

Efficacy of Food Industry By-Product β -Glucan/ Chitin–Chitosan on Lipid Profile of Overweight and Obese Individuals: Sustainability and Nutraceuticals

Victoria Santisteban ^{1,2}, Natàlia Muñoz-Garcia ¹, Anallely López-Yerena ¹, Montserrat Puentes ³, Lina Badimon ^{1,4,5,†} and Teresa Padro ^{1,4,*}

¹ Institut Recerca Sant Pau, Sant Antoni M^a Claret 167, 08025 Barcelona, Spain; vsantisteban@santpau.cat (V.S.); nmunoz@santpau.cat (N.M.-G.); naye.yerena@gmail.com (A.L.-Y.), lbadimon@santpau.cat (L.B.)

² School of Pharmacy and Food Sciences, University of Barcelona (UB), 08036 Barcelona, Spain

³ Medicament Research Center (CIM), Institut d'Investigació Biomèdica Sant Pau (IIB SANT PAU), 08041 Barcelona, Spain; mpuntes@santpau.cat

⁴ Centro de Investigación Biomédica en Red Cardiovascular (CIBER-CV), Instituto de Salud Carlos III, 28029 Madrid, Spain

⁵ Cardiovascular Research Chair, Universitat Autònoma de Barcelona (UAB), 08193 Barcelona, Spain

* Correspondence: tpadro@santpau.cat

† These authors contributed equally to this work.

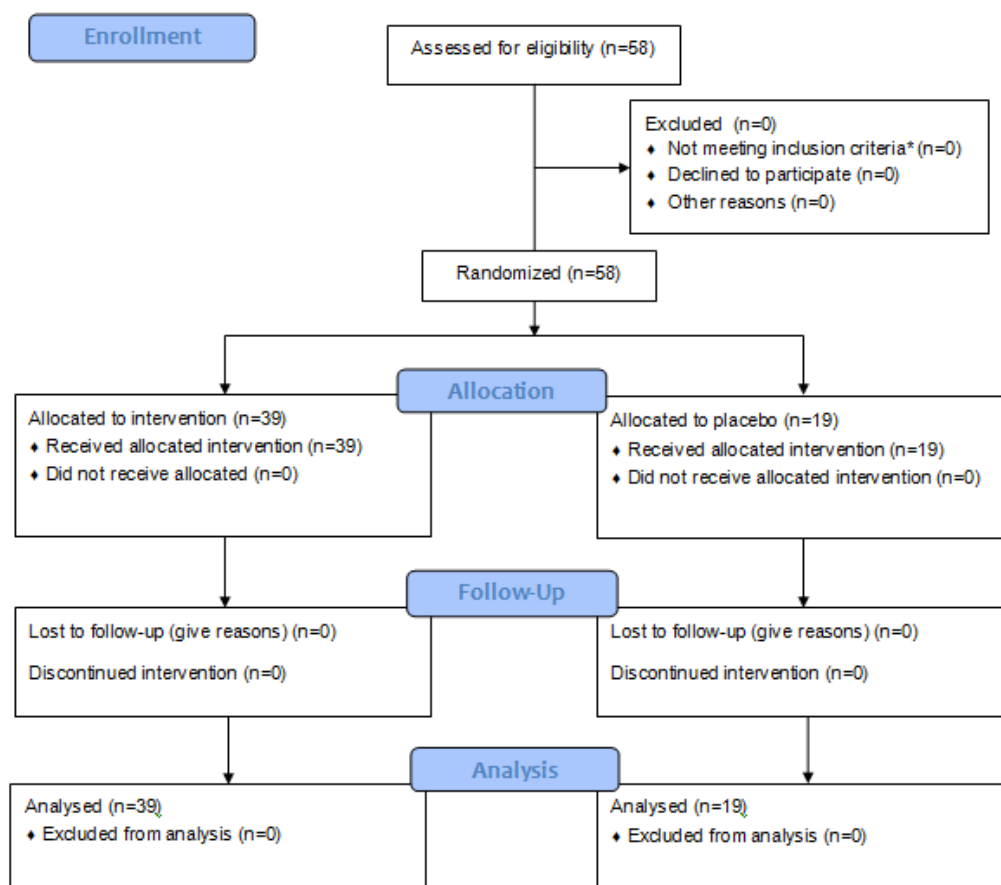


Figure S1. CONSORT diagram. *Subjects were excluded if eating disorders and illnesses as hyperlipidemia, hypertension, and diabetes or a history of ischemic heart disease, arrhythmia, previous strokes, or peripheral vascular disease and excessive alcohol consumption (> 60 g day of ethanol). Other exclusion criteria included the use of lipid-lowering drugs, β -blockers or diuretics, history of CVD, psychiatric illness or treatment of psychotropic drugs, being in a weight-loss program or had followed or were following a hypocaloric diet or consuming dietary supplements for weight or constipation control or for cholesterol control for at least 2 months earlier to study inclusion were excluded.

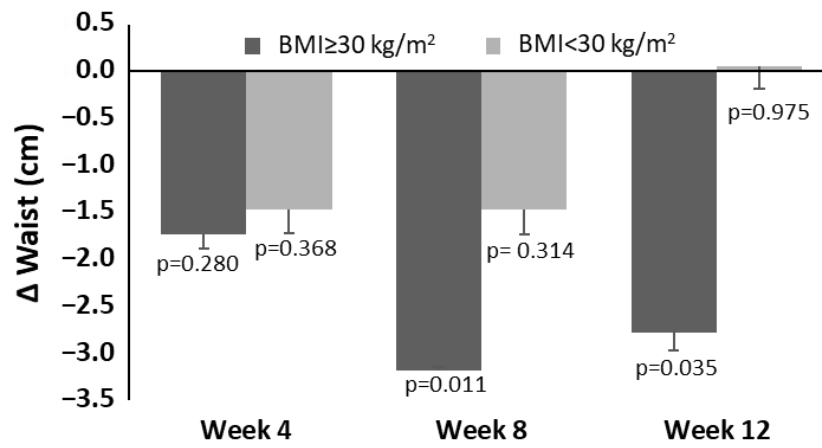


Figure S2. Effect of the β GluCnCs in waist circumference during intervention period. Bars represent changes (Δ) of waist circumference at week 4, 8 and 12 respect to baseline of subjects with BMI lower than 30 kg/m² and BMI equal or higher than 30 kg/m². p-Value: Paired samples T Test. Statistical significance: $p < 0.05$.

Table S1. Inclusion and exclusion criteria.

Inclusion criteria:

- Men and Women aged 25-60 years.
- BMI: 27-37 kg/m²

Exclusion criteria

- Eating disorders
 - History of ischaemic heart disease (and/or previous angina or AMI) or arrhythmia (current or previous)
 - Previous stroke and/or peripheral vascular disease
 - Alcohol consumption greater than 60 g/day
 - Renal insufficiency (creatinine > 2 mg/dL)
 - Presence of malignancy
 - Presence of systemic disease
 - Psychiatric disease in treatment with psychotropic drugs
 - Unestablished thyroid pathology
 - Having followed or being following a hypocaloric diet or regularly consumed dietary supplements for weight control or constipation (fiber supplements or psyllium seed extracts) or cholesterol control (plant sterols, soya lecithin, omega-3 fatty acids) since 2 months prior to inclusion in the study
 - Food allergies or sensitivities, especially to yeast.
 - Pregnancy or breastfeeding
 - Current treatment with non-steroidal anti-inflammatory drugs, antiplatelet drugs, fibrates or statins.
-

Table S2. Anthropometric, hemodynamic control and biochemical variables at baseline in β GluCnCs and placebo groups

	β GluCnCs	Placebo	p-Value
Age (years)	43 \pm 1.6	40 \pm 2.0	0.254
Sex (Women/men)	(18/21)	(12/7)	0.224*
<i><u>Anthropometric parameters</u></i>			
Weight (kg)	88.3 \pm 2.1	86.4 \pm 3.0	0.614
BMI (kg/m ²)	30.8 \pm 0.5	31.07 \pm 0.6	0.786
Waist (cm)	100.3 \pm 1.5	99.9 \pm 2.8	0.886
Hips (cm)	109.5 \pm 1.3	112.16 \pm 2.0	0.255
Waist/hips	0.92 \pm 0.01	0.89 \pm 0.02	0.329
<i><u>Hemodynamic control</u></i>			
SBP (mmHg)	123.8 \pm 1.7	119.1 \pm 2.4	0.118
DBP (mmHg)	70.1 \pm 1.3	65.6 \pm 2.0	0.065
Heart beat rate (bpm)	71.7 \pm 1.6	69.3 \pm 2.4	0.381
<i><u>Biochemical parameters</u></i>			
ALT (UI/L)	21.6 \pm 1.9	17.0 \pm 1.9	0.127
AST (UI/L)	19.7 \pm 1.0	15.5 \pm 0.5	0.006
GGT (UI/L)	22.7 \pm 2.5	23.9 \pm 3.7	0.792
Glucose (mmol/L)	4.8 \pm 0.1	4.6 \pm 0.1	0.130
Urate (μ mol/L)	320.5 \pm 13.8	280.4 \pm 14.6	0.078
Urea (mmol/L)	5.5 \pm 0.2	4.9 \pm 0.3	0.095
Creatinine (UI/L)	70.1 \pm 1.5	63.6 \pm 1.9	0.013
Total protein (g/L)	68.5 \pm 0.7	68.2 \pm 0.8	0.790

Values are expressed as mean \pm SEM. For numeric variables p-Value: Two-sample t-tests and; for categorical variables *p-Value: Chi-squared test. Statistical significance: p<0.05. β GluCnCs, beta-glucan/chitin-chitosan; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyltransferase.

Table S3. Anthropometric, hemodynamic control and biochemical variables during the 12-week intervention period in β GluCnCs and placebo groups

	Baseline	Week 4	Week 8	Week 12	p-Value
β GluCnCs					
<i>Anthropometric parameters</i>					
Weight (kg)	88.3 \pm 2.1	88.1 \pm 2.0	88.0 \pm 2.2	88.0 \pm 2.1	0.938
BMI (kg/m ²)	30.8 \pm 0.5	30.9 \pm 0.5	30.7 \pm 0.5	30.7 \pm 0.5	0.914
Waist (cm)	100.3 \pm 1.5	98.7 \pm 1.6	97.9 \pm 1.5	98.8 \pm 1.5	0.073
Hips (cm)	109.5 \pm 1.3	107.9 \pm 1.0	107.3 \pm 1.1	107.8 \pm 1.5	0.127
Waist / hips	0.92 \pm 0.01	0.91 \pm 0.01	0.91 \pm 0.01	0.92 \pm 0.01	0.952
<i>Hemodynamic control</i>					
SBP (mmHg)	123.8 \pm 1.7	122.7 \pm 2.0	121.33 \pm 1.7	120.7 \pm 1.6	0.059
DBP (mmHg)	70.1 \pm 1.3	69.6 \pm 1.6	69.67 \pm 1.2	68.4 \pm 1.3	0.512
Heart beat rate (bpm)	71.7 \pm 1.6	69.9 \pm 1.5	69.15 \pm 2.2	71.8 \pm 1.5	0.452
<i>Biochemical parameters</i>					
ALT (UI/L)	21.6 \pm 1.9	23.2 \pm 2.7	22.1 \pm 2.4	23.8 \pm 3.5	0.712
AST (UI/L)	19.7 \pm 1.0	20.7 \pm 1.3	20.4 \pm 1.3	21.3 \pm 1.4	0.428
GGT (UI/L)	22.8 \pm 2.5	28.2 \pm 6.2	25.9 \pm 3.8	34.8 \pm 11.5	0.211
Glucose (mmol/L)	4.8 \pm 0.1	4.9 \pm 0.1	4.8 \pm 0.1	4.8 \pm 0.1	0.320
Urate (μ mol/L)	320.5 \pm 13.8	327.8 \pm 13.0	319.1 \pm 13.7	333.3 \pm 13.6	0.262
Urea (mmol/L)	5.5 \pm 0.2	5.4 \pm 0.2	5.4 \pm 0.2	5.6 \pm 0.2	0.651
Creatinine (UI/L)	70.09 \pm 1.5	69.9 \pm 1.6	67.7 \pm 1.5	69.9 \pm 1.5	0.050
Total protein (g/L)	68.53 \pm 0.7	69.5 \pm 0.6	67.8 \pm 0.6	68.8 \pm 1.0	0.215
Placebo					
<i>Anthropometric parameters</i>					
Weight (kg)	86.4 \pm 3.0	86.2 \pm 3.1	86.7 \pm 3.1	86.1 \pm 3.1	0.889
BMI (kg/m ²)	31.1 \pm 0.6	31.0 \pm 0.7	31.2 \pm 0.8	31.0 \pm 0.7	0.858
Waist (cm)	99.9 \pm 2.8	99.2 \pm 2.7	99.4 \pm 2.9	99.8 \pm 2.1	0.983
Hips (cm)	112.1 \pm 2.0	107.7 \pm 2.5	108.3 \pm 2.1	110.37 \pm 2.1	0.046
Waist / hips	0.89 \pm 0.02	0.93 \pm 0.03	0.92 \pm 0.02	0.90 \pm 0.01	0.513
<i>Hemodynamic control</i>					
SBP (mmHg)	119.1 \pm 2.5	120.2 \pm 3.1	119.5 \pm 2.5	116.9 \pm 2.3	0.428
DBP (mmHg)	65.63 \pm 2.0	67.58 \pm 2.3	67.11 \pm 2.1	64.95 \pm 2.2	0.438
Heart beat rate (bpm)	69.3 \pm 2.4	70.3 \pm 2.3	72.1 \pm 2.7	66.3 \pm 2.1	0.073
<i>Biochemical parameters</i>					
ALT (UI/L)	17.0 \pm 1.9	16.4 \pm 1.6	15.7 \pm 1.2	18.4 \pm 2.7	0.480
AST (UI/L)	15.5 \pm 0.5	16.3 \pm 0.7	16.4 \pm 1.3	17.1 \pm 1.1	0.569
GGT (UI/L)	23.9 \pm 3.7	24.4 \pm 3.7	22.4 \pm 3.0	25.2 \pm 4.4	0.745
Glucose (mmol/L)	4.6 \pm 0.1	4.7 \pm 0.1	4.6 \pm 0.1	4.52 \pm 0.1	0.205
Urate (μ mol/L)	280.4 \pm 14.6	291.6 \pm 15.8	292.0 \pm 16.2	289.6 \pm 14.0	0.451
Urea (mmol/L)	4.9 \pm 0.3	5.2 \pm 0.3	5.1 \pm 0.3	5.2 \pm 0.3	0.563
Creatinine (UI/L)	63.6 \pm 1.9	65.4 \pm 2.3	63.3 \pm 1.9	65.2 \pm 2.1	0.135
Total protein (g/L)	68.2 \pm 0.8	69.5 \pm 0.8	67.7 \pm 0.8	68.6 \pm 0.8	0.072

Values are expressed as mean \pm SEM. p-Value: Repeated measures analysis of variance. Statistical significance: p<0.05.

Table S4. Concentration and diameter of circulating HDL lipoproteins analyzed by ¹H-NMR during the firsts 8 weeks of intervention in βGluCnCs and placebo groups.

	Baseline	Week 8	p-Value
βGluCnCs			
HDL particles (μmol/L)	30.0 ± 0.7	30.6 ± 0.8	0.254
HDL diameter (nm)	8.28 ± 0.01	8.27 ± 0.01	0.651
Placebo			
HDL particles (μmol/L)	27.6 ± 0.7	29.6 ± 1.2	0.036
HDL diameter (nm)	8.31 ± 0.02	8.30 ± 0.02	0.183

Values are expressed as mean ± SEM. p-Value: Paired Samples T Test. Statistical significance: p<0.05. HDL, high density lipoprotein particles; NMR, nuclear magnetic resonance.

Table S5. Lipoprotein functionality variables during the 12-week intervention period in β GluCnCs and placebo groups.

	Baseline	Week 12	p-Value
β GluCnCs			
CD max (nmol CD/mg protein of LDL)	356.2 \pm 6.7	353.0 \pm 6.9	0.671
Time to half maximum (min)	58.2 \pm 2.3	60.56 \pm 1.4	0.189
% of oxidation of LDL particles	23.2 \pm 1.3	19.9 \pm 1.1	0.671
Placebo			
CD max (nmol CD/mg prot LDL)	374.7 \pm 8.3	364.9 \pm 12.1	0.267
Time to half maximum (min)	56.4 \pm 2.0	58.1 \pm 3.14	0.458
% of oxidation of LDL particles	26.5 \pm 2.4	20.7 \pm 1.4	0.267

Values are expressed as mean \pm SEM. p-Value: Paired sample T-test. Statistical significance: $p < 0.05$. CDmax, maximum of conjugated dienes; LDL, low density lipoprotein.

Table S6. Lipid profile variables during the 12-week intervention period of β GluCnCs group stratified by sex. Values are expressed as mean \pm SEM. p-Value: Repeated measures analysis of variance. * indicates p-Value for comparisons

		Baseline	Week 4	Week 8	Week 12	p-Value
TC (mg/dL)	Women	196.5 \pm 7.0	197.8 \pm 6.5	199.5 \pm 7.5	205.6 \pm 8.3	0.202
	Men	190.3 \pm 6.9	189.1 \pm 7.9	189.2 \pm 6.8	195.5 \pm 6.7	0.367
HDLc (mg/dL)	Women	55.9 \pm 2.4*	60.3 \pm 2.7	60.6 \pm 2.81	63.1 \pm 3.1	0.000
	Men	48.3 \pm 2.1	49.2 \pm 2.0	51.2 \pm 2.3	50.3 \pm 2.1	0.285
Non-HDLc (mg/dL)	Women	140.6 \pm 7.0	137.6 \pm 6.4	138.8 \pm 7.3	142.5 \pm 8.3	0.574
	Men	142.0 \pm 7.0	139.9 \pm 8.0	138.0 \pm 6.6	145.3 \pm 7.0	0.219
HDLc/Non-HDLc	Women	0.42 \pm 0.03*	0.46 \pm 0.03	0.46 \pm 0.03	0.47 \pm 0.04	0.000
	Men	0.36 \pm 0.02	0.38 \pm 0.03	0.39 \pm 0.02	0.37 \pm 0.03	0.183
HDLc/TC	Women	0.29 \pm 0.01*	0.31 \pm 0.01	0.31 \pm 0.01	0.31 \pm 0.02	0.000
	Men	0.26 \pm 0.01	0.27 \pm 0.02	0.28 \pm 0.01	0.26 \pm 0.01	0.153
LDLc (mg/dL)	Women	124.5 \pm 5.6	121.8 \pm 5.4	123.0 \pm 6.5	127.6 \pm 7.4	0.450
	Men	120.9 \pm 6.6	113.4 \pm 6.5	114.5 \pm 6.1	121.2 \pm 5.3	0.082
VLDLc (mg/dL)	Women	16.1 \pm 1.8*	15.8 \pm 1.9	15.8 \pm 1.8	14.9 \pm 1.5	0.541
	Men	21.1 \pm 2.0	26.4 \pm 3.9	23.5 \pm 2.0	24.11 \pm 3.3	0.269
TG (mg/dL)	Women	81.6 \pm 9.0*	80.04 \pm 9.33	80.04 \pm 9.2	75.3 \pm 7.5	0.507
	Men	106.8 \pm 10.3	133.8 \pm 19.5	119.0 \pm 9.9	121.83 \pm 16.6	0.270

between men and women at baseline. Statistical significance: $p < 0.05$. TC, total cholesterol; HDLc, high density lipoprotein cholesterol; LDLc, low density lipoprotein cholesterol; VLDL, very low density lipoprotein cholesterol; TG, triglycerides.

Table S7. Changes in HDLc, ApoA1 and ApoB levels during the 12-week intervention period in β GluCnCs and placebo groups stratified by BMI and baseline LDLc levels.

	β GluQnQs	p-Value	Placebo	p-Value
Δ HDLc (mg/dL)				
BMI < 30 kg/m ²	4.2 \pm 1.2	0.003	3.5 \pm 2.1	0.152
BMI \geq 30 kg/m ²	3.1 \pm 1.6	0.073	1.9 \pm 1.2	0.155
Low-LDLc	5.6 \pm 1.2	0.000	2.3 \pm 1.2	0.078
High-LDLc	0.7 \pm 1.8	0.704	2.8 \pm 1.8	0.254
ApoA1/ApoB				
BMI < 30 kg/m ²	1.1 \pm 0.4	0.013	0.5 \pm 0.5	0.407
BMI \geq 30 kg/m ²	1.0 \pm 0.5	0.070	0.4 \pm 0.4	0.362
Low-LDLc	1.3 \pm 0.4	0.004	0.6 \pm 0.4	0.149
High-LDLc	0.6 \pm 0.5	0.298	-0.3 \pm 0.8	0.773

Values are expressed as mean \pm SEM. p-Value: One sample T-test. Statistical significance: p<0.05. Apo, Apolipoprotein. Low-LDLc, Baseline LDLc levels lower than 130 mg/dL; High-LDLc, Baseline LDLc levels equal or higher than 130 mg/dL.

Table S8. Lipid profile and lipoprotein functionality variables during the 12-week intervention period of β GluCnCs group stratified by baseline LDLc.

		Baseline	Week 4	Week 8	Week 12	p-Value
TC (mg/dL)	Low-LDLc	173.4 \pm 4.2*	181.8 \pm 6.8*	182.2 \pm 6.1*	187.5 \pm 6.4*	0.001
	High-LDLc	221.6 \pm 4.1	209.5 \pm 6.2	210.8 \pm 6.7	218.4 \pm 6.9	0.048
HDLc (mg/dL)	Low-LDLc	52.3 \pm 2.1	57.4 \pm 2.4*	57.6 \pm 2.5	58.7 \pm 2.5	0.000
	High-LDLc	51.0 \pm 2.8	49.9 \pm 2.7	52.6 \pm 3.0	52.6 \pm 3.6	0.378
Non-HDLc (mg/dL)	Low-LDLc	121.1 \pm 3.7*	124.4 \pm 6.2*	124.7 \pm 5.0*	128.8 \pm 5.9*	0.071
	High-LDLc	170.5 \pm 4.8	159.6 \pm 5.8	158.2 \pm 6.9	165.8 \pm 6.9	0.021
HDLc/Non-HDLc	Low-LDLc	0.44 \pm 0.02*	0.48 \pm 0.03*	0.47 \pm 0.02*	0.48 \pm 0.03*	0.009
	High-LDLc	0.31 \pm 0.02	0.32 \pm 0.02	0.34 \pm 0.03	0.33 \pm 0.03	0.058
HDLc/CT	Low-LDLc	0.30 \pm 0.01*	0.32 \pm 0.01*	0.32 \pm 0.01*	0.32 \pm 0.01*	0.014
	High-LDLc	0.23 \pm 0.01	0.24 \pm 0.01	0.25 \pm 0.01	0.24 \pm 0.02	0.067
LDLc (mg/dL)	Low-LDLc	104.3 \pm 3.3*	107.6 \pm 5.3*	106.8 \pm 4.4*	111.9 \pm 5.0*	0.058
	High-LDLc	148.8 \pm 4.0	131.2 \pm 5.8	135.1 \pm 6.9	141.7 \pm 5.8	0.002
VLDLc (mg/dL)	Low-LDLc	16.8 \pm 1.9	16.8 \pm 1.8*	17.8 \pm 1.8	16.9 \pm 1.9	0.853
	High-LDLc	21.7 \pm 2.0	28.3 \pm 4.8	23.1 \pm 2.3	24.1 \pm 3.9	0.165
TG (mg/dL)	Low-LDLc	84.9 \pm 9.5	85.0 \pm 9.1*	90.2 \pm 9.2	85.5 \pm 9.8	0.850
	High-LDLc	109.9 \pm 10.0	143.4 \pm 24.2	116.5 \pm 11.7	121.7 \pm 19.7	0.163
ApoA1 (mg/dL)	Low-LDLc	1.06 \pm 0.03			1.07 \pm 0.04	0.666
	High-LDLc	1.05 \pm 0.07			1.11 \pm 0.08	0.354
ApoB (mg/dL)	Low-LDLc	0.43 \pm 0.04			0.30 \pm 0.03	0.001
	High-LDLc	0.43 \pm 0.04			0.37 \pm 0.04	0.367
ApoA1/ApoB	Low-LDLc	2.78 \pm 0.27			4.05 \pm 0.41	0.004
	High-LDLc	2.60 \pm 0.26			3.15 \pm 0.33	0.298
CD max (nmol CD/mg protein of LDL)	Low-LDLc	345.9 \pm 7.4*			341.4 \pm 7.2*	0.626
	High-LDLc	376.9 \pm 10.5			376.4 \pm 11.1	0.968
Time to half maximum (min)	Low-LDLc	59.6 \pm 2.9			63.3 \pm 1.4*	0.122
	High-LDLc	55.3 \pm 3.4			55.1 \pm 2.1	0.930
% of oxidation of LDL particles	Low-LDLc	22.5 \pm 1.7			21.1 \pm 1.3	0.521
	High-LDLc	24.7 \pm 1.6			17.6 \pm 1.8	0.036

Values are expressed as mean \pm SEM. p-Value: Repeated measures analysis of variance. * indicates p-Value for comparisons between Low-LDLc and High-LDLc. Statistical significance: p<0.05.

Table S9. Insulin levels and HOMA-IR at baseline and the 12-week intervention period in β GluCnCs and placebo groups

	Baseline	Week 12	p-Value
β GluCnCs			
Insulin (μ U/mL)	21.19 \pm 1.48	22.41 \pm 2.57	0.457
HOMA-IR	4.45 \pm 0.29	4.83 \pm 0.54	0.353
Placebo			
Insulin (μ U/mL)	19.05 \pm 1.03	18.73 \pm 1.28	0.772
HOMA-IR	3.87 \pm 0.23	3.84 \pm 0.33	0.906

Values are expressed as mean \pm SEM. p-Value: Paired sample t-test. Statistical significance: $p < 0.05$. HOMA, homeostatic model assessment; IR, insulin resistance.