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An *In Vitro* Model to Probe the Regulation of Adipocyte Differentiation under Hyperglycemia (*Diabetes Metab J* 2013;37:176-80)

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We truly appreciate the interest and comments regarding our study, "An *in vitro* model to probe the regulation of adipocyte differentiation under hyperglycemia" which was published in *Diabetes & Metabolism Journal* 2013;37:176-180 [1]. Our response to the comments by Dr. In-Kyung Jeong is:

First, in *in vitro* studies, the preadipocytes are maintained and differentiated to mature adipocytes using DMEM supplemented with 25 mM glucose (ATCC® CL-173™) [2-4]. Han et al. [5,6] have studied the effect of 5 and 25 mM glucose on differentiation of 3T3-L1 adipocytes and reported that 3T3-L1 adipocytes when grown in 25 mM glucose were hypertrophic with large cells containing considerable numbers of lipid droplets in comparison to the cells maintained in 5 mM glucose. Similar results were observed by us upon treatment of 3T3-L1 adipocytes with 5, 25, 45, 65, 85, and 105 mM glucose concentrations, which showed less lipid accumulation at 5 mM measured using AdipoRed assay.

Our studies have been performed with hypertrophy inducing concentration of glucose (25 mM) as control, and the effect of higher concentrations of glucose on adipogenic markers following hypertrophy, were investigated.

Since the current study has been performed *in vitro*, the tolerance levels would vary in comparison to *in vivo* conditions. For instance, in healthy human subjects, LPS circulates in plasma at low concentrations between 1 and 200 pg/mL [7] but various *in vitro* studies are performed with LPS concentrations of 5, 25, 50, or 75 μg/mL [8,9], which are considered fatal *in vivo*. Serum tumor necrosis factor α (TNFα) levels is approximately 0.7 pg/mL in normal subjects and 5.1 pg/mL under obese condition [10,11]. The *in vitro* studies have been performed using 4, 5 ng/mL, and 100 pM TNFα which are considered high concentrations compared to the physiological concentration [8,12,13].

Second, hyperosmotic cell stress has been known to cause cell damage physically and is also known to induce apoptosis [14]. Glucose at 105 mM was not observed to cause cell death, investigated using MTT assay, whereas, glucose at 125 mM was found to be cytotoxic on 3T3L1 adipocytes. Hence the study focused on the non cytotoxic range of glucose concentration (25 to 105 mM glucose).

Overall, the study attempted to investigate the effect of glucose on adipogenesis, in the presence of concentrations exceeding 25 mM glucose, to study the fate of the cells above the hypertrophy inducing concentration of glucose. We hope studies to investigate the effect of mannitol as control, to confirm whether the increase of oxidative stress, cytokine gene expression, nuclear factor-κB, or TNFα, is due to very high concentration of glucose itself or high osmolarity can be performed through this effort. However, our aim was to explore the effect of high glucose concentrations on adipogenesis, which has not been reported earlier, to enable us to understand the mechanism of acute hyperglycemia. Thank you for the interest in this

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study and for your useful comments.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article is reported.

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