

# Internalization of intact $\gamma$ -conglutin, the lupin seed glucose-lowering glycoprotein, by Hep G2 cells: biochemical and microscopy studies

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Gamma-Conglutin ( $\gamma$ -C), a *Lupinus albus*, seed glycoprotein, has been shown to lower blood glucose in hyperglycaemic rats and increase glucose consumption in HepG2 cells (1). Aim of the present work was to monitor the localization of this protein in HepG2 cells in parallel to the glucose uptake and to study the mechanism of  $\gamma$ -C internalization by HepG2 cells. *Cell glucose uptake*: HepG2 cells were grown in DMEM supplemented with 0.2% BSA and 11.1 mM glucose. After 12 h, the medium was removed and the cells were treated with 2-NBDG (50  $\mu$ M), insulin (100 nM) w/wo  $\gamma$ -C in DMEM containing 11.1 mM glucose for 6 h. *Confocal microscopy*: experiments were carried out on cells after 0 h, 30', 3 h and 6 h incubation with DMEM w/wo  $\gamma$ -C (10 mM).  $\gamma$ -C localization was detected with specific antibodies. *Mechanism of  $\gamma$ -conglutin internalization*: HepG2 cells were pretreated for 30' w/wo inhibitors of caveolae/lipid raft-mediated endocytosis [filipin (5  $\mu$ g/ml) and genistein (200  $\mu$ M)], of clathrin-mediated pathway [chlorpromazine (25  $\mu$ M), methyl- $\beta$ -cyclodextrin (5 mM)] and of macropinocytosis [amiloride (5 mM)]. Cells were subsequently incubated with FICT- $\gamma$ -C (50  $\mu$ g/ml) for 4 h at 37°C in the presence of inhibitors as above reported, followed by FACS analysis. *The results obtained point out*: the key role of  $\gamma$ -Conglutin on cell glucose uptake (+ 64% alone and +84% with insulin); the localization of this food protein, in its intact form, in the cytosol compartment; the macropinocytosis pathway as mechanism of  $\gamma$ -conglutin internalization (69% inhibition by amiloride). We suggest that the biological activity of this lupin protein is exerted through a complex mechanism involving interaction with non-nuclear cell components that are the object of our on-going investigation.

A deeper knowledge of  $\gamma$ -Conglutin will make clear if this lupin protein can provide an alternative to drug treatment of glucose metabolism disorders, including diabetes, pre-diabetes, but also weight excesses, metabolic syndrome and others.

## References

1. MR Lovati, C Manzoni, A Parolari, S Castiglioni, et al., Lupin seed g-conglutin lowers blood glucose in hyperglycaemic rats and increases glucose consumption of HepG2 cells. *Brit. J Nutr.* 2012, 107: 67-73.