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Acute Administration of Unacylated Ghrelin Has No Effect on Basal or Stimulated Insulin Secretion in Healthy Humans

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Unacylated ghrelin (UAG) is the predominant ghrelin isoform in the circulation. Despite its inability to activate the classical ghrelin receptor, preclinical studies suggest that UAG may promote β-cell function. We hypothesized that UAG would oppose the effects of acylated ghrelin (AG) on insulin secretion and glucose tolerance. AG (1 μg/kg/h), UAG (4 μg/kg/h), combined AG+UAG, or saline were infused to 17 healthy subjects (9 men and 8 women) on four occasions in randomized order. Ghrelin was infused for 30 min to achieve steady-state levels and continued through a 3-h intravenous glucose tolerance test. The acute insulin response to glucose (AIRg), insulin sensitivity index (S_I), disposition index (DI), and intravenous glucose tolerance (k_g) were compared for each subject during the four infusions. AG infusion raised fasting glucose levels but had no effect on fasting plasma insulin. Compared with the saline control, AG and AG+UAG both decreased AIRg, but UAG alone had no effect. S_I did not differ among the treatments. AG, but not UAG, reduced DI and k_g and increased plasma growth hormone. UAG did not alter growth hormone, cortisol, glucagon, or free fatty acid levels. UAG selectively decreased glucose and fructose consumption compared with the other treatments. In contrast to previous reports, acute administration of UAG does not have independent effects on glucose tolerance or β-cell function and neither augments nor antagonizes the effects of AG.

The orexigenic peptide ghrelin is synthesized primarily in the stomach and has been implicated in the regulation of energy balance and glucose homeostasis (1,2). After translation, intracellular ghrelin is acylated at the serine-3 residue of the peptide (1), but acylated ghrelin (AG) and unacylated ghrelin (UAG) are both released to the circulation. Acylation of ghrelin is required for binding and activation of the growth hormone secretagogue receptor (GHSR) type-1a (3), the principle target for AG. A number of in vitro studies have demonstrated that UAG does not bind or activate the GHSR (1,4). Nonetheless, a case has been made for biologic activity for UAG (5). Ghrelin is the only orexigenic peptide known to circulate in the bloodstream and has been proposed to act as a hunger signal involved in body weight regulation through a GHSR-dependent mechanism (6).

Ghrelin and the GHSR are both expressed by cells in the pancreatic islets (7–9), raising the possibility of a novel system involved in islet hormone secretion through endocrine or paracrine mechanisms. AG inhibits glucose-stimulated insulin secretion in β-cell lines and in animal

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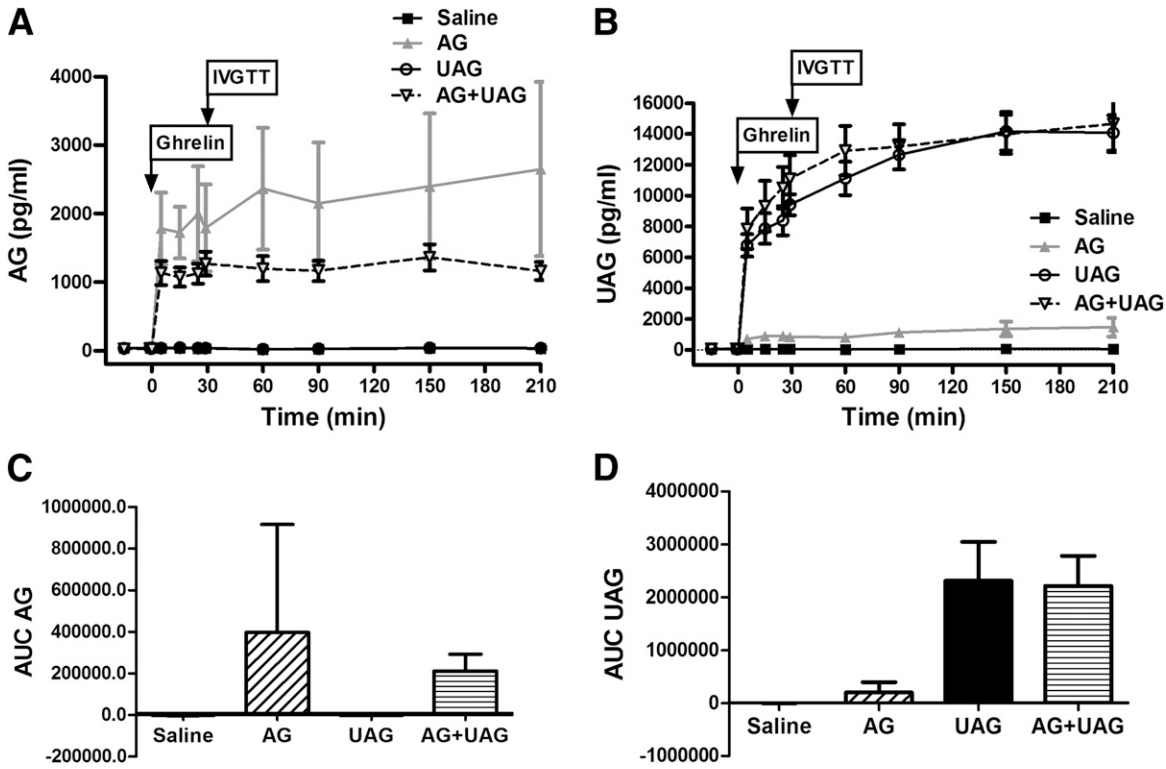

Figure 1—Plasma AG (*A*) and UAG (*B*) levels during continuous intravenous infusions (0 to 210 min) of AG (1 μg/kg/h), UAG (4 μg/kg/h), combined AG (1 μg/kg/h) and UAG (4 μg/kg/h), or saline in healthy men and women. A 180-min frequently sampled intravenous glucose tolerance test (IVGTT) was conducted between 30 and 210 min after the ghrelin infusion began. The area under the curve (AUC) is shown for AG (*C*) and UAG (*D*).

models (9–11). In humans, AG administration suppresses insulin secretion, induces peripheral insulin resistance, and impairs glucose tolerance (12–15). These findings raise the possibility that the ghrelin-GHSR system contributes to the regulation of β-cell function and could be adapted to therapeutic uses.

UAG is the predominant form of ghrelin in the circulation, where UAG and AG exist in variable ratios reported as anywhere from 2:1 to 9:1 (4,16,17). Several groups have recently reported that UAG can counteract the effect of AG on glucose metabolism and has "antidiabetic" properties (18–21). For example, UAG stimulated insulin secretion in INS-1E cells (22) and inhibited glucose output from porcine hepatocytes (18). Overexpression of UAG in adipose tissue is associated with improved glucose tolerance in mice (23). UAG also dose-dependently increases insulin secretion in rats, an effect that was abolished by the coadministration of AG (24). In humans, UAG (when given with AG) has been reported to counteract the actions of AG to impair glucose tolerance, suppress insulin secretion, and promote lipolysis (19,20). Overnight infusion of UAG to healthy subjects improved glucose tolerance, increased postprandial insulin secretion, and decreased free fatty acid levels (25). Improvement in glucose tolerance and insulin sensitivity was also observed in obese subjects with type 2 diabetes receiving pharmacologic doses of UAG (26). However, these results have not been consistent, and in several studies conducted by the same investigators, no effects of UAG on insulin or glucose levels were observed when the peptide was administered alone (19,20,27). Therefore, despite the potential importance of understanding a potential role of UAG in the regulation of glucose homeostasis, this area remains unclear.

The objective of this study was to determine whether UAG has an independent effect on insulin secretion and glucose tolerance and/or acts to antagonize the effects of AG. We hypothesized that UAG alone would enhance insulin secretion and improve glucose tolerance in healthy humans and that coadministration of UAG would blunt the effects of AG to suppress β-cell secretion. To test this hypothesis, synthetic human AG, UAG, a combination of AG and UAG, and saline (control) were administered intravenously to healthy lean subjects on four separate days. Insulin secretion, whole-body insulin sensitivity, and glucose tolerance were determined using the frequently sampled intravenous glucose tolerance test. As secondary end points, the effects of AG and UAG on appetite, thirst, caloric intake, and macronutrient preference were also examined.

RESEARCH DESIGN AND METHODS

Subjects

Healthy volunteers between the ages of 18 and 45 years, with a BMI between 18 and 29 kg/m^2, were recruited

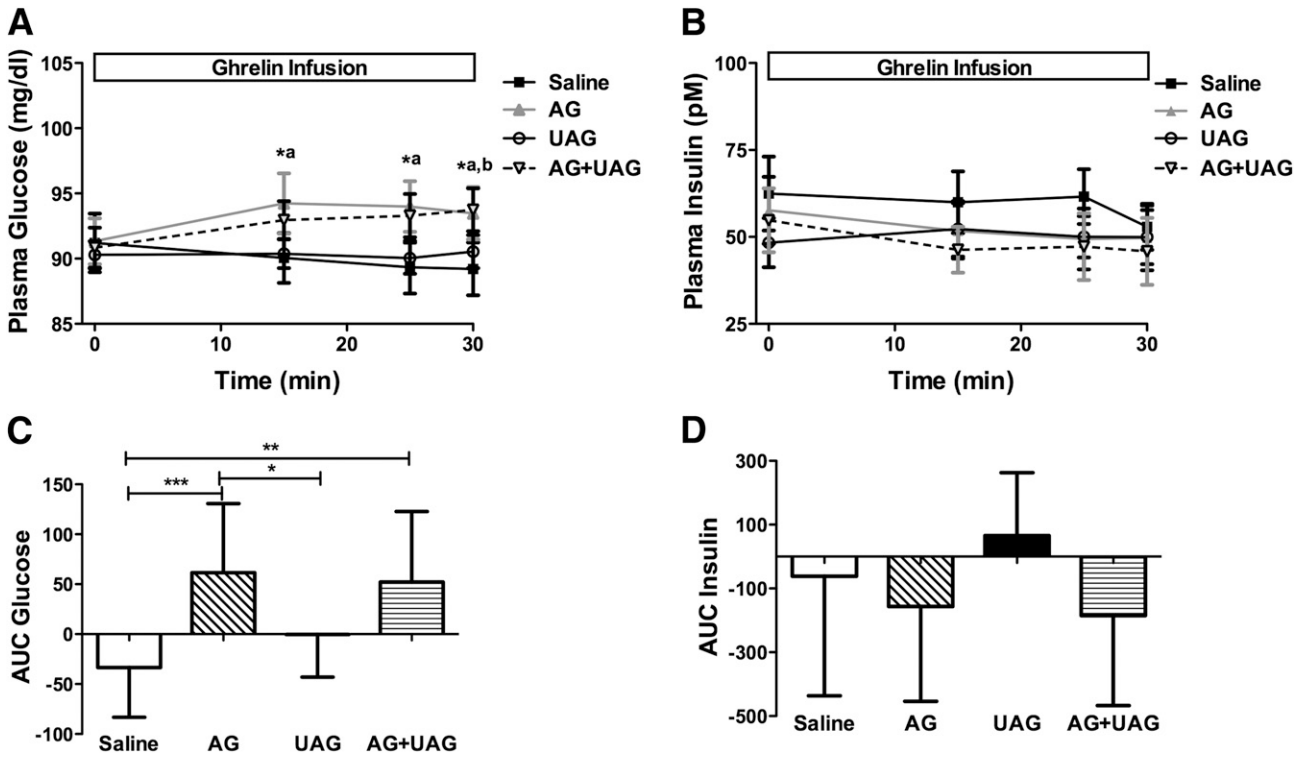

Figure 2—Fasting plasma glucose (*A*) and insulin levels (*B*) during AG (1 μg/kg/h), UAG (4 μg/kg/h), combined AG (1 μg/kg/h) and UAG (4 μg/kg/h), or saline infusions in healthy men and women. *$P < 0.05$; a, AG vs. saline; b, AG+UAG vs. saline. The area under the curve (AUC) is shown for glucose (*C*) and insulin (*D*). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

from the greater Cincinnati area. Excluded were subjects with a history of impaired fasting glucose or diabetes mellitus, recent myocardial infarction, congestive heart failure, active liver or kidney disease, growth hormone deficiency or excess, neuroendocrine tumor, anemia, or who were on medications known to alter insulin sensitivity.

All study procedures were conducted at the Clinical and Translational Research Center (CTRC) at Cincinnati Children's Hospital Medical Center. All study participants gave informed consent for the study by signing a form approved by University of Cincinnati and Cincinnati Children's Hospital Medical Center Institutional Review Boards (protocol number 10071904).

Experimental Protocol

Subjects arrived at the CTRC between 0730 and 0800 after a 10–12 h fast on four occasions separated by at least 5 days. Intravenous catheters were placed in veins of both forearms for blood sampling and infusion of test substances. The arm with the sampling catheter was placed in a 55°C chamber to maintain consistent blood flow. After fasting blood samples were withdrawn, a bolus dose of synthetic human AG (0.28 μg/kg; Bachem Americas, Torrance, CA), synthetic human UAG (1.1 μg/kg; CS Bio, Menlo Park, CA), or the combination of AG and UAG were given, followed by a continuous intravenous infusion with AG at 1.0 μg/kg/h, UAG at 4.0 μg/kg/h, or the combination of AG (1.0 μg/kg/h) and UAG (4.0 μg/kg/h) for the duration of the study. Steady-state ghrelin levels in the circulation were expected within 30 min of ghrelin infusion based on the pharmacokinetic data collected from our previous studies (17). AG and UAG levels were measured at −15, 0, 5, 15, 25, 30, 60, 90, 150, and 210 min of infusion.

After 30 min of peptide/saline infusion, an intravenous bolus of 50% dextrose solution (11.4 g/m^2 body surface area) was given as the commencement of an insulin-modified frequently sampled intravenous glucose tolerance test (28). Subsequently, regular insulin (0.025 units/kg body wt) was infused intravenously over 5 min, starting 20 min after the glucose injection. Blood samples were drawn for glucose, insulin, and C-peptide measurement at 32 time points over the 3 h after glucose administration. Blood was collected into 4 mmol/L 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride, a protease and esterase inhibitor, and 200 μL 1 N HCl was added to every milliliter of plasma for ghrelin measurements (16,29). Blood samples were placed on ice, and plasma and serum were separated by centrifugation within 1 h and stored at −80°C until assay. Blood pressure, respiration, heart rate, and body surface temperature were monitored every 15 min during the study procedure.

At the end of each procedure, subjects were given a meal of their choice, and the same meal was repeated for all subsequent visits. Portions of the main entrée were doubled to ensure that subjects had sufficient food to

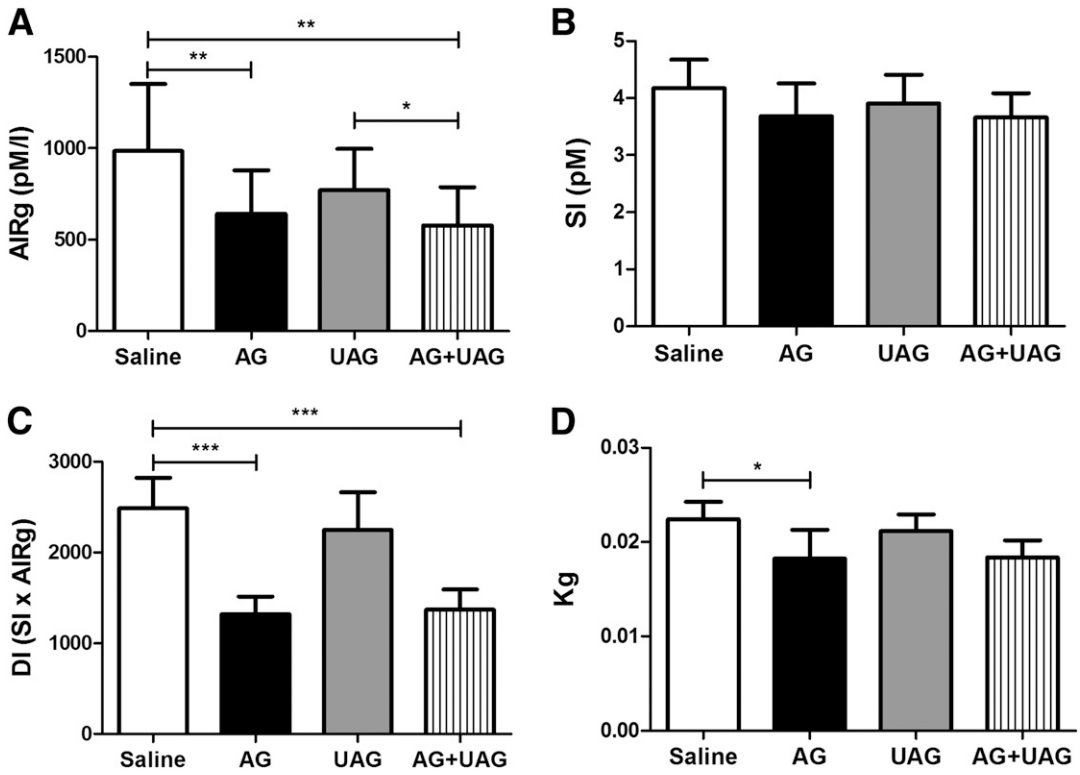

Figure 3—An intravenous glucose tolerance test was performed between 30 and 210 min during ghrelin or saline infusion. The AIRg (*A*), S_I (*B*), DI (*C*), and k_g (*D*) were determined during infusions of AG, UAG, AG+UAG, or saline. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

fulfill their appetite. Each food item was carefully weighed before and after serving by the study dietitian, and nutrient intake of foods consumed (gram weight, kilocalories, fat, protein, and carbohydrates) was calculated using Nutrition Data System for Research (NDSR) 2011 (Nutrition Coordinating Center, Minneapolis, MN). Subjects were unaware that their food intake was monitored. Hunger and thirst were assessed at 14 min before and 165 min after ghrelin/saline infusion using a 100-mm visual analog scale developed by Flint et al. (30).

Assays

Details of biochemical assays were described previously (31). Briefly, blood glucose concentrations were determined by the glucose oxidase method using a glucose analyzer (YSI 2300 STAT Plus; Yellow Springs Instruments, Yellow Springs, OH). Plasma immunoreactive insulin levels were measured using a double-antibody radioimmunoassay (Millipore, St. Charles, MO) (32). Plasma AG and desacylated ghrelin (DAG) levels were measured using separate sensitive and specific two-site sandwich ELISAs (16). The sensitivity of the AG assay was 6.7 pg/mL with intra- and interassay coefficients of variation of ~14 and 18% (16). The sensitivity of the DAG assay was 4.6 pg/mL with intra- and interassay coefficients of variations of ~13 and 20% (16). Glucagon was measured by radioimmunoassay (Millipore Life Sciences, Billerica, MA), and serum concentrations of human growth hormone were measured by a sandwich immunoassay using the automated Immulite 2000 chemiluminescent assay system (Siemens, Bad Nauheim, Germany) (31). Plasma free fatty acids were measured using a specific colorimetric assay (Wako Diagnostics, Richmond, VA). All samples were assayed in duplicate, and specimens from the four studies in each participant were run in the same assay.

Calculations

Fasting values of insulin and glucose were designated as the mean of samples drawn before ghrelin infusion. Baseline levels were designated as the mean of samples at −15 to 0 min before the intravenous glucose tolerance test. The acute insulin response to glucose (AIRg) was calculated as the average of plasma insulin increments above basal at 2, 3, 4, 5, 6, 8, and 10 min after intravenous glucose administration. Insulin sensitivity was quantified as the insulin sensitivity index (S_I) using the minimal model of glucose kinetics (33). The disposition index (DI), which provides a measure of β-cell function adjusted for insulin sensitivity, was calculated as $S_I \times$ AIRg based on the hyperbolic relationship of the two measures (34). The glucose disappearance constant, k_g, is an estimate of intravenous glucose tolerance (35) and was computed as the slope of the natural logarithm of glucose from 10 to 19 min.

Statistical Analysis

Main outcomes of interest (AIRg, S_I, DI, and k_g) and secondary outcomes (nutrient intake) for the four treatments

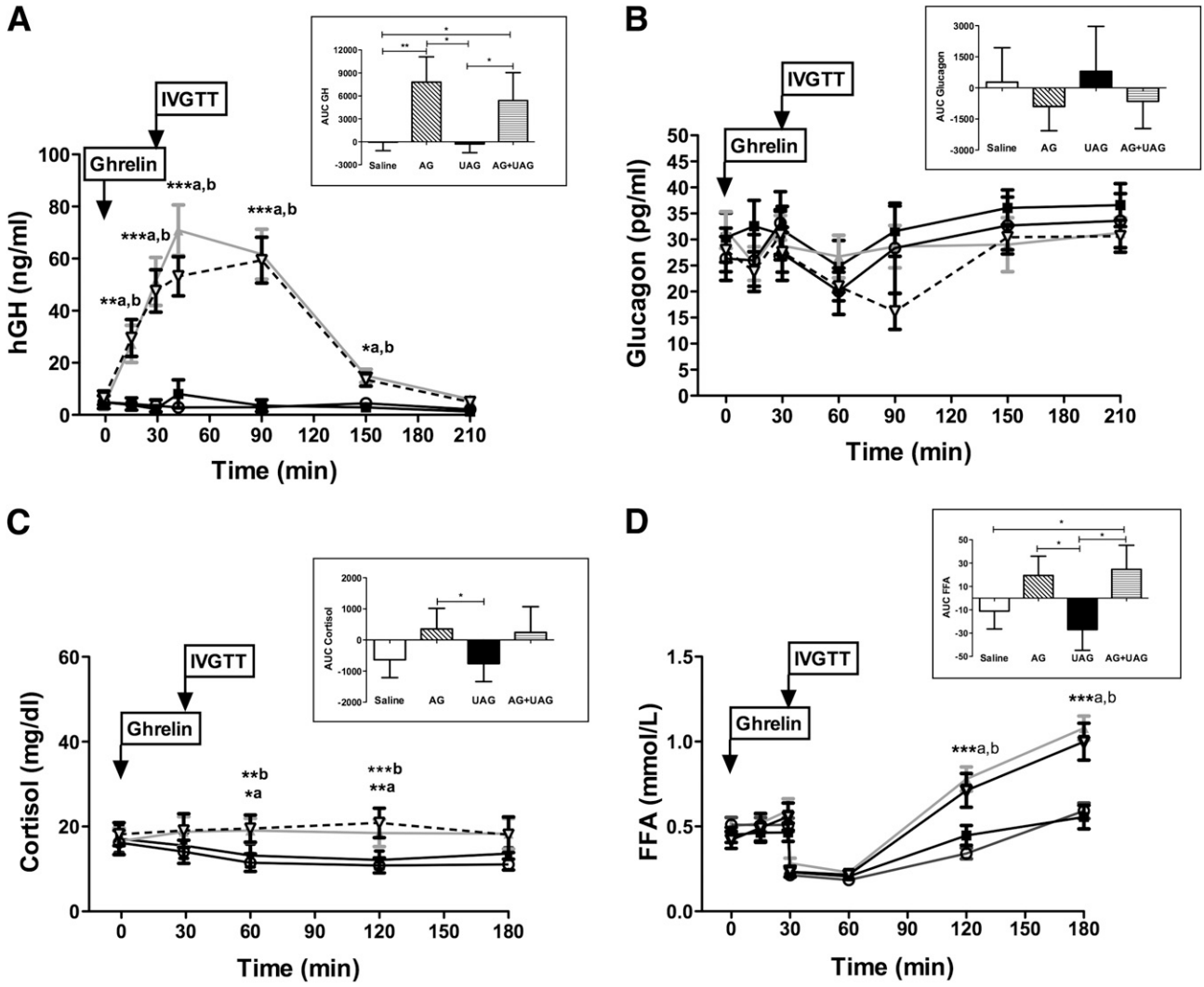

Figure 4—Plasma human growth hormone (hGH) (*A*), glucagon (*B*), cortisol (*C*), and free fatty acid (FFA) (*D*) concentrations during a 210-min infusion of AG, UAG, AG+UAG, or saline in healthy men and women. An intravenous glucose tolerance test (IVGTT) was performed between 30 and 210 min. Intravenous glucose was administered after 30 min of ghrelin or saline infusion. Insulin at 0.025 units/kg was given as a short intravenous infusion between 50 and 55 min. The *insets* show the respective areas under the curve (AUC). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; a, AG vs. saline; b, AG+UAG vs. saline.

(control, AG, UAG, AG+UAG) were compared within subjects using one-way ANOVA. In addition, insulin, glucose, ghrelin, growth hormone, glucagon, cortisol, and free fatty acid concentrations were analyzed using repeated-measures two-way ANOVA, where treatment and time effects were both assessed. Post hoc analysis to control for multiple comparisons was performed using the Dunnett test. Data were analyzed using GraphPad Prism 5.0 software (GraphPad Software). All results are expressed as mean ± SEM unless otherwise noted.

RESULTS

Subject Characteristics

The study enrolled 20 healthy subjects, and 17 (9 men and 8 women) aged 26 ± 1 year, with BMI of 24 ± 1 kg/m^2, completed the study; 3 subjects who had one or more studies but did not complete the four-infusion protocol were not included in the analysis.

Ghrelin Pharmacokinetics

After a bolus and infusion, the peak plasma AG concentration (C_{max}) was ~40-fold higher than during the saline infusion (1.9 ± 1.3 vs. 0.045 ± 0.02 ng/mL; Fig. 1*A*). A similar fold increase was seen with the AG+UAG infusion (C_{max} = 1.7 ± 0.6 ng/mL; Fig. 1*A* and *C*). The AG infusion also raised plasma UAG concentration by ~17-fold (1.3 ± 1.1 vs. 0.078 ± 0.03 ng/mL for AG infusion vs. saline; Fig. 1*B*) consistent with some deacylation in the plasma. When UAG was given alone or combined with AG, the C_{max} was ~200-fold higher than the saline control (UAG: 15.9 ± 4.9, UAG+AG: 15.5 ± 3.6 ng/mL, saline: 0.078 ± 0.03 ng/mL; Fig. 1*B* and *D*). UAG infusion did not alter

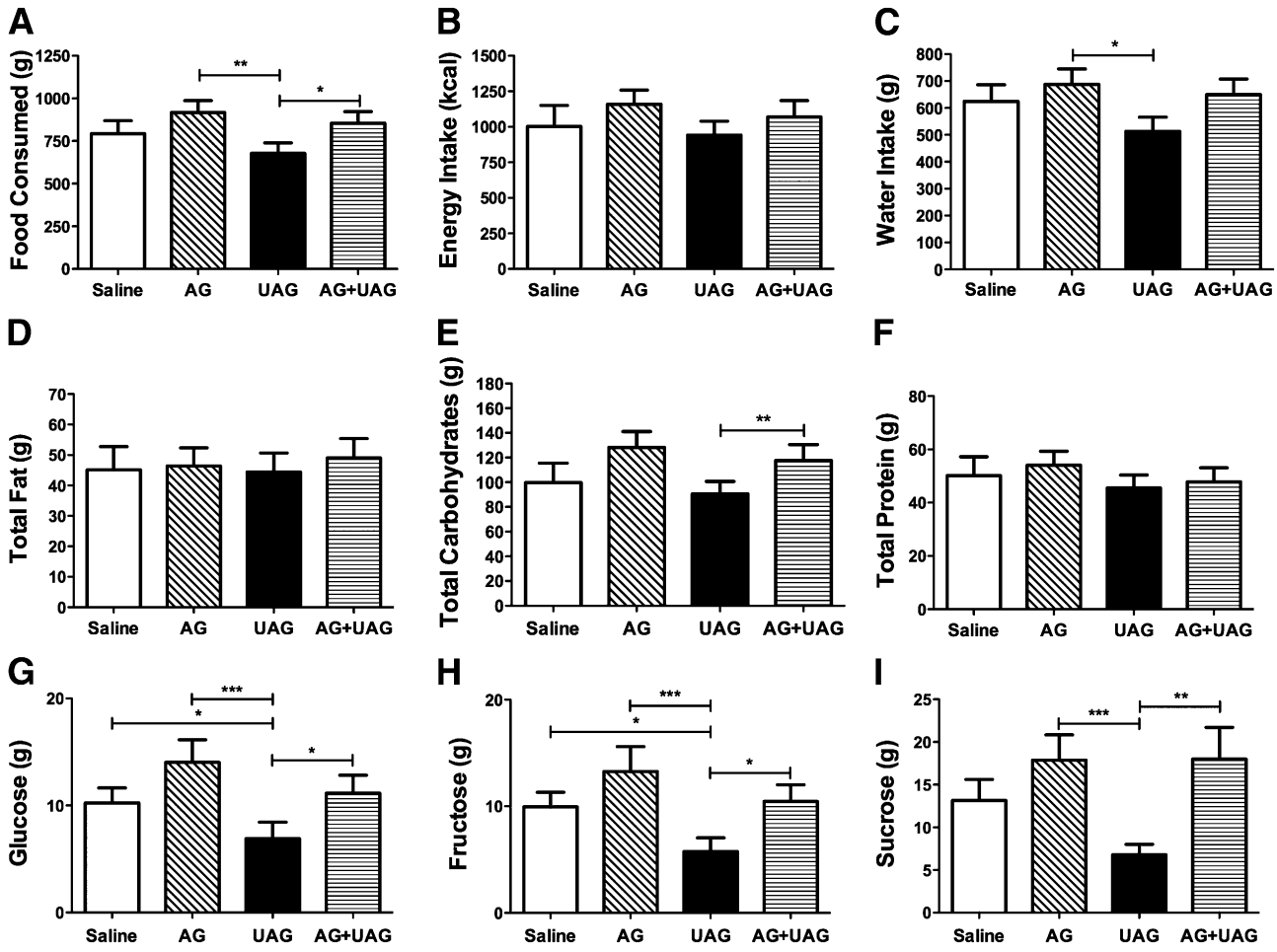

Figure 5—Nutrients consumption was measured in the poststudy meal provided at the end of each infusion. Subjects selected the meal at their first visit and were given the same meal at each subsequent visit. The consumption of food (*A*), total calorie intake (*B*), and water (*C*), fat (*D*), carbohydrates (*E*), protein (*F*), glucose (*G*), fructose (*H*), and sucrose (*I*) intake from meals were compared between AG, UAG, AG+UAG, and saline infusions. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

plasma AG concentration (C_{max}: 0.05 ± 0.02 vs. 0.045 ± 0.02 ng/mL for UAG infusion vs. saline; Fig. 1*A*).

Effects of Exogenous Ghrelin on β-Cell Function and Glucose Tolerance

AG infusion raised fasting glucose levels ($P < 0.05$ for overall effect; Fig. 2*A* and *C*), but none of the treatments changed concentrations of fasting plasma insulin (Fig. 2*B* and *D*). Compared with the saline control, AG and AG+UAG both decreased AIRg, but UAG alone had no effect (saline: 986 ± 366, UAG: 772 ± 226, AG: 642 ± 237, AG+UAG: 578 ± 210 pmol/L; $P < 0.01$ for both AG treatments vs. saline; Fig. 3*A*). S_I was not affected by any of the ghrelin treatments (Fig. 3*B*). The adjusted insulin secretion, DI, was significantly lower when AG or AG+UAG were infused but did not change when UAG was given (saline: 2,486 ± 340, AG: 1,321 ± 195, UAG: 2,248 ± 416, AG+UAG: 1,370 ± 223 pmol/L; $P < 0.001$ for both AG treatments vs. saline; Fig. 3*C*). Intravenous glucose tolerance (k_g) was lower during AG infusion (0.018 ± 0.003 vs. 0.022 ± 0.002 for AG vs. saline; $P < 0.05$) but not during UAG and AG+UAG infusions (Fig. 3*D*).

Effects of Exogenous Ghrelin on Other Hormones and Substrates

AG and the AG+UAG infusion increased serum growth hormone 20-fold from baseline, whereas UAG and saline had no effect (Fig. 4*A*). None of the ghrelin infusions changed plasma glucagon levels significantly during fasting or the glucose tolerance test (Fig. 4*B*). Serum cortisol concentration, however, was elevated during AG and AG+UAG infusion compared with saline or UAG (Fig. 4*C*). Neither AG nor UAG altered fasting serum fatty acid concentrations in the first 30 min of infusion. Intravenous glucose suppressed fatty acids by ~50% with ghrelin or saline treatment, but levels were higher at 120 and 180 min after intravenous glucose injection with AG treatment compared with saline and UAG (Fig. 4*D*).

Effects of Exogenous Ghrelin on Nutrient Intake, Hunger, and Thirst Ratings

Subjects consumed more food at the end of a 3.5-h AG or AG+UAG infusion then after the UAG infusion (Fig. 5*A*). The pattern of caloric intake was similar to that seen with food intake by weight but did not reach significance

(Fig. 5*B*). Water intake during the meal was lower with UAG than with AG (Fig. 5*C*). When macronutrient consumption was compared, fat and protein intake were similar across treatments; however, less carbohydrate was consumed during UAG infusion than during AG+UAG infusion but did not differ from AG infusion (Fig. 5*D–F*). Interestingly, UAG treatment decreased glucose and fructose consumption compared with saline, AG, or AG+UAG (Fig. 5*G* and *H*). The intake of disaccharides and polysaccharides (lactose, galactose, maltose, starch, or fiber) was not different between groups (data not shown) except for sucrose, the consumption of which was lower after UAG compared with AG or AG+UAG infusions (Fig. 5*I*). The decreased in food intake and carbohydrate intake with UAG than with AG did not correlate with decreased hunger, increased satiation (Fig. 6), or the desire for "eating something sweet" (Fig. 7). All four treatments led to a similar change in these parameters (Figs. 6 and 7). We did not observe any between-group difference on a visual analog scale for preference for sweet, salty, fatty foods, or alcohol, or their feeling of thirst before and after ghrelin infusion (Figs. 7 and 8). Furthermore, ghrelin did not affect the intake of essential amino acids, fatty acids (saturated, monounsaturated, or polyunsaturated fatty acids), cholesterol, or sodium (data not shown).

Side Effects

AG and UAG infusions were both well tolerated. No serious adverse events occurred during the study. One subject, who was later diagnosed with hypertension, withdrew from study due to elevated blood pressure during the first study visit (UAG infusion). This subject was asymptomatic during the UAG infusion.

DISCUSSION

Since its discovery in 1999, several biological functions have been ascribed to ghrelin. Besides its well-known stimulatory effect on growth hormone secretion, ghrelin has been implicated in the regulation of energy and more recently on glucose metabolism. UAG is the predominant form in the circulation, and there it has been proposed that UAG has biologic activity, possibly through a receptor distinct from GHSR (5). Our study was designed to clarify the role of UAG in the regulation of β-cell function in healthy individuals. We found that UAG did not alter insulin secretion, insulin sensitivity, or intravenous glucose

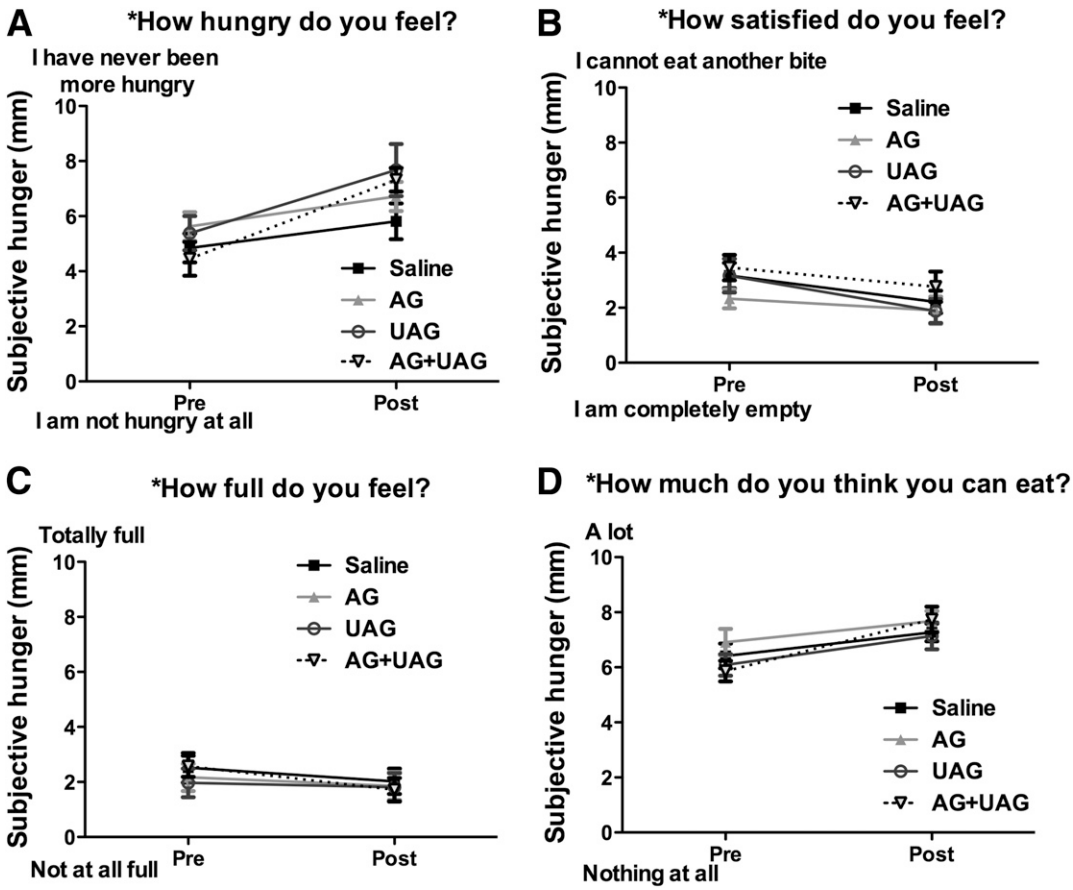

Figure 6—The ratings of hunger (*A*), satisfaction (*B*), fullness (*C*), and appetite (*D*) were measured using a visual analog scale in healthy men and women 14 min before and 165 min after ghrelin/saline infusion.

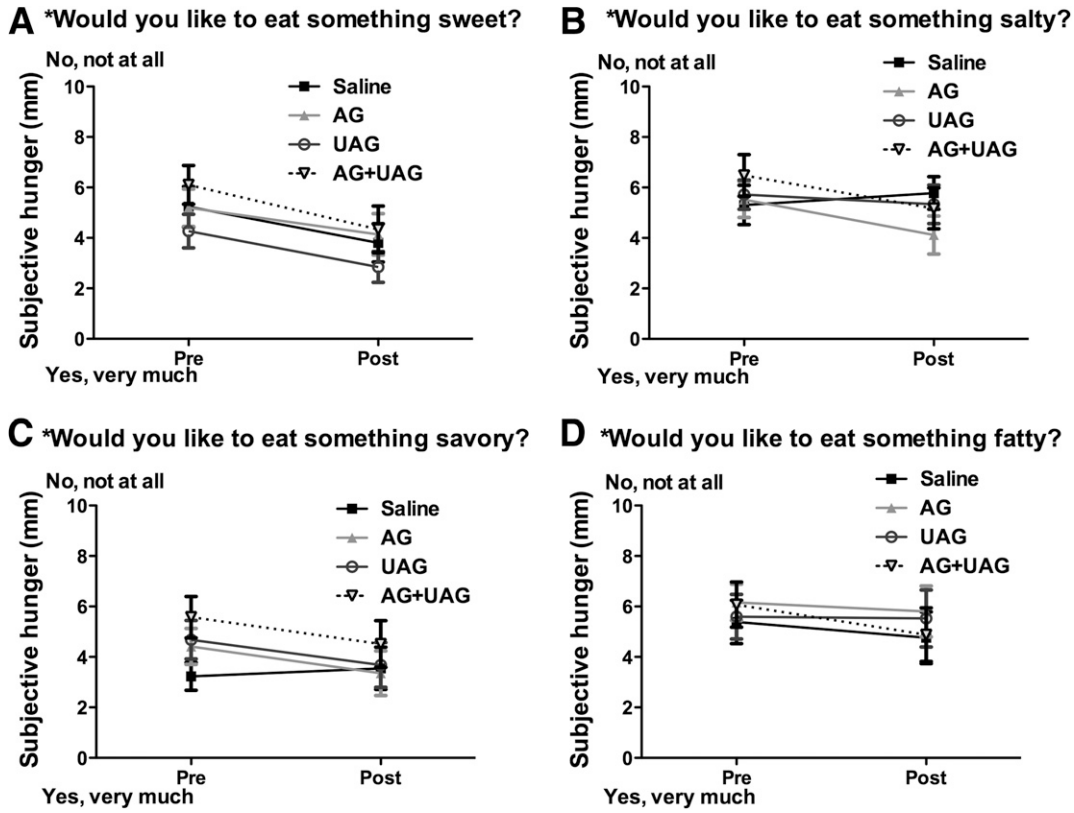

Figure 7—The rating of the desire for sweet (*A*), salty (*B*), savory (*C*), and fatty foods (*D*) were measured using a visual analog scale in healthy men and women 14 min before and 165 min after AG, UAG, AG+UAG, and saline infusions.

tolerance when administered alone or combined with AG acutely. Unlike AG, UAG did not stimulate growth hormone or cortisol secretion or increase lipolysis. On one hand, these findings indicate that in healthy humans, UAG does not affect the key parameters of glucose tolerance or alter counterregulatory hormone secretion. On the other hand, UAG had effects on feeding behavior, selectively reducing glucose and fructose consumption—a novel finding that warrants further investigation.

The AG-to-UAG ratio in the circulation had been reported to be 1:2 to 1:9, depending on the assay used, the species, and the nutritional state at the time of measurement (4,16). At the time this study was initiated, the best estimate of the ratio of AG-to-UAG in humans was 1:4 (16), and this was the rationale for the choice of the UAG dose in our study. We used a supraphysiologic amount of AG that we have previously shown provides a reliable effect on insulin secretion (29,31). Although the plasma concentrations of ghrelin peptides achieved in our study were much higher than those occurring naturally, our goal was to maximize the ability to detect any effects of UAG. It is worth considering that concentrations of ghrelin are likely to be much higher in the islet, such that elevated plasma levels may have some physiologic relevance. A major difference between previous human studies of ghrelin (25,26) and ours was that we assessed the effects of steady state UAG on both basal and stimulated β-cell function. Work by Bergman et al. (36) and others has demonstrated the importance of taking into account tissue insulin sensitivity when evaluating β-cell function. Therefore, we measured insulin secretion and insulin sensitivity simultaneously with the intravenous glucose tolerance test to obtain an unconfounded assessment of β-cell function.

Our finding of a lack of effect of UAG on insulin secretion is consistent with the findings reported by Broglio et al. (19) and Gauna et al. (20) using a lower dose of UAG (1 μg/kg i.v. bolus). No effect of UAG on fasting or postprandial insulin or glucose levels was seen in those studies. However, when these investigators gave UAG together with AG, the actions of AG to increase blood glucose were attenuated. We did not observe this counterbalancing effect of UAG on AG when they were administered together, even with a UAG dose that was higher than that used in previous studies. UAG has very low affinity for GHSR-1a but, in the high nanomolar to low micromolar range, can activate the receptor and functions as a full agonist in vitro (37). However, our findings suggest that circulating UAG at concentrations, even at supraphysiologic amounts, does not antagonize activation of GHSR by AG in vivo. We cannot rule out possible paracrine/neurocrine effects of UAG because there may be settings

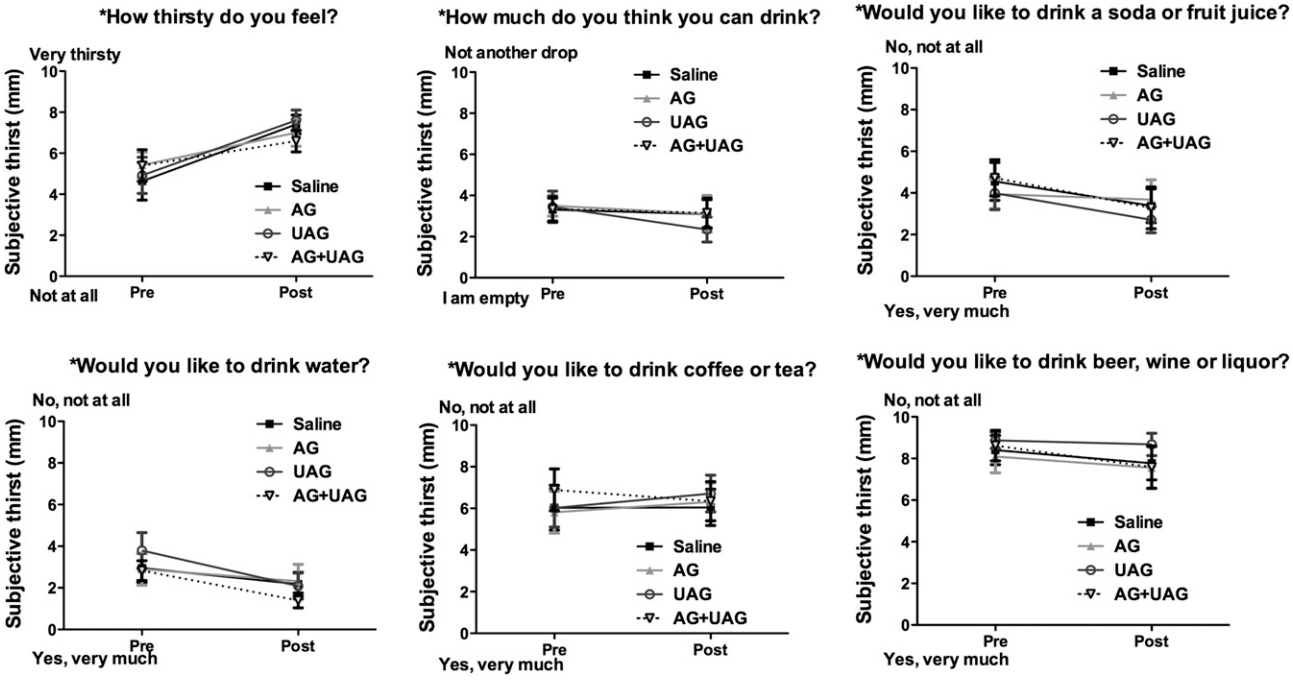

Figure 8—A visual analog scale was used to measure the ratings of thirst and fluid preference in healthy men and women 14 min before and 165 min after AG, UAG, AG+UAG, and saline infusions.

where local concentrations are even higher than the plasma levels achieved in this study.

The observations reported here differ from those of several other groups. Benso et al. (25) gave UAG overnight at a dose of 1 μg/kg/h and demonstrated a decrease in glucose and fatty acids over the ensuing 16 h, with a transient increase of postprandial plasma insulin. The same duration of UAG infusion at 3 and 10 μg/kg/h in obese subjects with well-controlled type 2 diabetes decreased average blood glucose, as reflected by continuous glucose monitoring, but did not have any effect on postprandial insulin (26). Differences in study design (meal tolerance test vs. intravenous glucose tolerance test), duration of peptide administration (16-h vs. 3.5-h infusion), and subject characteristics may explain some of the discordance between these findings and what we report here. For example, it is plausible that the high plasma concentration of UAG during a 16-h administration of peptide leads to activation of brain centers that do not occur with shorter infusions. However, on the basis of our results, it seems unlikely that circulating UAG, in contrast to AG, has immediate effects on islet function or insulin sensitivity.

The effects of ghrelin on growth hormone, cortisol, and prolactin secretion are likely to be GHSR-dependent (19). For example, the effect of ghrelin to stimulate growth hormone is absent in GHSR knockout mice (38). The lack of an acute stimulatory effect of UAG on growth hormone and cortisol in our study is consistent with the absence of GHSR activation that has been previously reported (39). In comparison, activation of pituitary function by AG, in our study and others (19,29,31,40), is likely to be an endocrine action of this peptide mediated acutely. So, too, the acute effect of intravenous AG to enhance lipolysis and increase fatty acid levels. Our findings are consistent with endocrine actions of AG, but not UAG, during 3- to 4-h administrations.

Ad libitum food intake is increased by AG in humans (41), but its effect on macronutrient intake has not been studied. UAG has been shown to either decrease or have no effect on food intake in rodents (41,42), but this has not been studied previously in humans. We found that food intake was decreased after UAG infusion compared with AG or AG+UAG infusions, but not different from saline control. Interestingly, UAG reduced the consumption of monosaccharides (glucose and fructose) as well as sucrose, a disaccharide that is composed of glucose and fructose (Fig. 5*I*). This finding is compatible with previous work showing the ghrelin system is involved in the consumption of sweets (43). Peripheral injection of AG increases, whereas a GHSR-1a antagonist reduces, the intake of sucrose in rats (43). In our study, AG showed a trend toward higher food intake and higher carbohydrate intake but did not reach statistical significance. Although the effect of ghrelin peptides on food intake was a secondary aim in this study, and not powered adequately to make definitive conclusions, the results here are suggestive and bear further investigation.

In conclusion, acute administration of a pharmacological dose UAG does not alter glucose-stimulated insulin secretion, insulin sensitivity, or glucose tolerance. Moreover,

the combination treatment of UAG with AG showed similar effects as the single AG infusion, indicating that the nonacylated form is not a significant antagonist of the GHSR in vivo. These findings indicate that although UAG is the predominant ghrelin species in the human circulation, it does not have important effects on insulin secretion or plasma glucose and fatty acid regulation.

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Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. J.T. designed the study, collected and analyzed the data, and was the primary author. H.W.D., S.S., and A.H. assisted with data collection, contributed to discussion, and reviewed and edited the manuscript. S.C.B., M.H.T., and D.D. contributed to discussion and reviewed and edited the manuscript. M.B. assisted with data collection and reviewed and edited the manuscript. J.T. 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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