the estimate. Insulin secretion rate (ISR) values were calculated using ISEC software as described previously (27–29) and expressed as picomoles of insulin secreted per minute per kilogram of body weight.

Results

Levels of infused peptides

Steady-state plasma concentrations of GLP-1, glucagon, and oxyntomodulin during the infusions (Figure 1, B–D) were 120 (117, 123) and 117 (114, 121) pmol/L (GLP-1 infusions, mean level between 30 and 240 min), 63 (61, 66), and 58 (55, 61) pmol/L (glucagon infusions, mean level between 30 and 240 min) and 295 (283, 306) pmol/L (oxyntomodulin infusion, mean level from 75 to 240 min).

Acetaminophen

During the infusion of saline, the serum acetaminophen concentration increased briskly (mean time to peak [95% CI]; 80 [69, 90] min, Figure 2A) as an indirect measure of a fast GE rate of the liquid meal. During glucagon infusion, the GE rate was almost identical with that of the saline infusion (time to peak 91 [−46, 24] min), whereas the infusions with GLP-1, oxyntomodulin, and glucagon+

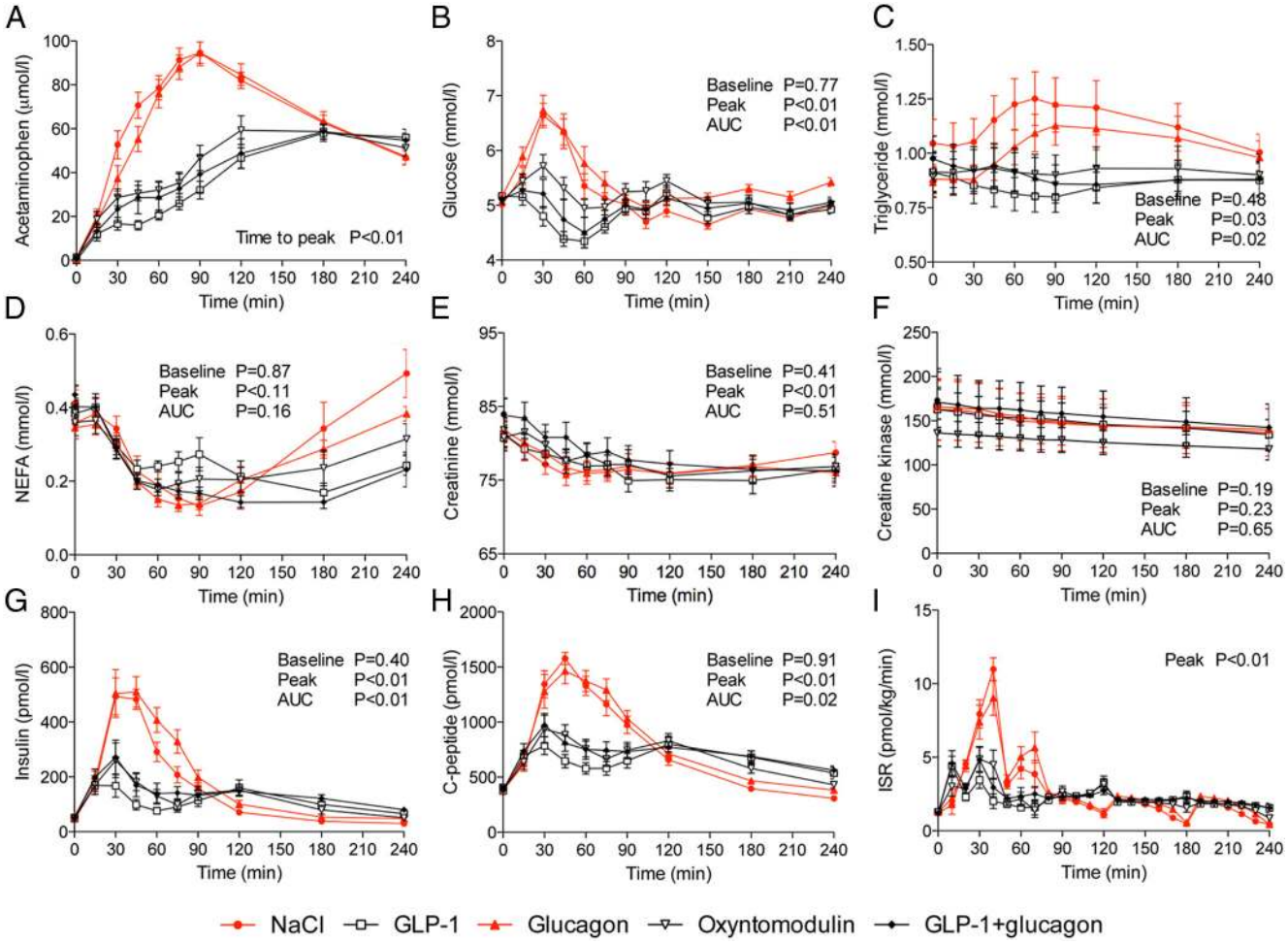

Figure 2. Postprandial plasma/serum excursions and the levels of significance from the overall models of acetaminophen (A), glucose (B), triglyceride (C), NEFAs (D), creatinine (E), CK (F), insulin (G), C-peptide (H), and insulin secretion rate (I) during iv infusions with saline (dot), GLP-1 (square), glucagon (upright triangle), oxyntomodulin (downright triangle), and GLP-1+glucagon (diamond), respectively. Red lines indicate rapid gastric emptying and black lines indicate slow gastric emptying as predicted by acetaminophen levels (panel A).

Table 1. Baseline, Peak, and AUC Values

	Saline	GLP-1	Glucagon	Oxyntomodulin	GLP-1+Glucagon
GLP-1					
Baseline, pmol/L	13 [11, 15]		13 [11, 15]	12 [10, 14]	
Saline			[-13, 16]%	[−19, 9]%	
Glucagon				[−19, 9.]%	
Peak, pmol/L	30 [24, 34]		29 [22, 32]	18 [15, 22]	
Saline			[−25, 15]%	[−48, −21]%	
Glucagon				[−44, −15]%	
AUC, min × mmol/L	4446 [3709, 5015]		4049 [3382, 4573]	3467 [2897, 3918]	
Saline			[−23, 9]%	[−34, −7]%	
Glucagon				[−28, 2]%	
Glucagon					
Baseline, mmol/L	11 [9, 12]	12 [9, 13]		11 [9, 13]	
Saline		[−6, 23]%		[−11, 17]%	
GLP-1				[−17, 9]%	
Peak, mmol/L	19 [15, 21]	14 [11, 16]		18 [15, 21]	
Saline		[−34, −14]%		[−12, 15]%	
GLP-1				[17, 53]%	
AUC, min × mmol/L	2974 [2485, 3408]	2288 [1785, 2708]		3576 [3061, 3984]	
Saline		[−1171, −228]		[105, 1048]	
GLP-1				[805, 1747]	
Glucose					
Baseline, mmol/L	5.0 [4.9, 5.2]	5.1 [5.0, 5.2]	5.1 [5.0, 5.2]	5.1 [5.0, 5.2]	5.1 [5.0, 5.2]
Saline		[−0.1, 0.2]	[−0.1, 0.2]	[−0.1, 0.2]	[−0.1, 0.2]
GLP-1			[−0.1, 0.1]	[−0.2, 0.1]	[−0.1, 0.1]
Glucagon				[−0.2, 0.1]	[−0.2, 0.1]
Oxyntomodulin					[−0.1, 0.1]
Peak, mmol/L	7.0 [6.5, 7.4]	5.6 [5.3, 6.0]	7.2 [7.1, 7.5]	6.1 [5.7, 6.4]	5.9 [5.4, 6.1]
Saline		[−24, −13]%	[−4, 10]%	[−18, −6]%	[−22, −11]%
GLP-1			[18, 35]%	[1, 16]%	[−4, 10]%
Glucagon				[−19, −8]%	[−24, −13]%
Oxyntomodulin					[−12, 1]%
AUC, min × mmol/L	1234 [1197, 1270]	1164 [1127, 1200]	1307 [1270, 1343]	1227 [1190, 1262]	1176 [1140, 1212]
Saline		[−109, −32]	[34, 111]	[−46, 31]	[−97, −20]
GLP-1			[105, 182]	[24, 101]	[−26, 51]
Glucagon				[−119, 42]	[−170, −93]
Oxyntomodulin					[−89, −12]
Triglycerides					
Baseline, mmol/L	1.0 [0.8, 1.2]	0.9 [0.7, 1.0]	0.9 [0.7, 1.0]	0.9 [0.7, 1.0]	1.0 [0.7, 1.1]
Saline		[−28, 8]%	[−30, 5]%	[−30, 5]%	[−23, 14]%
GLP-1			[−20, 19]%	[−20, 19]%	[−13., 29]%
Glucagon				[−18, 22]%	[−11, 33]%
Oxyntomodulin					[−11, 33]%
Peak, mmol/L	1.3 [1.1, 1.5]	1.0 [0.8, 1.2]	1.2 [1.0, 1.4]	1.1 [0.9, 1.3]	1.1 [0.9, 1.3]
Saline		[−0.5, −0.1]	[−0.3, 0.1]	[−0.4, 0.0]	[−0.4, 0.0]
GLP-1			[0.0, 0.4]	[−0.1, 0.3]	[−0.1, 0.3]
Glucagon				[−0.3, 0.1]	[−0.3, 0.1]
Oxyntomodulin					[−0.2, 0.2]
AUC, min × mmol/L	273 [213, 308]	205 [160, 233]	250 [195, 283]	221 [171, 248]	218 [167 242]
Saline		[−37, −9]%	[−23, 10]%	[−33, −3]%	[−34, −6]%
GLP-1			[1, 43]%	[−12, 28]%	[−14, 25]%
Glucagon				[−27, 5]%	[−29, 3]%
Oxyntomodulin					[−19, 17]%
NEFAs					
Baseline, mmol/L	0.4 [0.3, 0.5]	0.4 [0.3, 0.5]	0.4 [0.3, 0.4]	0.4 [0.3, 0.4]	0.4 [0.4, 0.5]
Saline		[−0.1, 0.1]	[−0.1, 0.1]	[−0.1, 0.1]	[−0.1, 0.1]
GLP-1			[−0.1, 0.1]	[−0.1, 0.1]	[−0.1, 0.1]
Glucagon				[−0.1, 0.1]	[−0.1, 0.1]
Oxyntomodulin					[0.0, 0.1]
Peak, mmol/L	0.6 [0.5, 0.7]	0.4 [0.4, 0.5]	0.5 [0.4, 0.5]	0.5 [0.4, 0.5]	0.5 [0.4, 0.5]
Saline		[−0.2, 0.0]	[−0.2, 0.0]	[−0.2, 0.0]	[−0.2, 0.0]
GLP-1			[−0.1, 0.1]	[−0.1, 0.1]	[−0.1, 0.1]
Glucagon				[−0.1, 0.1]	[−0.1, 0.1]
Oxyntomodulin					[−0.1, 0.1]

(*Continued*)

Table 1. Continued

	Saline	GLP-1	Glucagon	Oxyntomodulin	GLP-1+Glucagon
AUC, min × mmol/L	68 [51, 86]	57 [48, 66]	61 [52, 70]	58 [49, 68]	47 [39, 57]
Saline		[−31, 9]	[−27, 13]	[−30, 10]	[−41, −1]
GLP-1			[−10,16]	[−12, 14]	[−22, 3]
Glucagon				[−15, 10]	[−27, −1]
Oxyntomodulin					[−24, 2]
Creatinine, mmol/L					
Baseline, mmol/L	81 [78, 84]	81 [78, 84]	82 [79, 85]	81 [78, 84]	84 [78, 89]
Saline		[−3, 3]	[−2, 4]	[−2, 3]	[0, 6]
GLP-1			[−2, 4]	[−2, 3]	[−1, 7]
Glucagon				[−3, 2]	[−1, 6]
Oxyntomodulin					[−1, 6]
Peak, mmol/L	82 [78, 85]	82 [79, 86]	83 [79, 86]	83 [80, 86]	86 [83, 90]
Saline		[-2, 3]	[−2, 4]	[−1,4]	[2, 8]
GLP-1			[−2, 3]	[−2, 4]	[1, 7]
Glucagon				[−2, 3]	[1, 7]
Oxyntomodulin					[1, 6]
AUC, min × mmol/L	18.5 [17.8, 19.2]	18.3 [17.6, 19.0]	18.4 [17.7, 19.1]	18.5 [17.8, 19.2]	18.7 [18.0, 19.5]
Saline		[−0.7, 0.3]	[−0.6, 0.5]	[−0.5, 0.6]	[−0.3, 0.8]
GLP-1			[−0.3, 0.7]	[−0.3, 0.8]	[−0.1, 1.0]
Glucagon				[−0.4, 0.6]	[−0.2, 0.8]
Oxyntomodulin					[−0.3, 0.7]
CK					
Baseline, U/L	163 [94, 194]	164 [109, 189]	166 [100, 186]	136 [96, 159]	171 [105 189]
Saline		[−20, 44]%	[−26, 41]%	[−29, 21]%	[−23, 43]%
GLP-1			[−25, 19]%	[−24, −1]%	[−21, 20]%
Glucagon				[−26, −10]%	[−21, 32]%
Oxyntomodulin					[−5, 35]%
Peak, U/L	164 [95, 194]	166 [111, 192]	169 [102, 188]	139 [98, 162]	175 [107, 192]
Saline		[−19, 45]%	[−26, 42]%	[−29, 23]%	[−22, 45]%
GLP-1			[−24, 19]%	[-25, −1]%	[−20, 20]%
Glucagon				[−25, 11]%	[−19, 33]%
Oxyntomodulin					[−5, 36]%
AUC, min × mmol/L	35 [20, 43]	35 [24, 41]	36 [22, 39]	30 [21, 36]	37 [24, 41]
Saline		[−21, 47]%	[−25, 39]%	[−31, 29]%	[−22, 46]%
GLP-1			[−21, 14]%	[−27, 6]%	[−17, 19]%
Glucagon				[−23, 26]%	[−13, 26]%
Oxyntomodulin					[−6, 36]%
Insulin					
Baseline, pmol/L	48 [38, 55]	49 [39, 56]	55 [44, 63]	51 [39, 57]	54 [43, 61]
Saline		[−15, 21]%	[−10, 38]%	[−15, 24]%	[−6, 34]%
GLP-1			[−5, 36]%	[−15, 22]	[−8, 32]%
Glucagon				[−25, 7]%	[−19, 16]%
Oxyntomodulin					[−10, 29]%
Peak, pmol/L	617 [469, 752]	256 [179, 286]	672 [493, 790]	345 [243, 389]	349 [235, 376]
Saline		[−71, −49]%	[−21, 39]%	[−61, −31]%	[−62, −33]%
GLP-1			[108, 248]%	[3, 80]%	[−1, 74]%
Glucagon				[−63, −35]%	[−64, −37]%
Oxyntomodulin					[−27, 28]
AUC, min X mmol/L	35 [30, 40]	27 [22, 32]	43 [39, 48]	29 [24, 35]	34 [28, 39]%
Saline		[−14, −2]	[2, 14]	[−11, 0]	[−7, 4]
GLP-1			[10, 22]	[−4, 8]	[1, 12]
Glucagon				[−20, −8]	[−15, −4]
Oxyntomodulin					[−1, 10]
C-peptide					
Baseline, pmol/L	386 [337, 435]	391 [342, 440]	395 [346, 443]	391 [342, 440]	373 [324, 422]
Saline		[−44, 53]	[−40, 57]	[−44, 54]	[−61, 36]
GLP-1			[−45, 52]	[−49, 49]	[−67, 31]
Glucagon				[−53, 45]	[−70, 27]
Oxyntomodulin					[−67, 31]
Peak, pmol/L	1725 [1548, 1876]	962 [795, 1082]	1719 [1420, 1933]	1160 [944,1285]	1128 [917,1248]
Saline		[−53, −36]%	[−17, 13]%	[−45, −25]%	[−46, 27]%
GLP-1			[67, 117]%	[−2, 44]%	[−5, 40]%

(*Continued*)

Table 1. Continued

	Saline	GLP-1	Glucagon	Oxyntomodulin	GLP-1+Glucagon
Glucagon				[−45, −19]%	[−47, −21]%
Oxyntomodulin					[−20, 18]%
AUC, min × mmol/L	178 [160, 195]	160 [142, 177]	189 [171, 207]	163 [145, 180]	172 [155, 180]
Saline		[−36, 1]	[−7, 30]	[−33, 4]	[−24, 13]
GLP-1			[11, 48]	[−15, 22]	[−6, 31]
Glucagon				[−44, −8]	[−35, 2]
Oxyntomodulin					[−9, 27]
ISR					
Peak, pmol/kg · min	12 [11, 14]	6 [5, 7]	12 [10, 14]	7 [5, 9]	7 [5, 9]
Saline		[−7, 5]	[−2, 2]	[−7, −3]	[−7, −3]
GLP-1			[4, 8]	[0, 3]	[0, 3]
Glucagon				[−7, −2]	[−7, −2]
Oxyntomodulin					[−2, 2]

Mean baseline, peak, and AUC values in response to the initial liquid mixed meal of GLP-1, glucagon, PG, triglycerides, NEFAs, insulin, C-peptide, creatinine, and CK. Infused levels of GLP-1 and glucagon are not shown here but referred to in the text. First row are mean values and model adjusted 95% CIs of the mean in brackets. The following lines represents the post hoc analysis as 95% CIs of the respective differences from the saline, GLP-1, glucagon, and oxyntomodulin infusion (as predicted by the model).

GLP-1, respectively, resulted in delayed GE (time to peak 188 [−143, −73] forGLP-1, 146 [−101, −31] for oxyntomodulin, and 159 [−114, −44] min for glucagon+GLP-1).

Glucagon-like peptide-1

Plasma levels of GLP-1 after the initial liquid meal are shown in Figure 1B1 and Table 1. During the infusion of saline and glucagon, plasma levels of GLP-1 doubled after meal ingestion. Plasma levels returned to baseline after 3 hours. We observed no changes during the oxyntomodulin infusion.

Glucagon

Plasma levels of glucagon after the initial liquid meal are shown in Figure 1C1 and Table 1. Meal-induced rises in plasma levels of glucagon were observed during the infusions with saline and oxyntomodulin, respectively. However, during the saline infusion, plasma glucagon levels started to decline at 90 minutes and gradually returned to baseline after 3 hours, whereas plasma glucagon continued to be elevated during the 4 hours of oxyntomodulin infusion, probably caused by the reduced GE and the subsequently continued stimulation of the epithelium and absorption of amino acids. The GLP-1 infusion effectively reduced postprandial plasma glucagon levels (compared with saline infusion), which never rose above baseline plasma glucagon levels.

Glucose

Mean PG concentrations are displayed in Figure 2B. Baseline PG did not differ between study days (Table 1). The postprandial PG profiles were almost identical during saline and glucagon infusions, although the AUC was significantly greater after the glucagon infusion (Table 1 and Figure 2B). Compared with the saline infusion, infusion of GLP-1 resulted in a clear reduction in PG after meal ingestion (during the first 60 min of the GLP-1 infusion), whereas the infusion of oxyntomodulin induced a more moderate reduction in postprandial PG excursions (Table 1 and Figure 2B). The GLP-1+glucagon infusion resulted in a PG profile similar to what was seen with GLP-1, although with a slightly delayed lowering of PG (Figure 2B).

Triglycerides

Fasting plasma triglyceride concentrations did not differ between study days (Table 1). During the first hour of saline infusion, the postprandial triglyceride levels increased slowly, peaked at 75 minutes, and then slowly decreased to baseline levels at 240 minutes (Table 1 and Figure 2C). The glucagon infusion resulted in a similar postprandial response, whereas the GLP-1 and GLP-1+ glucagon infusions induced significantly lower postprandial triglyceride responses. Infusion of oxyntomodulin kept triglyceride levels constant during the entire experiment (Table 1 and Figure 2C).

Nonesterified free fatty acids

Baseline levels of NEFAs did not differ between the test days (Table 1). After the meal ingestion, NEFA levels decreased during all experimental days (Figure 2D). The peptide infusions resulted in a delayed return to baseline compared with the saline infusion, although clearly the most after the GLP-1 and GLP-1+glucagon infusions.

Creatinine and CK

Only peak creatinine values differed significantly, related to minor difference in baseline levels rater than the infusions Figure 2E. We observed no other differences in the levels of creatinine or CK between the infusions. We observed steady decreases from baseline throughout all experiments (Figure 2, E and F, and Table 1).

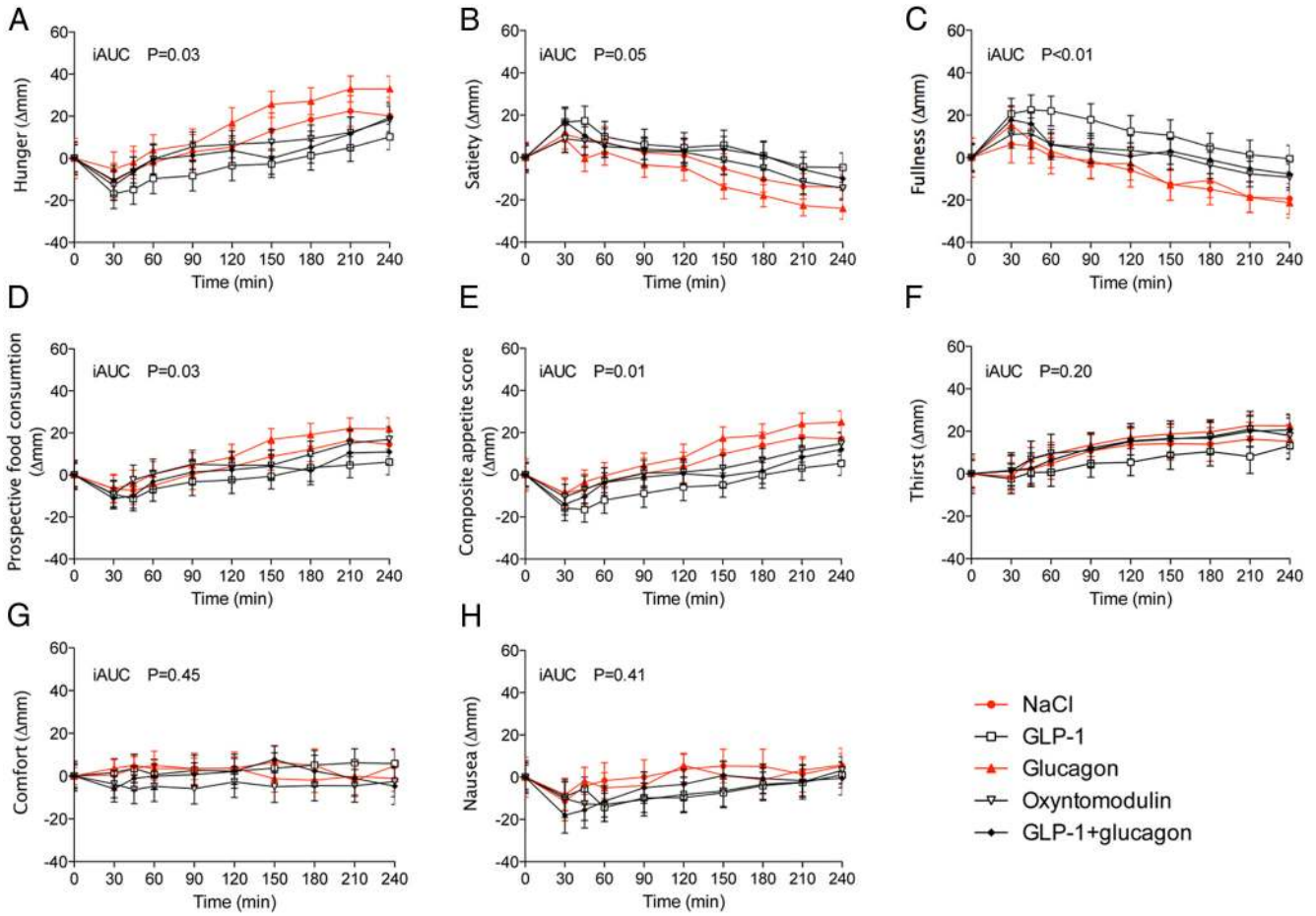

Figure 3. Baseline-subtracted postprandial VAS scores (measured in millimeters) as a response over time and the levels of significance from the overall models during infusion of saline (dot), GLP-1 (square), glucagon (upright triangle), oxyntomodulin (downright triangle), and GLP-1+glucagon (diamond), respectively. A, hunger. B, satiety. C, fullness. D, prospective food consumption. E, composite appetite score. F, thirst. G, comfort. H, nausea. Red lines indicate rapid gastric emptying and black indicate slow gastric emptying as predicted by acetaminophen levels (see Figure 2A).

Insulin, C-peptide, and ISR

The ingestion of the liquid meal resulted in brisk and similar β-cell responses in all groups within the first 15 minutes (Figure 2, D–F). Thereafter the excursions diverged in a manner closely related to the GE (Table 1, Figure 2, G–I).

Appetite, thirst, and comfort

In general, the VAS scores for appetite sensations decreased after the ingestion of the liquid meal and then increased gradually for the remainder of the experiments (opposite for satiety sensations) (Figure 3, A–E). We found an overall significant difference in the development in hunger, satiety, fullness, and CAS scores between the infusions (Table 2). This overall difference seems to be driven primarily by the remarkable differences between scores obtained with the GLP-1 and glucagon infusion, which is even greater than the difference between the GLP-1 and saline infusion (Table 2 and Figure 3, A–C and E). Only with respect to CAS and fullness did the GLP-1 infusion result in values significantly different from those obtained after saline infusion (Table 2). We found no differences with respect to thirst, comfort, or nausea (Figure 3, F–H).

Food intake and palatability

Overall, the ad libitum meal was considered palatable with ingested food portions ranging from 347 to 984 g. A consistent and significantly decreased food intake was seen after all peptide infusions compared with the saline infusion (Table 2). No differences in food intake or palatability were observed between the different peptide infusions (differences from saline ranging from −121 to −141 g [Table 2]).

Calorimetry

Baseline VO_2 was similar at all experimental days (Figure 4). At the second registration, the saline infusion was associated with a slight decrease in VO_2 from baseline, whereas slight increases were observed in response to all peptide infusions (Figure 4) with increases ranging from 1% to 19%. However, there were no significant changes in VO_2 between the baseline and final measurements on any day of the pro-

Table 2. Appetite Scores and Food Intake

Infusion	NaCl	GLP-1	Glucagon	OXM	GLP-1+Glucagon
Hunger, (min × mm)/100	53 [31, 75]	32 [15,49]	67 [44, 89]	47 [30, 63]	39 [23, 55]
Saline		[−43, 2]	[−13, 41]	[−29, 16]	[−36, 9]
GLP-1			[12, 57]	[−3, 32]	[−10, 24]
Glucagon				[−42, 2]	[−50, −5]
Oxyntomodulin					[−24, 9]
Satiety	−12 [−28, 5]	2 [−16, 18]	−24 [−41, −8]	−4 [−21, 13]	−5 [−21, 12]
Saline		[−4, 31]	[−30, 5]	[−10, 25]	[−11, 24]
GLP-1			[−43, −8]	[−23, 12]	[−24, 11]
Glucagon				[3, 37]	[2, 37]
Oxyntomodulin					[−18, 17]
Fullness	−19 [−38, 1]	10 [−5, 24]	−21 [−41, −2]	−2 [−16, 13]	−6 [−21, 9]
Saline		[10, 47]	[−25, 20]	[−2, 36]	[−6, 31]
GLP-1			[−50, −12]	[−25, 2]	[−29, −2]
Glucagon				[1, 38]	[−3, 34]
Oxyntomodulin					[−17, 9]
Prospective Food Consumption	43 [24, 61]	29 [15, 43]	54 [40, 68]	45 [31, 59]	38 [24, 52]
Saline		[−33, 6]	[−8, 31]	[−17, 22]	[−24, 15]
GLP-1			[10, 41]	[1, 32]	[−6, 25]
Glucagon				[−24, 6]	[−31, −1]
Oxyntomodulin					[−22, 8]
CAS	46 [28, 64]	28 [15, 41]	57 [39, 75]	39 [26, 53]	37 [24, 50]
Saline		[−35, −2]	[−10, 31]	[−24, 10]	[−26, 8]
GLP-1			[12, 46]	[0, 23]	[−2, 21]
Glucagon				[−35, −1.]	[−37, −4]
Oxyntomodulin					[−14, 9]
Thirst	48 [33, 63]	40 [25, 55]	61 [46, 75]	52 [38, 67]	52 [37, 67]
Saline		[−26, 9]	[−5, 30]	[−13, 22]	[−13, 21]
GLP-1			[3, 38]	[−5, 30]	[−5, 30]
Glucagon				[−25, 9]	[−26, 9]
Oxyntomodulin					[−18, 17]
Comfort	26 [9, 43]	29 [11,46]	20 [3, 37]	14 [−3, 31]	27 [10, 44]
Saline		[−15, 20]	[−24, 11]	[−29, 5]	[−17, 18]
GLP-1			[−26, 9]	[−32, 3]	[−19, 16]
Glucagon				[−23, 12]	[−10, 24]
Oxyntomodulin					[−5, 30]
Nausea	37 [17, 57]	16 [−4, 36]	33 [14, 53]	21 [1, 40]	32 [13, 52]
Saline		[−47, 5]	[−29, 22]	[−42, 1 0]	[−30, 21]
GLP-1			[−8, 43]	[−21, 30]	[−9, 42]
Glucagon				[−38, 13]	[−26, 24]
Oxyntomodulin					[−13, 37]
Food intake (g)	811 [729, 892]	669 [586, 750]	686 [604, 768]	689 [608, 771]	688 [606, 769]
Saline		[−241, −44]	[−222, −27]	[−219, −24]	[−221, −25]
GLP-1			[−80, 116]	[−77, 119]	[−79, 118]
Glucagon				[−95, 101]	[−95, 99]
Oxyntomodulin					[−99, 96]

Appetite score by VAS tabled by incremental AUC ([minutes × centimeters]/100) and food intake in weight (grams). For each measure the first row are mean values and model adjusted 95% CIs of the mean are in brackets. The following lines represents the post hoc analysis as 95% CIs of the respective differences from the saline, GLP-1, glucagon, and oxyntomodulin infusion (as predicted by the model).

tocol. CO_2 exchange reflected VO_2 and we found no differences in urinary nitrogen excretion (data not shown).

Fibroblast growth factor 21

Baseline levels varied considerably (saline 157 [77–6039] [median with range in brackets] pg/mL GLP-1 122 [80–5853] pg/mL; glucagon 142 [75–5723] pg/mL; oxyntomodulin 168 [77–6382] pg/mL; GLP-1+glucagon 110 [76–6527] pg/mL), with no differences between the experimental days. After 240 minutes of infusion, there were neither significant changes between the infusions nor differences compared with baseline (saline 105 [76–5953] pg/mL; GLP-1 107 [79–5836] pg/mL; glucagon 107 [75–5645] pg/mL; oxyntomodulin 107 [77–6250] pg/mL; GLP-1+glucagon 105 [75–6668] pg/mL).

Discussion

In the present study, we show that an infusion of oxyntomodulin and separate or combined infusions of GLP-1 and glucagon inhibited food intake similarly in young,

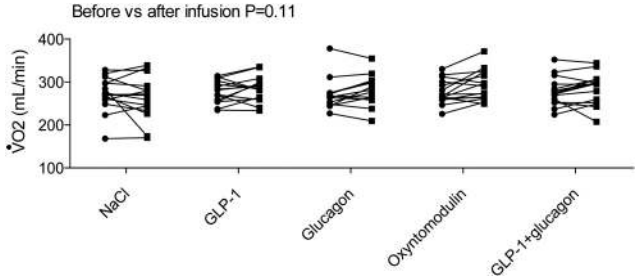

Figure 4. The measured gas exchange of O_2 ($\dot{V}O_2$) at steady state before infusion start (circles) and after 210 minutes of infusion (squares) during the five different experimental days. The gas exchange is displayed as the means from the 20-minute measurement on the individual level linked with lines. The *P* value is the significance level from the overall model testing the differences in the change in gas exchange between the infusions.

lean, healthy male subjects, with no additive effect of the combined infusion. We confirm the inhibitory effects of oxyntomodulin and GLP-1, respectively, on GE observed previously, but by adding glucagon to the infusion of GLP-1, we found no additive effects. Surprisingly, glucagon alone had no effect on GE and appetite scores, but food intake decreased to the same extent as during oxyntomodulin, GLP-1, and GLP-1+glucagon infusions.

The positive effects of the peptide infusions reported here with respect to the second calorimetric measurement of VO_2 are confounded by a residual meal-induced thermogenesis (driven by absorption and deposition nutrients [30]). Especially during the GLP-1, oxyntomodulin, and the GLP-1+glucagon infusions, which all delayed GE, the measurement of VO_2 was performed relatively soon after the serum acetaminophen peak, indicating that considerable nutrient absorption was still going on compared with saline. Flint et al (13) previously concluded from a protocol very similar to the present that the observed increases in energy expenditure during GLP-1 infusions most likely were linked to the meal. However, robust reflections of the actual nutrient absorption, ie, glucose levels, triglyceride levels, and insulin responses, were rather similar between the five infusions at the time of the second calorimetry (31, 32), suggesting that the differences could be due to the different infusions. On the other hand, there were no significant changes in VO_2 from baseline in any of the experiments (Figure 4). The lack of a clear effect on VO_2 contrasts to recent reported results of infusions of glucagon and GLP-1 (33). But the dose of glucagon used in that particular study was more than 15-fold higher than ours and associated with large changes in glucose and insulin levels. Such increases are likely to influence REE and offer an explanation for the reported additive effect of combinations of GLP-1 and glucagon (33). Our observation that the peptides did not have consistent effects on energy expenditure is consistent with recent findings showing no increases after short term native GLP-1 infusions (34). Long-term treatments with the GLP-1 analog liraglutide using 24-hour chamber calorimetry has, so far, shown no differences in energy expenditure after the treatment (35, 36). In a single study, infusions of oxyntomodulin were associated with increased energy expenditure related to physical activity (as determined with the Actiheart device) in humans (but had no effect on basal metabolic rate). This finding is very difficult to translate to the general experience with peptide infusions, where decreases (because of malaise) but not increases (4) may be observed. It has been suggested that stimulation of the production/levels of the metabolic regulator, FGF21, could constitute the link by activation of specific metabolic pathways such as improved glucose metabolism and activation of brown adipose tissue (19, 37). However, none of the peptide infusions tested in the present study lead to consistent changes in circulating FGF21. Our data do not support this link. This may, however, be due to insufficient statistical power, although we did use relatively high doses of the peptides.

In the present study, the infusion of glucagon did not change GE. This finding was unexpected because glucagon previously has been shown to inhibit bowel motility (38, 39). However, the dose used to inhibit bowel motility was more than 3000-fold higher than the dose used in the present study (38), and such doses might activate the GLP-1 receptor pathway (in vitro EC_{50} of glucagon on the GLP-1 receptor is about 100-fold higher compared with GLP-1) (20). The rate of glucagon infusion used in our trial was specifically chosen to avoid marked increases in PG but was expected to be high enough to impact food intake (18). We did observe a small but significant increase in PG toward the end of the experiment with glucagon infusion compared with the other infusions. Nevertheless, the glucagon infusion did result in decreased food intake to the same extent as the other peptide infusions (despite having no impact on GE and appetite scores). These findings suggest that glucagon receptor agonists could be considered for the treatment of obesity. By adding a GLP-1 receptor agonist, any deleterious effect of glucagon on glucose homeostasis (as observed in the present study; ie, slightly elevated PG levels after separate glucagon infusion) may be prevented. On the other hand, no additional effects on any measure were observed by adding glucagon to the GLP-1 infusion. The doses of both GLP-1 and oxyntomodulin used have both (like glucagon) previously been shown to inhibit food intake (13, 40). Furthermore, the dose of oxyntomodulin was chosen to be sufficiently high to potentially impact both the glucagon and GLP-1 receptor (because oxyntomodulin has lower potency on both receptors compared with GLP-1 and glucagon, respectively) to be comparable with the combined infusion of glucagon and GLP-1. However, we did not find any sig-

nificant superior effect of oxyntomodulin on any of the measures in the present protocol as was suggested by preclinical interventions in rodents (10, 19).

The present protocol focused on the effects of 4-hour continuous infusions of three potent peptides with short half-lives (2–12 min), and whether the observed effects would persist for days or even weeks as suggested by preclinical interventions in mice (19) cannot be concluded from this study. Nevertheless, we found a mean 180 kcal (120 g) difference in food intake after infusions of all the peptides compared with saline. This would approximately sum up to a body weight loss of 402 g/wk (using the energetic value of fat 9.4 kcal/g), which is in the range of what previously has been found in overweight and obese humans with sc injections of oxyntomodulin (3). The relative weak signal observed in appetite scores when comparing saline and GLP-1 might reflect the limited number of participants included (21), although we did show highly significant differences between appetite scores during the glucagon and GLP-1 infusion. The dissociation between appetite scores and GE indicates differences between the mode of actions of GLP-1 and glucagon in relation to the inhibition of food intake.

In conclusion, the infusion of oxyntomodulin, GLP-1, and glucagon reduced food intake similarly but with no additional effect of adding glucagon to the GLP-1 infusion. Surprisingly, in these near physiological doses, glucagon lacked inhibitory effects on gastric emptying as well as appetite scores.

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Author contributions include the following: J.I.B., J.J.H., F.K.K., and T.V. designed the study; J.I.B. preformed the experiments and the data analysis; J.I.B., J.J.H., F.K.K., and T.V. wrote the manuscript; B.H. and B.A. contributed to the measurement of peptides, to the interpretation of the results, and writing of the manuscript. J.I.B. and T.V. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Disclosure Summary: J.J.H. has served as a consultant or adviser to Novartis Pharmaceuticals, Novo Nordisk, Merck, Sharp, and Dome, and Roche and has received fees for lectures from Novo Nordisk, Merck, Sharp, and Dome, and GSK. T.V. has received fees for being part of an advisory board from AstraZeneca, Boehringer Ingelheim Pharmaceuticals, Bristol-Myers Squibb, Eli Lilly and Co, GI Dynamics, Inc, Merck Sharp, and Dohme, Novo Nordisk, Sanofi, and Takeda; has received fees for lectures from AstraZeneca, Boehringer Ingelheim Pharmaceuticals, Bristol-Myers Squibb, Eli Lilly and Co, Merck, Sharp, and Dohme, Novo Nordisk, Novartis, Sanofi, Takeda, and Zealand Pharma; and has received research support from Novo Nordisk. F.K.K. has received fees for consultancy or being part of an advisory board from AstraZeneca, Bristol-Myers Squibb/AstraZeneca, Eli Lilly and Co, Gilead Sciences, Ono Pharmaceuticals, Sanofi-Aventis, and Zealand Pharma; has received fees for lectures from AstraZeneca, Boehringer Ingelheim Pharmaceuticals, Bristol-Myers Squibb, Eli Lilly and Co, Gilead Sciences, Merck, Sharp, and Dohme, Novo Nordisk, Ono Pharmaceuticals, Sanofi, and Zealand Pharma; and has received research support from Sanofi-Aventis. B.A. is employed at Novo Nordisk A/S. The remaining authors (J.I.B. and B.H.) have nothing to disclose.

References

1. **Schjoldager BT, Baldissera FG, Mortensen PE, Holst JJ, Christiansen J.** Oxyntomodulin: a potential hormone from the distal gut. Pharmacokinetics and effects on gastric acid and insulin secretion in man. *Eur J Clin Invest*. 1988;18(5):499–503.
2. **Dakin CL, Gunn I, Small CJ, et al.** Oxyntomodulin inhibits food intake in the rat. *Endocrinology*. 2001;142(10):4244–4250.
3. **Wynne K, Park AJ, Small CJ, et al.** Subcutaneous oxyntomodulin reduces body weight in overweight and obese subjects: a double-blind, randomized, controlled trial. *Diabetes*. 2005;54(8):2390–2395.
4. **Wynne K, Park AJ, Small CJ, et al.** Oxyntomodulin increases energy expenditure in addition to decreasing energy intake in overweight and obese humans: a randomised controlled trial. *Int J Obes*. 2006; 2005 30(12):1729–1736.
5. **Gros L, Thorens B, Bataille D, Kervran A.** Glucagon-like peptide-1-(7–36) amide, oxyntomodulin, and glucagon interact with a common receptor in a somatostatin-secreting cell line. *Endocrinology*. 1993;133(2):631–638.
6. **Baldissera FG, Holst JJ, Knuhtsen S, Hilsted L, Nielsen OV.** Oxyntomodulin (glicentin-(33–69)): pharmacokinetics, binding to liver cell membranes, effects on isolated perfused pig pancreas, and secretion from isolated perfused lower small intestine of pigs. *Regul Pept*. 1988;21(1–2):151–166.
7. **Holst JJ.** Enteroglucagon. *Annu Rev Physiol*. 1997;59:257–271.
8. **Holst JJ.** Gut glucagon, enteroglucagon, gut glucagonlike immunoreactivity, glicentin — current status. *Gastroenterology*. 1983;84(6): 1602–1613.
9. **Holst JJ.** Evidence that enteroglucagon (II) is identical with the C-terminal sequence (residues 33–69) of glicentin. *Biochem J*. 1982; 207(3):381–388.
10. **Druce MR, Minnion JS, Field BCT, et al.** Investigation of structure-activity relationships of Oxyntomodulin (Oxm) using Oxm analogs. *Endocrinology*. 2009;150(4):1712–1722.
11. **Nauck MA, Niedereichholz U, Ettler R, et al.** Glucagon-like peptide

1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. *Am J Physiol*. 1997;273(5 Pt 1):E981–E988.
12. **Astrup A, Rössner S, Van Gaal L, et al.** Effects of liraglutide in the treatment of obesity: a randomised, double-blind, placebo-controlled study. *Lancet*. 2009;374(9701):1606–1616.
13. **Flint A, Raben A, Ersbøll AK, Holst JJ, Astrup A.** The effect of physiological levels of glucagon-like peptide-1 on appetite, gastric emptying, energy and substrate metabolism in obesity. *Int J Obes Relat Metab Disord*. 2001;25(6):781–792.
14. **Zander M, Madsbad S, Madsen JL, Holst JJ.** Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and β-cell function in type 2 diabetes: a parallel-group study. *Lancet*. 2002;359(9309):824–830.
15. **Baggio LL, Huang Q, Brown TJ, Drucker DJ.** Oxyntomodulin and glucagon-like peptide-1 differentially regulate murine food intake and energy expenditure. *Gastroenterology*. 2004;127(2):546–558.
16. **Penick SB, Hinkle LE Jr.** Depression of food intake induced in healthy subjects by glucagon. *N Engl J Med*. 1961;264:893–897.
17. **Schulman JL, Carleton JL, Whitney G, Whitehorn JC.** Effect of glucagon on food intake and body weight in man. *J Appl Physiol*. 1957;11(3):419–421.
18. **Geary N, Kissileff HR, Pi-Sunyer FX, Hinton V.** Individual, but not simultaneous, glucagon and cholecystokinin infusions inhibit feeding in men. *Am J Physiol*. 1992;262(6 Pt 2):R975–R980.
19. **Pocai A, Carrington PE, Adams JR, et al.** Glucagon-like peptide 1/glucagon receptor dual agonism reverses obesity in mice. *Diabetes*. 2009;58(10):2258–2266.
20. **Kosinski JR, Hubert J, Carrington PE, et al.** The glucagon receptor is involved in mediating the body weight-lowering effects of oxyntomodulin. *Obes (Silver Spring Md)*. 2012;20(8):1566–1571.
21. **Flint A, Raben A, Blundell JE, Astrup A.** Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *Int J Obes Relat Metab Disord*. 2000; 24(1):38–48.
22. **Gregersen NT, Flint A, Bitz C, Blundell JE, Raben A, Astrup A.** Reproducibility and power of ad libitum energy intake assessed by repeated single meals. *Am J Clin Nutr*. 2008;87(5):1277–1281.
23. **Orskov C, Rabenhøj L, Wettergren A, Kofod H, Holst JJ.** Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans. *Diabetes*. 1994;43(4):535–539.
24. **Albrechtsen NJW, Hartmann B, Veedfald S, et al.** Hyperglucagonaemia analysed by glucagon sandwich ELISA: nonspecific interference or truly elevated levels? *Diabetologia*. 2014;57(9):1919–1926.
25. **Sloth B, Due A, Larsen TM, Holst JJ, Heding A, Astrup A.** The effect of a high-MUFA, low-glycaemic index diet and a low-fat diet on appetite and glucose metabolism during a 6-month weight maintenance period. *Br J Nutr*. 2009;101(12):1846–1858.
26. **Verbeke G, Molenberghs G.** *Linear Mixed Models for Longitudinal Data*. New York: Springer; 2000.
27. **Van Cauter E, Mestrez F, Sturis J, Polonsky KS.** Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes*. 1992;41(3):368–377.
28. **Kjems LL, Christiansen E, Vølund A, Bergman RN, Madsbad S.** Validation of methods for measurement of insulin secretion in humans in vivo. *Diabetes*. 2000;49(4):580–588.
29. **Kjems LL, Vølund A, Madsbad S.** Quantification of β-cell function during IVGTT in type II and non-diabetic subjects: assessment of insulin secretion by mathematical methods. *Diabetologia*. 2001; 44(10):1339–1348.
30. **D'Alessio DA, Kavle EC, Mozzoli MA, et al.** Thermic effect of food in lean and obese men. *J Clin Invest*. 1988;81(6):1781–1789.
31. **Shalev A, Holst JJ, Keller U.** Effects of glucagon-like peptide 1 (7–36 amide) on whole-body protein metabolism in healthy man. *Eur J Clin Invest*. 1997;27(1):10–16.
32. **Christin L, Nacht CA, Vernet O, Ravussin E, Jéquier E, Acheson KJ.** Insulin. Its role in the thermic effect of glucose. *J Clin Invest*. 1986; 77(6):1747–1755.
33. **Tan TM, Field BCT, McCullough KA, et al.** Coadministration of glucagon-like peptide-1 during glucagon infusion in humans results in increased energy expenditure and amelioration of hyperglycemia. *Diabetes*. 2013;62(4):1131–1138.
34. **Schmidt JB, Gregersen NT, Pedersen SD, et al.** Effects of PYY3–36 and GLP-1 on energy intake, energy expenditure and appetite in overweight men. *Am J Physiol Endocrinol Metab*. 2014;306(11): E1248–E1256.
35. **Harder H, Nielsen L, Thi TDT, Astrup A.** The effect of liraglutide, a long-acting glucagon-like peptide 1 derivative, on glycemic control, body composition, and 24-h energy expenditure in patients with type 2 diabetes. *Diabetes Care*. 2004;27(8):1915–1921.
36. **Van Can J, Sloth B, Jensen CB, Flint A, Blaak EE, Saris WHM.** Effects of the once-daily GLP-1 analog liraglutide on gastric emptying, glycemic parameters, appetite and energy metabolism in obese, non-diabetic adults. *Int J Obes*. 2014;38(6):784–793.
37. **Xu J, Lloyd DJ, Hale C, et al.** Fibroblast growth factor 21 reverses hepatic steatosis, increases energy expenditure, and improves insulin sensitivity in diet-induced obese mice. *Diabetes*. 2009;58(1):250–259.
38. **Taylor I, Duthie HL, Cumberland DC, Smallwood R.** Glucagon and the colon. *Gut*. 1975;16(12):973–978.
39. **Dotevall G, Kock NG.** The effect of glucagon on intestinal motility in man. *Gastroenterology*. 1963;45:364–367.
40. **Cohen MA, Ellis SM, Le Roux CW, et al.** Oxyntomodulin suppresses appetite and reduces food intake in humans. *J Clin Endocrinol Metab*. 2003;88(10):4696–4701.