Response to inquiry by Gaylinn *et al.* on 'Administration of UAG improves glycemic control in obese subjects with diabetes'

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Dear Editor,

In a recent Letter to the Editor Gaylinn *et al.* (1) discuss the outcomes of a study that we published in the *European Journal of Endocrinology* in which we reported that the administration of unacylated ghrelin (UAG) improves glycemic control in obese subjects with diabetes and (2) that this effect might be mediated by a decrease in acylated ghrelin (AG) levels in response to UAG administration (2). They comment that, while we clearly demonstrated the anti-diabetogenic potential of UAG, our observation that AG levels are suppressed may be an assay artefact. They refer to a similar UAG infusion study by Tong *et al.* (3) in which a decline in AG was not observed. Gaylinn *et al.* attribute this to technical differences in the antibody used to capture AG in the sandwich assay. Tong *et al.* used an assay in which the capture antibody is an N-terminal AG-specific antibody whereas in our study a C-terminal specific antibody was used. Gaylinn *et al.* argue that when high UAG concentrations are present, as during an infusion study where they can reach 10–20 ng/ml, the UAG may compete for capture at the C-terminal assay antibody and interfere with the detection of AG.

We would like to point out a few critical points which may address the concerns raised by Gaylinn *et al.*

1. We have assessed the interference in the AG assay under high concentration of UAG (up to 10 000 pg/ml): in this experiment, we spiked in either 20 or 200 pg/ml of AG to solutions with various concentration of UAG up to 10 000 pg/ml. The recoveries of the AG under these conditions are well within the regulatory clinical biomarker requirements (i.e. 70–130%) (4). Furthermore, in the UAG infusion experiment, our average

peak plasma UAG concentration is 10 900 pg/ml. A 20% lower than expected recovery of AG at the highest concentration of UAG 10 000 pg/ml, might be indicative of a small degree of signal suppression under these conditions (Table 1). However, even if taken for face value, this would not explain >80% lower level of AG in patients infused with UAG. Consequently, our original finding on the possibility that high concentration of circulating UAG may lead to lower plasma AG remains valid.

2. There are some technical differences that differentiate the assay we used from that used by Tong *et al.*: in the study by Ozcan *et al.* (2), we used a new immunoassay technology, by Meso Scale Discovery, Inc. (Rockville, MD, USA), using electro-chemoluminescence (ECL) readout. This immunoassay platform has better sensitivity (~1 pg/ml) and a wider dynamic range (i.e. five orders of magnitudes). With this wide dynamic range, samples with wide range concentrations of analyte can be loaded to the immunoassays without any dilution. In contrast to our method, the study by Tong *et al.* used a traditional ELISA with fluorescent detection method, which is known to have narrower dynamic range than that of the ECL readout.
3. Additionally, the blood samples in those two studies were stabilized in a slightly different way. In the study by Ozcan *et al.* (2), whole blood was equilibrated in an acidic isotonic solution containing high concentration of AEBSF and NaF, whereas in Tong *et al.* AEBSF was dissolved in the blood samples and only after preparation of plasma was the sample acidified.
4. Perhaps the most important difference, also pointed out by Gaylinn *et al.* (1), is that while in the study by

Table 1 Fixed concentrations of AG was added to various concentration of UAG, the AG concentration in each sample was measured in the AG assay. All spike recovery experiments were conducted in human plasma.

UAG concentration	Spiked 20 pg/ml AG to indicated concentration of UAG				Spiked 200 pg/ml AG to indicated concentration of UAG			
	Average measured AG (pg/ml) 20 pg/ml AG was spiked in	S.D.	Recovery of spiked 20 pg/ml AG (%)	S.D. recovery (%)	Average measured AG (pg/ml) 200 pg/ml AG was spiked in	S.D.	Recovery of spiked 200 pg/ml AG (%)	S.D. recovery (%)
10 000	15.8	0.3	79	1	163.7	0.5	82	0.3
5000	19.7	0.4	99	2	199.6	1.1	100	0.6
2500	21.8	0.1	109	0.3	221.8	0.6	111	0.3
1250	23.5	0.1	117	0.3	223.8	1.4	112	0.7
625	22.1	0.4	110	2	227.7	0.5	114	0.3
312.5	22.7	0.2	113	1	240.9	4.1	120	2
156.3	22.7	0.2	113	1	232.3	1.9	116	1
78.1	22.7	0.1	114	0.6	235.7	2.1	118	1
39.1	22.7	0.2	113	1	232.5	0.1	116	0.7
19.5	22.2	0.3	111	2	227.8	3.8	114	1.9

Tong *et al.* (3) healthy volunteers were used, we studied the effects of UAG administration in obese subjects with diabetes (2).

Although we value the concern expressed by Gaylinn *et al.*, the administration of UAG does improve glycemic control in obese diabetes patients. Based on our data, the high concentration of circulating UAG appears to results in lower level of circulating AG. However, we acknowledge that the reduction of circulating AG followed by UAG infusion may not be able to fully explain the therapeutic benefit of UAG. The detailed mechanism(s) of the beneficial effects of UAG on the improvement of glycemic control warrant farther studies. Consequently, we used a cautious title to the study: does des-acyl ghrelin improve glycemic control in obese diabetic subjects by decreasing AG levels? (2).

Declaration of interest

A R Miller, H-C Yang, and V Lucaites are employees of the Eli Lilly Company (Indianapolis, IN, 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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