Anti-Obesity and Anti-Hyperglycemic Effects of Cinnamaldehyde via altered Ghrelin Secretion and Functional impact on Food Intake and Gastric Emptying.

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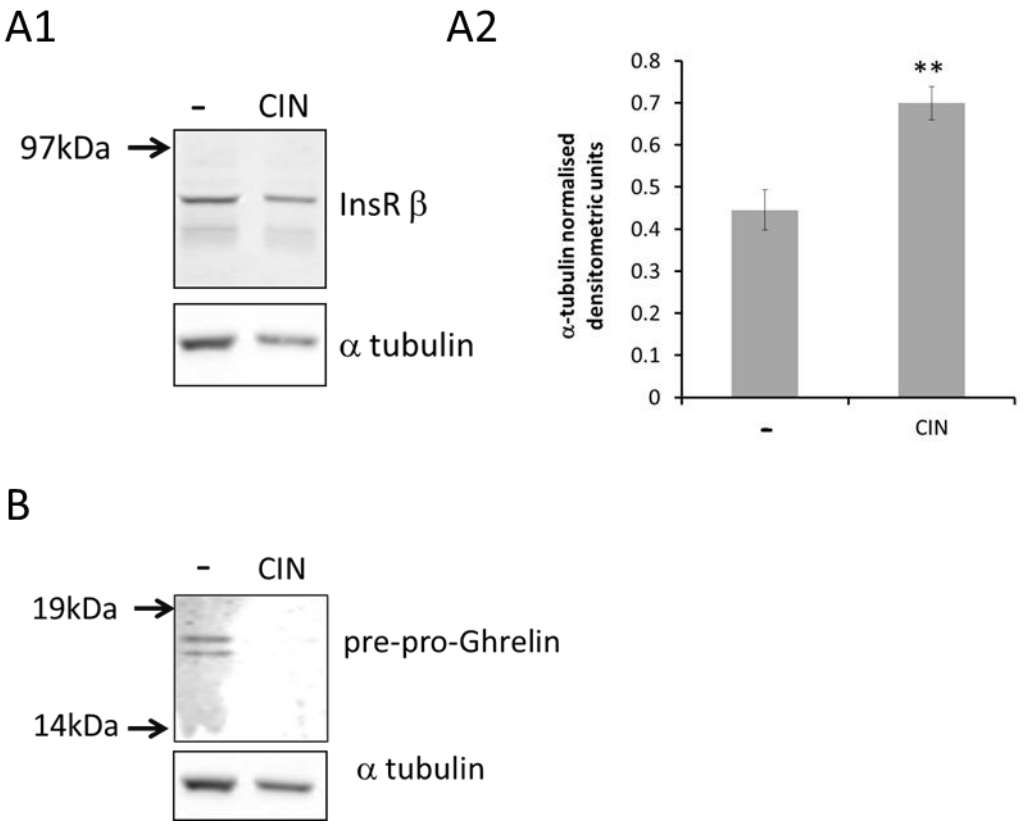
Supplementary data

taq man primers	genes	tissue			
Mm00441473_m1	GLUT1(Slc2a1)	liver	BAT	WAT	muscle
Mm00446229_m1	GLUT2(Slc2a2)	liver			
Mm00441483_m1	GLUT3(Slc2a3)			WAT	
Mm01245502_m1	GLUT4(Slc2a4)		BAT	WAT	muscle
Mm00600311_m1	GLUT5(Slc2a5)			WAT	muscle
Mm00554217_m1	GLUT6(Slc2a6)				
Mm00444634_m1	GLUT8 (Slc2a8)			WAT	muscle
Mm00455122_m1	GLUT9(Slc2a9)	liver			
Mm01249519_m1	GLUT10(Slc2a10)	liver			muscle
Mm00619244_m1	GLUT12(Slc2a12)			WAT	muscle
Mm00446190_m1	IL-6	liver		WAT	muscle
Mm01343606_m1	adiponectin			WAT	
Mm00434759_m1	leptin			WAT	
Mm01244861_m1	UCP1		BAT		
Mm00627598_m1	UCP2		BAT		
Mm00494077_m1	UCP3		BAT		
Mm01135198_m1	CD36		BAT		
Mm01211875_m1	insulin receptor			WAT	muscle
Mm01173149_m1	SGLT3(SLC54)	liver		WAT	muscle
Mm00490331_m1	ACSL4		BAT	WAT	
Mm01231183_m1	Cpt1a		BAT	WAT	
Mm00440939_m1	Ppara		BAT	WAT	

Mm01246831_m1	Acox 1		BAT	WAT	
Mm00607939_s1	b-actin	liver	BAT	WAT	muscle
Mm01352366_m1	SDHA	liver	BAT	WAT	muscle
Mm01227437_m1	TRPA1	liver	BAT	WAT	muscle

Supplementary table: List of additional genes has been studied in the following tissues: Liver, Brown adipose tissue (BAT), White adipose tissue (WAT) and muscle.



Supplementary Figure 1: Representative Western Blot (A1) of Insulin receptor β (InsR β) and ghrelin (B) proteins expression in MGN 3-1 cells stimulated or not with cinnamaldehyde (CIN) for 24 hours. The corresponding α-tubulin Western blots are represented underneath. Quantitative densitometric analysis of InsR β (A2) is performed by normalization with α-tubulin. Quantification is represented as mean ± SEM (**: $p<0,01$, Student t-test; n=5)

SUPPLEMENTARY METHODS

Western blot analysis:

Cell were grown and stimulated with cinnamaldehyde as described in paragraph***: "Ghrelin secretion in MGN3-1 cells: measurements in culture media and gene expression analysis".*** Cells were collected in NP40 cell Lysis Buffer (Invitrogen) supplemented with PMSF (Sigma-Aldrich) and protease inhibitor (P-2714; Sigma). The equivalent of 1/16 of the total lysate of each well (12 well plate) were resolved by SDS-PAGE and transferred on nitrocellulose membrane. Membranes were incubated overnight in primary antibodies, anti-Insulin receptor β (sc-711; Santa Cruz Biotechnology), anti- ghrelin (sc-10368; Santa Cruz Biotechnology) and anti-GOAT (H-032-12; Phoenix pharmaceuticals) diluted 1/200 in blocking buffer 0.1% Tween 20 (Invitrogen) or α-tubulin (926-42213; LI-COR) diluted 1/10'000. Primary antibody detection was done using IRDye secondary antibodies (926-32411, 926-32210, 926-32211; LI-COR) diluted 1/10'000 in blocking buffer 0.1% Tween 20 and incubated during 1 hour. To perform quantification, α-tubulin (926-42213; LI-COR) was detected on membranes stripped (stripping buffer from LI-COR) after the detection of each protein of interest. Detection of the signal was done using ODYSSEY classic Infrared Imaging System (LI-COR).