Does des-acyl ghrelin improve glycemic control in obese diabetic subjects by decreasing acylated ghrelin levels?

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Abstract

Objective: The objective of this study was to assess the effects of a continuous overnight infusion of des-acyl ghrelin (DAG) on acylated ghrelin (AG) levels and glucose and insulin responses to a standard breakfast meal (SBM) in eight overweight patients with type 2 diabetes. Furthermore, in the same patients and two additional subjects, the effects of DAG infusion on AG concentrations and insulin sensitivity during a hyperinsulinemic–euglycemic clamp (HEC) were assessed.

Research design and methods: A double-blind, placebo-controlled cross-over study design was implemented, using overnight continuous infusions of 3 and 10 µg DAG/kg per h and placebo to study the effects on a SBM. During a HEC, we studied the insulin sensitivity.

Results: We observed that, compared with placebo, overnight DAG administration significantly decreased postprandial glucose levels, both during continuous glucose monitoring and at peak serum glucose levels. The degree of improvement in glycemia was correlated with baseline plasma AG concentrations. Concurrently, DAG infusion significantly decreased fasting and postprandial AG levels. During the HEC, 2.5 h of DAG infusion markedly decreased AG levels, and the M-index, a measure of insulin sensitivity, was significantly improved in the six subjects in whom we were able to attain steady-state euglycemia. DAG administration was not accompanied by many side effects when compared with placebo.

Conclusions: DAG administration improves glycemic control in obese subjects with type 2 diabetes through the suppression of AG levels. DAG is a good candidate for the development of compounds in the treatment of metabolic disorders or other conditions with a disturbed AG:DAG ratio, such as type 2 diabetes mellitus or Prader–Willi syndrome.

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Introduction

Ghrelin is a gut hormone that can be found as two isoforms in the circulation: acylated ghrelin (AG) and des-acyl ghrelin (DAG) (1). Acylation is catalyzed by the enzyme ghrelin O-acyl transferase (GOAT). GOAT is mainly expressed in the stomach and the intestine, but it can also be found in other tissues (1). While AG is known as the hunger hormone that induces obesity and insulin resistance (1), DAG appears to be a modulator of the effects

of AG. For example, DAG can inhibit AG-induced glucose output by hepatocytes *in vitro* (2) and prevents the hyperglycemic effects of AG when co-administered in healthy volunteers (3). This initial observation was followed by several reports on the anti-diabetogenic activity of DAG (2, 4, 5, 6), and recently it has been shown that a continuous infusion of DAG in healthy volunteers improves glucose metabolism (7).

Obesity is characterized by a tendency for decreased levels of DAG but unchanged AG levels, indicating a state of relative DAG deficiency where DAG levels are regulated by body weight (8, 9). Moreover, insulin-resistant obese subjects have an elevated AG:DAG ratio when compared with insulin-sensitive obese subjects (10, 11, 12). These findings suggest that the inhibition of ghrelin might be beneficial for glycemic control and body composition.

Herein, we report the results of an interventional clinical study on the effects of a continuous overnight infusion of two doses of DAG (3 and 10 µg DAG/kg per h) vs placebo using a double-blind, cross-over design in eight overweight patients with controlled type 2 diabetes mellitus. End points include glucose and insulin responses to a standard breakfast meal (SBM) and AG concentrations.

We also report for the same group of patients, together with two additional subjects, the effects of a continuous infusion of DAG (10 µg/kg per h) vs placebo on AG concentrations and insulin sensitivity parameters during a hyperinsulinemic–euglycemic clamp (HEC) using a double-blind, cross-over design.

Subjects and methods

Study 1: SBM study design

A single-center, investigator-initiated, double-blind and placebo-controlled, randomized cross-over study design was used. Eight patients were selected from our hospital outpatient clinic. After obtaining written informed consent, all the subjects underwent a physical examination and serum samples were collected. During three overnight hospital admissions, all the subjects received placebo, 3, or 10 µg DAG/kg per h in a randomized order as an i.v. continuous infusion. A wash-out period of at least 7 days was used between the admissions.

All the subjects were admitted in the afternoon, prior to the overnight infusion, and provided dinner, and then they fasted until breakfast. After admission, glucose levels were assessed by continuous glucose monitoring (CGM). After a run-in period, CGM was active from 1800 until 1300 h the next day.

Placebo or DAG was administered for 15 h by continuous infusion from 2200 to 1300 h the next day. At 0800 h, a SBM was served (three slices of wheat bread, three portions of margarine, two slices of cheese (48% fat), one portion of jam, one cup of whole milk, and one boiled egg, representing 714 kcal (17% proteins, 46% fat, and 37% carbohydrates)).

Study 2: HEC study design

In summary, the eight subjects from the SBM study and two additional subjects (see below) underwent a HEC for 4 h in total (from 0 to +240 min), during which they received an infusion of DAG at a dose of 10 µg/kg per h (after a bolus injection of 100 µg DAG intravenously) for 2.5 h (from +90 to +240 min) or saline (after a bolus injection of saline). A wash-out period of at least 1 week was used between the treatment periods and a wash-out period of at least 1 month was used between the SBM and HEC studies.

The test began at 0800 h after a 12-h overnight fast, as described previously (13). Indwelling catheters were placed into two different antecubital veins ~30 min prior to the test: the first line was used for the infusion of insulin, glucose, and DAG, and the second line was used for blood sampling. The catheters were kept patent by the infusion of isotonic saline.

A 10-min priming dose infusion of regular human insulin, calculated to raise plasma insulin levels to 100 µU/ml, was followed by a constant infusion of insulin at 40 mU/m² per min for the remaining 230 min. The glucose (20%) infusion rate was adjusted manually throughout the clamp procedure to maintain blood glucose levels at 99 mg/dl (5.5 mmol/l). Blood glucose levels were determined at bedside every 5 min. Blood samples for the measurement of insulin levels were collected at baseline (0 min), 90 min, and then every 10 min from 210 to 240 min. AG and DAG levels were assessed at baseline, 90, and 240 min. Following the session, the subjects were kept under observation in a clinical setting. If a 90-min steady state was not reached (either the glucose infusion rate was 0 or normoglycemia was not achieved), the subjects were retested using an infusion of 80 mU/m² per min insulin during a HEC on another day. The M-index, or mean glucose infusion rate (mg/kg per min), was calculated over the last 60 min of the clamp, i.e. 180–240 min after the start of the clamp and 90–150 min after the start of DAG or placebo infusion.

Subjects

Ten subjects were enrolled (eight participating in both SBM studies and at least one HEC study): three females and seven males, with a mean age of 53 years (range 30–64 years). Mean BMI was 30.7 (range 26.0–40.1) kg/m². All the subjects used a fixed dose of metformin monotherapy for at least 3 months prior to enrollment. Metformin therapy was temporarily stopped at least 24 h before the tests. All the subjects were diagnosed with type 2 diabetes

according to the criteria defined by the American Diabetes Association at least 3 months prior to enrollment (14). Mean HbA1c levels were 49 (41–55) mmol/mol (6.6 (5.9–7.2) %).

Exclusion criteria included history or presence of diabetic complications, clinical laboratory abnormalities at screening, systemic corticosteroid use for <60 days prior to screening, pregnancy or breast feeding, drug or alcohol dependence or abuse, participation in another trial at the same time, and a BMI <25 or >40 kg/m². Both studies were approved by the local human ethical review board and registered as clinical trials.

Study drug

Synthetic human DAG was obtained from Bachem AG (Bubendorf, Switzerland), as a lyophilized powder (5 mg/vial) and stored according to the manufacturer's specifications. Prior to infusion, DAG was diluted in 0.9% saline. Placebo was 0.9% saline alone.

Laboratory assessments

Upon initiation of each of the five visits (three for the SBM study and two for the HEC study), baseline serum samples were collected for the measurement of hematological and biochemical safety parameters.

During the SBM study, s.c. blood glucose levels were measured using a continuous glucose monitor (Medtronic CGM iPro Continuous Glucose Recorder, Medtronic, MN, USA) for each of the three overnight admission periods (15).

AG and DAG measurements

To stabilize AG, blood samples were collected directly into EDTA tubes, and then 1 ml of EDTA blood was immediately added to 1 ml of a preservative solution on ice (0.0295 M HCl containing 72 mM NaCl, 58 mM NaF, and 4 mM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride, pH 3.0, 295 mOsm/kg). Plasma was prepared by centrifugation at 4 °C, and then 1 ml was acidified with 100 µl 1 M HCl and stored at –80 °C until assays were performed. For the ghrelin assay, 96-well ELISA plates (Meso Scale Discovery (MSD), Rockville, MD, USA) coated overnight at room temperature (RT) with 30 ng/well of D4 antibodies (1 µg/ml in PBS; mouse MAB raised against RKESKKPP) were utilized. After removal of D4 antibodies, the wells were blocked with 150 µl casein buffer (Pierce, Thermo Fisher Scientific, Rockford, IL, USA) for 1 h at RT with shaking.

Standards for AG and DAG were prepared using eight 4× serial dilutions in the casein buffer starting at 8 and 30 ng/ml respectively. Plasma was diluted 1:1 in the casein buffer. Separate plates were used for the detection of AG and DAG. Standards and samples (25 µl/well) were loaded onto coated ELISA plates, incubated at RT while shaking for 2 h, and then washed thrice with PBS-T (150 µl/well). The C2-4a1 and E8 detection antibodies (N-terminal AG and N-terminal DAG respectively) were sulfo-tagged using the standard protocol from MSD and then diluted 1:10 000 in 0.2× casein/0.05% Tween 20 and 25 µl/well were added to the AG or DAG plates. The plates were incubated at RT for 1 h with shaking followed by three washes with PBS-T (150 µl/well). Finally, 150 µl of 1× Read Buffer (MSD) were added to each well, and the plates were measured on a MSD Sector Imager 6000. AG and DAG values for the samples were calculated by interpolation from their respective standard curves using the Sector Imager Software (Meso Scale Discovery, Rockville, MD, USA).

Statistical analyses

Data analyses were performed using Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). Results are reported as mean or median (±s.d. or s.e.m. as indicated). Comparisons were calculated using Wilcoxon matched-pairs signed-rank tests and ANOVAs. Differences were considered significant if $P < 0.05$.

Results

Overnight infusion of DAG and SBM

Glucose and insulin levels ► DAG infusion significantly decreased the glucose response to a SBM as assessed by CGM. The area under the curve (AUC, 0–180 min after the SBM study) decreased from 1618 mmol/3 h for placebo infusion to 1601 mmol/3 h ($P < 0.01$) and 1540 mmol/3 h ($P < 0.01$) for the 3 and 10 µg DAG/kg per h infusions respectively.

When depicted as changes from baseline, the AUCs decreased from 419 mmol/3 h (placebo) to 273 and 311 mmol/3 h for the 3 and 10 µg DAG/kg per h infusions (both $P < 0.01$; Fig. 1). A significant decrease in postprandial peak glucose levels was observed during the 10 µg/kg per h infusion ($P < 0.05$).

DAG infusion did not significantly affect postprandial insulin AUCs (790.1 pmol/l/3 h for placebo vs 976.0 and 855.9 pmol/l/3 h for 3 and 10 µg DAG/kg per h respectively).

Figure 1

Changes from baseline glucose levels (mmol/l) at the start of the standardized test meal study measured by CGM in eight obese diabetic subjects during infusions of either placebo or 3 or 10 µg DAG/kg per h. Areas under the curve: repeated-measures ANOVA $P < 0.0001$. Using Bonferroni's multiple comparisons: placebo vs 3 µg DAG, $P < 0.05$; placebo vs 10 µg DAG, $P < 0.001$; and 3 vs 10 µg DAG, $P < 0.001$. SBM, standard breakfast meal.

Ghrelin levels ► Owing to technical difficulties with blood sampling, AG and DAG levels were measured in only five of the eight subjects. However, in these five subjects, preprandial AG levels dropped significantly from 21.0 ± 8.9 pg/ml (mean $\pm$ s.d.) during placebo infusion to 3.0 ± 6.7 and 1.4 ± 3.2 pg/ml during the 3 and 10 µg DAG/kg per h infusions respectively (both $P < 0.001$; Fig. 2). DAG infusion was also associated with markedly decreased postprandial AG levels following the SBM study (14.0 ± 9.4 pg/ml for placebo vs 0.8 ± 1.8 and 0.8 ± 1.8 for 3 and 10 µg DAG/kg per h infusions respectively; $P < 0.01$). DAG levels increased from 105.9 ± 31.4 (mean $\pm$ s.d.) pg/ml during placebo infusion and before the start of the SBM study to 10.998 ± 2.6 and 12.085 ± 1.6 pg/ml during the 3 and 10 µg DAG/kg per h infusions respectively. Intriguingly, fasting AG levels correlated with the degree of decline in postprandial glucose concentrations measured by CGM during the 10 µg DAG/kg per h infusion (Fig. 3).

The reported adverse events (AEs) during the SBM study were mild abdominal discomfort in subject 1 (all three infusion periods) and subject 7 (3 µg DAG/kg per h infusion only). Subject 6 complained of headache during all three infusions. Hematological and biochemical

parameters were unchanged either during or between the infusions.

Hyperinsulinemic-euglycemic clamp

Glucose infusion rate ► Insulin infusion at 40 mU/m^2 per min led to steady-state glucose levels at 90 min in only four of the initial eight subjects tested, while in the remaining four either the glucose infusion rate was 0 or glucose levels remained elevated. Therefore, the HEC was repeated using an infusion of $80 \text{ mU insulin/m}^2$ per min in two new subjects, while two subjects who declined to be retested were replaced by two new subjects. On using this insulin dose, two additional patients reached a steady state. On combining data from these six subjects, it was found that DAG infusion significantly improved the M-index, which increased from 2.97 ± 0.64 to $3.46 \pm 0.87 \text{ mg/kg per min}$ ($P = 0.047$, mean $\pm$ s.e.m.).

During 40 mU/m^2 per min insulin infusion, serum insulin levels increased from $98.3 \pm 35 \text{ pmol/l}$ ($13.7 \pm 4.9 \text{ mU/l}$) at baseline to $314.1 \pm 52.3 \text{ pmol/l}$ ($43.8 \pm 7.3 \text{ mU/l}$) at 240 min during placebo administration

Figure 2

Changes in AG levels (pg/ml) in five obese diabetic subjects during overnight infusions of either placebo or 3 or 10 µg DAG/kg per h. * $P < 0.01$ and ** $P < 0.001$. SBM, standard breakfast meal; NS, nonsignificant. Bonferroni's multiple comparisons.

Figure 3

Correlation between fasting AG levels and the amount of postprandial decrease in glucose levels (mmol/l) during the 10 µg DAG/kg per h infusion (Spearman's $r=0.9$; $n=5$).

and to 289.4 ± 9.8 pmol/l (7.3 ± 1.4 mU/l) during DAG administration (nonsignificant).

Ghrelin levels ► Data on the DAG and AG levels were derived from all the clamps (ten subjects in total). Baseline AG levels before the start of the HEC study dropped significantly from 10.6 (6.6–18.1; median, 25 and 75% percentiles) to 8.4 (4.9–12.1) pg/ml at 90 min, just before the start of the DAG or placebo infusions ($P=0.02$; Fig. 4). DAG infusion was associated with markedly decreased AG levels at the end of the HEC study as AG levels fell from 8.4 (4.9–12.1) pg/ml at 90 min to 2.5 (0–4.9) pg/ml at 240 min ($P=0.005$ vs (DAG) at 240 min during placebo infusion). During placebo infusion, however, AG levels increased slightly from 8.4 (4.9–12.1) pg/ml at 90 min to 11.8 (6.4–15.1) pg/ml at 240 min ($P=0.03$; Fig. 4). DAG levels increased from 27.6 (16.7–87.3) pg/ml just before DAG infusion to 12.253 (10.1–13.6) pg/ml after 240 min of infusion ($P<0.0001$).

During the HEC studies, no AEs were recorded. Hematological and biochemical parameters did not change either during or between the clamps.

Discussion

We report for the first time that DAG significantly improves glycemic control in obese patients with

controlled type 2 diabetes following both an overnight infusion of DAG and during a HEC and coincides with a rapid and marked suppression of AG levels. The consistency of this effect in both studies has convinced us that DAG can be added to the list of compounds and methods that improve glycemic control, possibly through a novel mechanism involving the suppression of AG activity. Although we cannot exclude that changes in gastric/gut motility, induced by DAG, might also have been a factor that could change the postprandial glycemia, none of the subjects in the present study had reported any nausea or other complaints after DAG injections that might be related to a decrease in gut motility.

The *in vivo* inhibitory effect of DAG on AG levels in diabetic obese subjects is significant, particularly in relation to the rapidity of the response in the clamp study where AG levels decreased within 3 h of the initiation of DAG infusion. The decline in AG levels observed 90 min after the start of the HEC study is also of interest, which was probably induced by the hyperinsulinemia, either directly or indirectly. However, unlike that observed during DAG treatment, AG levels in placebo controls normalized by the end of the clamp period. The observation that HEC can decrease total ghrelin levels has

Figure 4

Box-whisker plots of AG levels during a hyperinsulinemic-euglycemic clamp ($n=10$). Differences were calculated using Wilcoxon matched-pairs signed-rank tests. Note that at 90 min both the placebo and DAG samples reflect placebo situation as DAG infusion started at 90 min, immediately after the 90-min samples were collected.

been reported before (10, 16, 17), although only St-Pierre *et al.* (10) reported a decrease in AG levels.

The observed inhibition of AG levels coincides with an improvement in postprandial glycemic control, as assessed by CGM, together with a reduction in peak glucose levels. Similar glycemic effects of DAG administration have recently been reported in normal individuals as well (7). Moreover, the correlation between the effects of DAG infusion on postprandial glycemia and baseline AG levels before the start of the meal in the SBM protocol warrants further study, as it suggests the clinical benefits of DAG administration in subjects with relatively high levels of AG. Appropriate subjects to study might be those with Prader–Willi syndrome (PWS), who not only are hyperphagic and morbidly obese but have elevated ghrelin levels (18, 19). This shows that there may be a suppressive (or upregulating) controlling mechanism of ghrelin secretion in PWS children. However, confirmation of our observations in a larger number of subjects is warranted before strong conclusions can be drawn.

Another population to investigate might be patients with the metabolic syndrome, especially since reports suggest that diabetes and obesity are linked with a relative DAG deficiency or an increased AG:DAG ratio (20, 21, 22, 23).

Recently, Barnett *et al.* (24) have characterized GO-CoA-Tat, a peptide-based GOAT antagonist. Interestingly, the degree of AG suppression induced in mice by GO-CoA-Tat was less than that observed in the present study during DAG administration in humans. The administration of GO-CoA-Tat improved glucose tolerance and reduced weight gain in wild-type (WT) mice (24). Interestingly, GO-CoA-Tat did not suppress weight gain in ghrelin-deficient mice that lack both AG and DAG, suggesting that these animals are less responsive because of their DAG deficiency (24). Reports suggesting that high DAG levels might be linked to positive metabolic effects include a report by Cederberg *et al.* (25). They found that 6 months of intensive exercise in 552 young men increased DAG levels, which was associated with decreased body weight and improved body composition.

Several reports support the concept that the blockade of AG action is beneficial for metabolic regulation. Maletinska *et al.* (26) reported that treatment with the ghrelin receptor antagonist [D-Lys³]GHRP-6 markedly decreased food intake in ovariectomized mice fed both high-fat and standard diets. Furthermore, [D-Lys³]GHRP-6 reduced body weight and blood glucose, insulin, and leptin levels and increased β -hydroxybutyrate levels and *Ucp1* mRNA in brown adipose tissue. They

concluded that the antagonism of ghrelin can be beneficial in obese individuals (26). Landgren *et al.* (27, 28) showed that GHS-R1a antagonism reduces the intake and self-administration of sucrose in rats as well as saccharin intake in mice. They also reported that AG administration increases the intake of sucrose in rats. They concluded that the ghrelin signaling system provides a novel target for the treatment of addictive behaviors (29).

Kirchner *et al.* reported on the potential role of AG and DAG in the regulation of glucose homeostasis, using *Mboat4*^{−/−} *ob/ob* mice. This targeted deletion of AG did not improve glucose homeostasis in this model, which suggests that neither ghrelin nor the increased ratio of DAG:AG is crucial for controlling glucose homeostasis in mice with massive obesity induced by leptin deficiency (30).

Zhao *et al.* also reported on *Mboat4*^{−/−} mice. On normal or high-fat diets, *Mboat4*^{−/−} mice grew and maintained the same weights as WT littermates. When subjected to 60% calorie restriction, both the WT and *Mboat4*^{−/−} mice lost 30% of body weight and 75% of body fat within 4 days. In both lines, fasting blood glucose levels initially declined equally. After 4 days, glucose levels stabilized in WT mice, while in *Mboat4*^{−/−} mice, glucose levels continued to decline. On day 7, WT mice exhibited normal physical activity, whereas *Mboat4*^{−/−} mice were moribund. Interestingly, GH levels rose progressively in calorie-restricted WT mice but less in *Mboat4*^{−/−} mice. Life-saving infusions of either ghrelin or GH normalized blood glucose levels in *Mboat4*^{−/−} mice. Apparently, in mice, an essential function of ghrelin is the elevation of GH levels during severe calorie restriction, thereby preserving blood glucose levels and preventing death (31).

Previous reports suggest that higher DAG levels might be important in glycemic control. For example, Asakawa *et al.* examined the effects of DAG on energy balance by studying DAG-overexpressing mice. These exhibited a decrease in body weight, food intake, and fat pad mass weight accompanied by moderately decreased linear growth. This indicates that in contrast to AG, DAG induces a negative energy balance by decreasing food intake and delaying gastric emptying via the hypothalamus (32).

Finally, Zhang *et al.* also examined the effect of DAG driven from the fatty acid-binding protein-4 (*Fabp4*) promoter on adiposity and glucose metabolism. A high level of DAG expression was detected in the adipose tissue in *Fabp4*-ghrelin transgenic mice, but not in WT littermates. Circulating DAG levels were significantly higher in *Fabp4*-ghrelin transgenic mice than in WT mice. Glucose tolerance tests showed glucose levels to be

significantly lower in *Fabp4*-ghrelin transgenic mice than in the controls after glucose administration. Insulin sensitivity testing showed that *Fabp4*-ghrelin transgenic mice exhibited a significant greater hypoglycemic response to insulin (33).

The inhibition of AG action by GHS-R1a blockade might, however, have unwanted side effects. For example, Granata *et al.* (34) showed that AG and DAG exert similar beneficial effects on pancreatic β -cells, suggesting that GHS-R1a blockade of AG would have negative effects on pancreatic function. DAG does not antagonize GHS-R1a even in the micromolar range (6). Furthermore, although it activates GHS-R1a at micromolar concentrations, circulating levels of DAG achieved during the infusions only reached the nanomolar range, meaning its effects on AG levels cannot be explained by a GHS-R1a-mediated mechanism. No specific receptor for DAG has been identified to date, but there is indirect evidence for its existence. For example, we found that DAG treatment of *Ghsr1a* knockout mice rapidly affected the expression of genes involved in glycemic control and lipid metabolism, indicating a mechanism of action independent of GHS-R1a (35).

Another feature that distinguishes DAG from the classic GHS-R1a blocking agents is that DAG directly reduces body weight, independently of AG. This is indicated by the more potent effects of GOAT inhibitors in WT mice than in ghrelin knockout mice in the studies of Barnett *et al.* (24).

Our observation that DAG administration improves the glycemic response to a SBM might be explained by the reduction in AG levels, especially since the magnitude of this effect is correlated with basal AG levels. However, direct metabolic effects of DAG cannot be excluded in view of the reported GHS-R1a-independent (AG-independent) effects of DAG, which include improved glycemic control through the modulation of glucose and medium-chain fatty acid uptake (2, 6, 35, 36). Another indication of the importance of DAG levels as a potential physiological inhibitor of AG action has been reported already by the above-mentioned studies of transgenic mice overexpressing DAG (32, 33, 37), which were lighter and shorter compared with controls. In another example of its antagonistic effects, DAG was found to suppress AG-induced neuronal activity in the arcuate nucleus of rats, thus suppressing the induction of food intake by AG (21).

No changes in insulin levels were observed during either low- or high-dose overnight DAG infusions compared with placebo, suggesting that the improved

glycemic control was the result of improved insulin sensitivity. This is in line with other observations on its insulin-sensitizing effects in rodents (35) and humans (3, 5). Although we observed significant improvements in the M-index during DAG infusion in the HEC study, it is difficult to draw strong conclusions since this was assessed in only six subjects. It is possible that earlier initiation of the DAG and placebo infusions, before the start of the clamps, might have better revealed the effects of DAG on glucose infusion rates.

Further studies are needed to show that the short-term effects of DAG administration on the improvement of postprandial glycemia persist and can be translated into long-term improvement of glycemic control and insulin sensitivity. Also, whether or not chronic DAG administration reduces body weight in humans is still unknown.

In conclusion, our data show for the first time that DAG administration might lead to an improvement in postprandial glucose levels compared with placebo, without an increase in insulin levels, in obese diabetic humans. This suggests an insulin-sensitizing effect rather than an insulin secretagogue effect, although at this stage the number of subjects in whom we could reliably assess glycemic control is too small to draw firm conclusions. This improvement in glycemic control during DAG infusion coincides with a potent and rapid decrease in AG levels. Clearly, studies in larger populations are necessary to confirm the potential beneficial effects of DAG-suppressed AG levels on glycemic control. Nevertheless, the present study suggests that DAG might be a good candidate for the development of compounds for the treatment of metabolic disorders or other conditions with a disturbed AG:DAG ratio, such as type 2 diabetes mellitus or PWS.

Declaration of interest

A R Miller, H-C Yang, and V Lucaites are employees of the Eli Lilly Company (Indianapolis, USA); T Abribat and S Allas are employees of Alizé Pharma (Ecully, France); and A J van der Lely is a scientific advisor, shareholder of Alizé Pharma, and guarantor of this work, had full access to all the data, and had full responsibility for the integrity of data and the accuracy of the data analyses.

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Author contribution statement

B Özcan, S J C M M Neggers, and A J van der Lely researched the data and wrote the manuscript; A R Miller, H-C Yang, V Lucaites, M Huisman,

J A Visser, and P J D Delhanty researched the data and reviewed/edited the manuscript; and A P N Themmen, E J G Sijbrands, T Abribat, and S Allas reviewed/edited the manuscript.

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