Cocoa-carob blend acute intake modifies miRNAs related to insulin sensitivity in type 2 diabetic subjects: a randomised controlled nutritional trial

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SUPPLEMENTARY MATERIAL

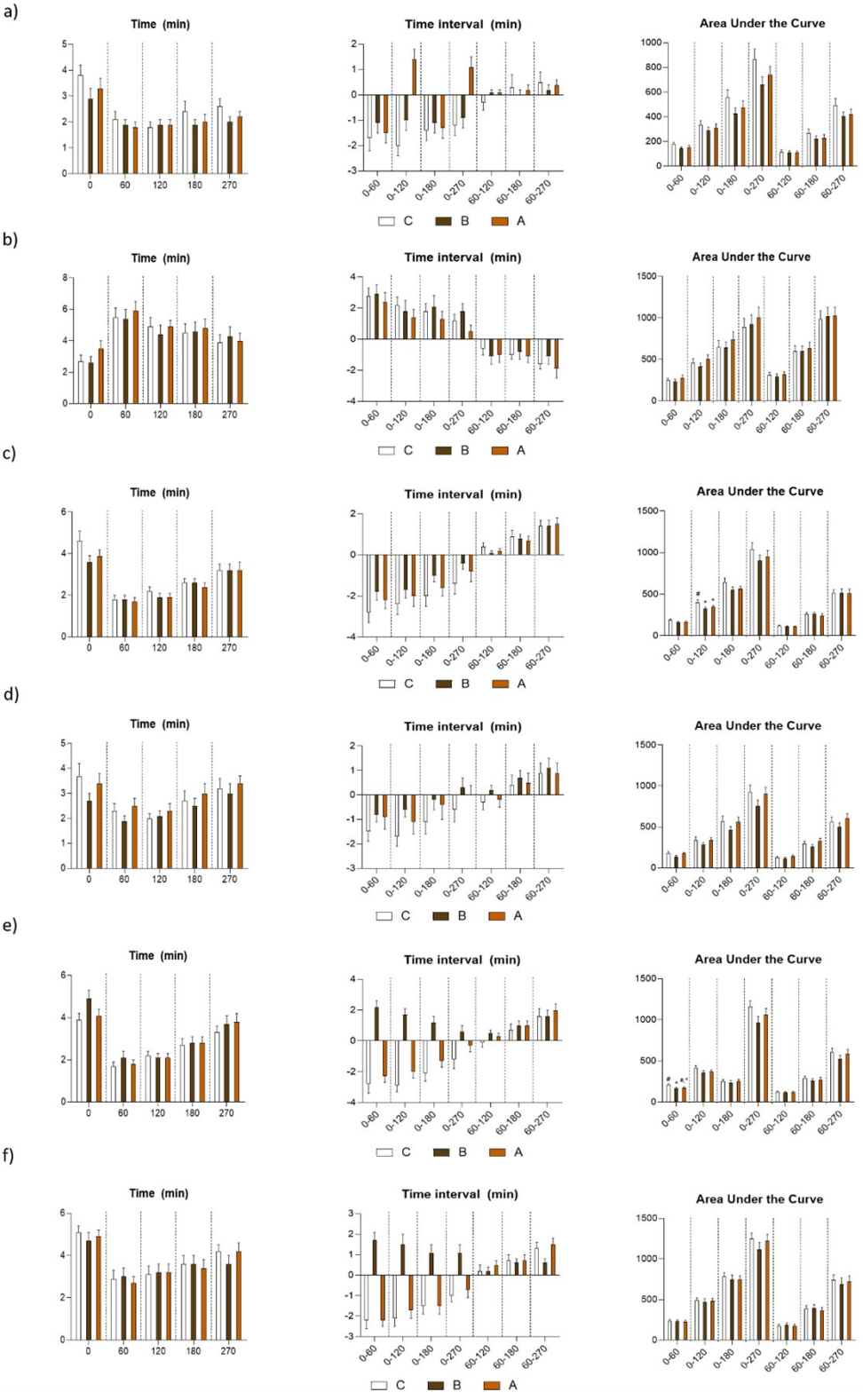


Fig. S1.- Satiety perception at fasting state and the associated Area Under the Curve (AUC) at all times in the clinical trial on the postprandial effect of a cocoa-carob blend (CCB) in the whole group (*n=20)*. Questions: a) How strong is your desire to eat something sweet? b) How full do you feel? c) How hungry do you feel? d) How strong is your desire to eat something salty? e) How strong is your desire to eat? f) How much food do you think you could eat? The three interventions were: A) hypercaloric breakfast accompanied by CCB (10g), B) hypercaloric breakfast having consumed it the night before, and C) hypercaloric breakfast (control). Data represents means ± SEM. Statistical differences marked with different symbols (# or *) were found for AUC values of questions **c)** and **e)** using Friedman non-parametric test with Wilcoxon post-hoc test, and one-way ANOVA with Tukey post-hoc test, respectively ($p<0.05$).

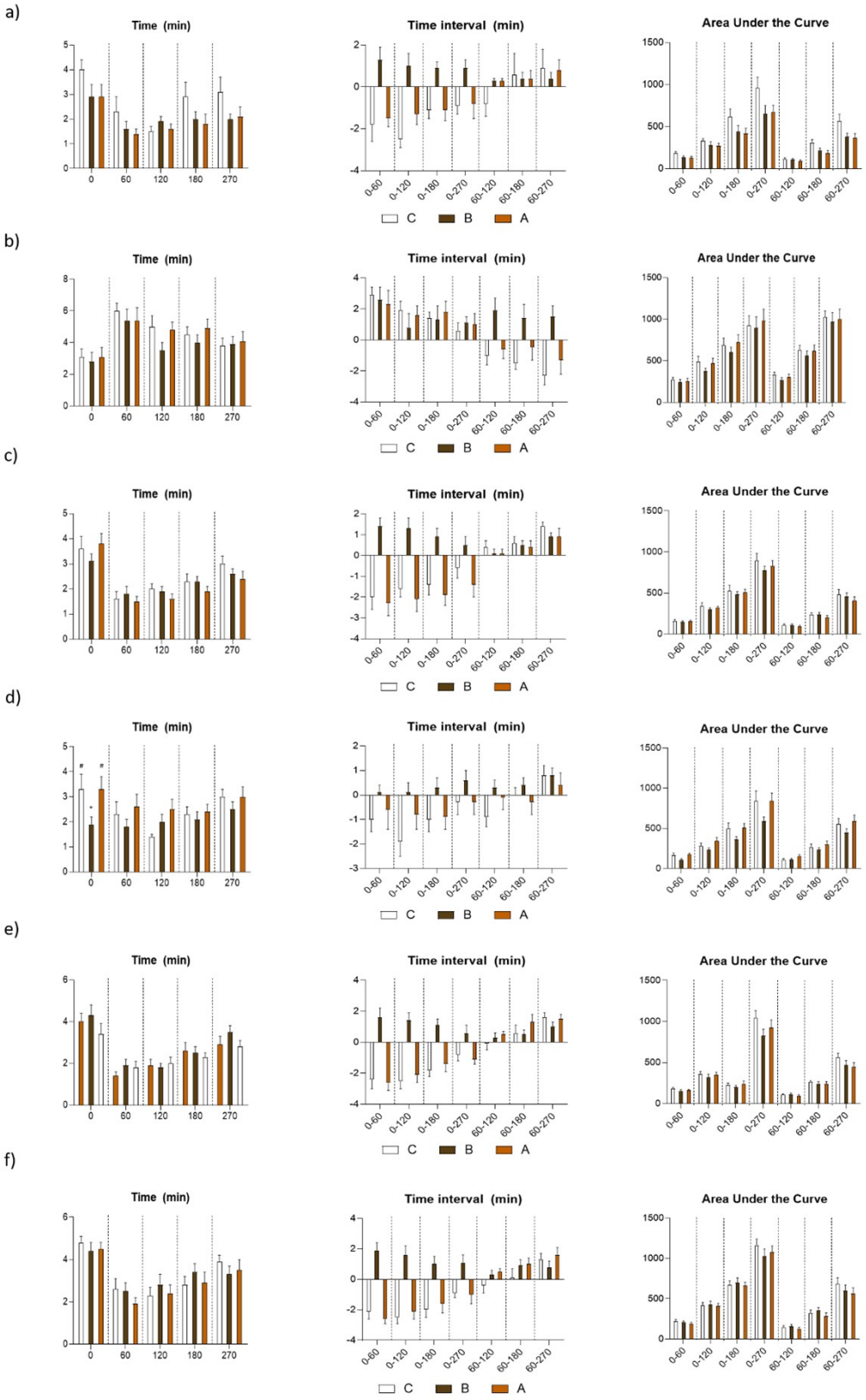


Fig. S2.- Satiety perception at fasting state and the associated Area Under the Curve (AUC) at all times in the clinical trial on the postprandial effect of a cocoa-carob blend (CCB) in those

subjects with overweight (Group Ovw, BMI≤30, group 0, *n=8*). Questions: a) How strong is your desire to eat something sweet? b) How full do you feel? c) How hungry do you feel? d) How strong is your desire to eat something salty? e) How strong is your desire to eat? f) How much food do you think you could eat? The three interventions were: A) hypercaloric breakfast accompanied by CCB (10g), B) hypercaloric breakfast having consumed it the night before, and C) hypercaloric breakfast (control). Data represents means ± SEM. No significant differences were found.

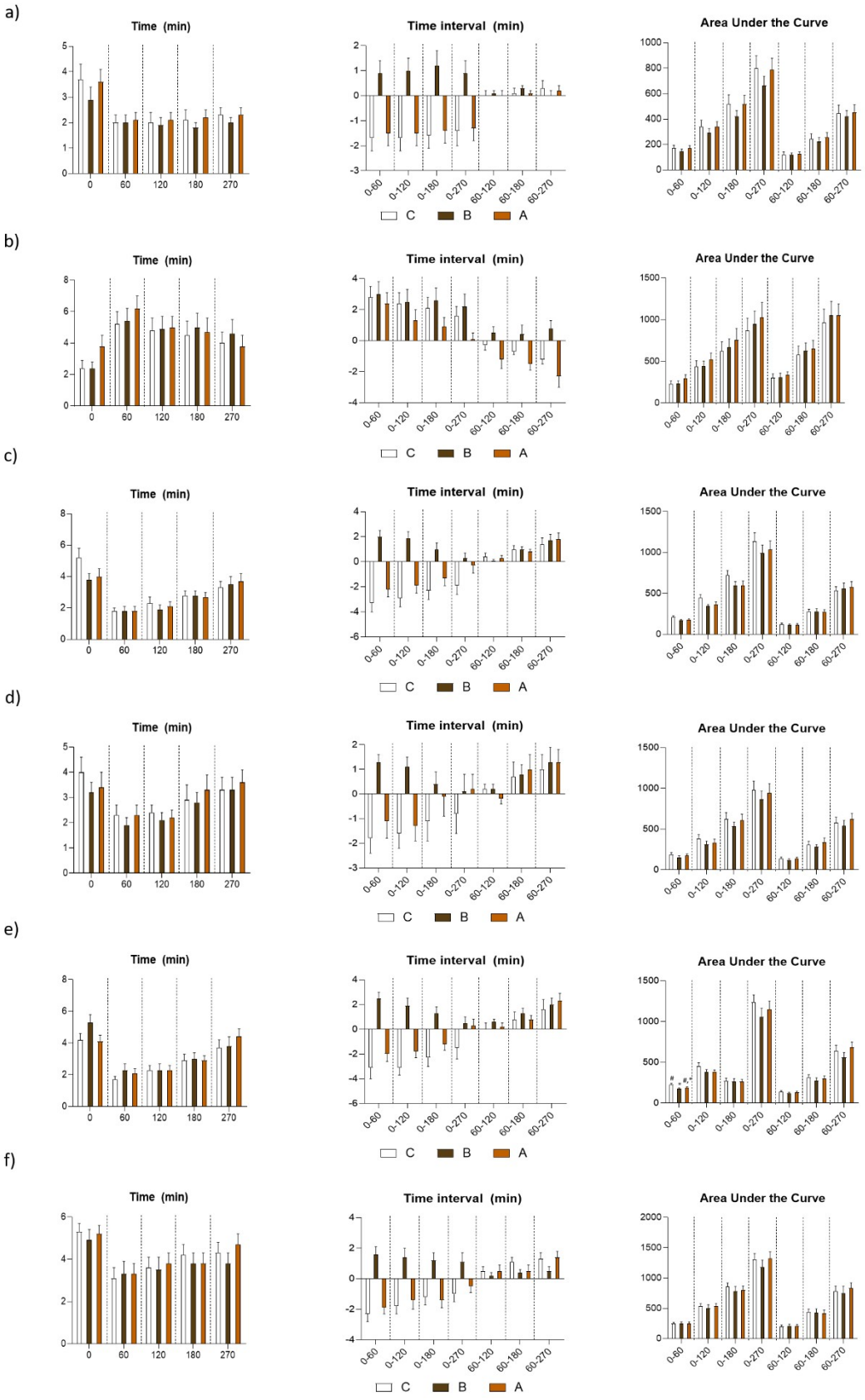
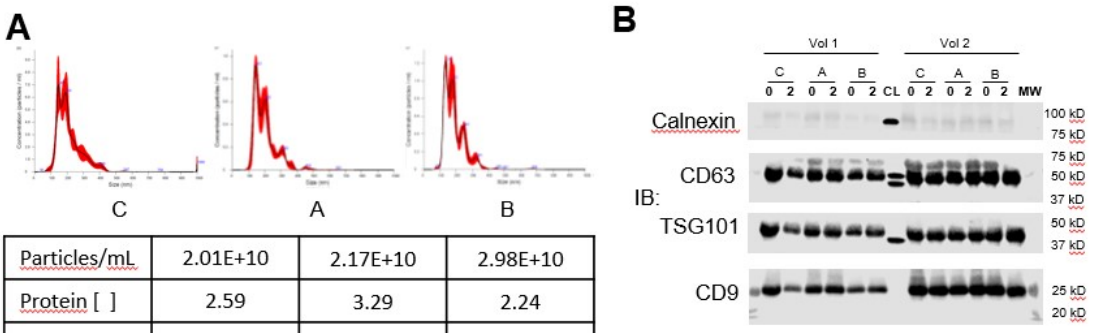


Fig. S3.- Satiety perception at fasting state and the associated Area Under the Curve (AUC) at all times in the clinical trial on the postprandial effect of a cocoa-carob blend (CCB) in those

subjects with obesity (Group Obe, BMI>30, group 1, *n=12*). Questions: a) How strong is your desire to eat something sweet? b) How full do you feel? c) How hungry do you feel? d) How strong is your desire to eat something salty? e) How strong is your desire to eat? f) How much food do you think you could eat? The three interventions were: A) hypercaloric breakfast accompanied by CCB (10g), B) hypercaloric breakfast having consumed it the night before, and C) hypercaloric breakfast (control). Statistical differences marked with different symbols (# or *) were found for AUC values of questions **e)** using one-way ANOVA with Tukey post-hoc test ($p<0.05$).



Particles/mL	2.01E+10	2.17E+10	2.98E+10
Protein [ ]	2.59	3.29	2.24
Mean	210.5 ± 6.1	200.9 ± 5.2	186.7 ± 3.2
Mode	155.6 ± 11.2	142.4 ± 1.8	158.9 ± 11.6
SD	76.6 ± 12.6	68.9 ± 2.8	62.1 ± 5.1

Fig. S4. Example of an isolated exosomes characterized by a Nanoparticle Tracking Analysis (NTA) from a volunteer after 2 hours of receiving each breakfast (interventions C, B, A). The three interventions were: A) hypercaloric breakfast accompanied by CCB (10g), B) hypercaloric breakfast having consumed it the night before, and C) hypercaloric breakfast (control). Figure S5A Determination of Extracellular vesicles (EVs) concentration, size distribution and protein content. Figure S5B Western blot of EV proteins. (CL: Cell lysate).

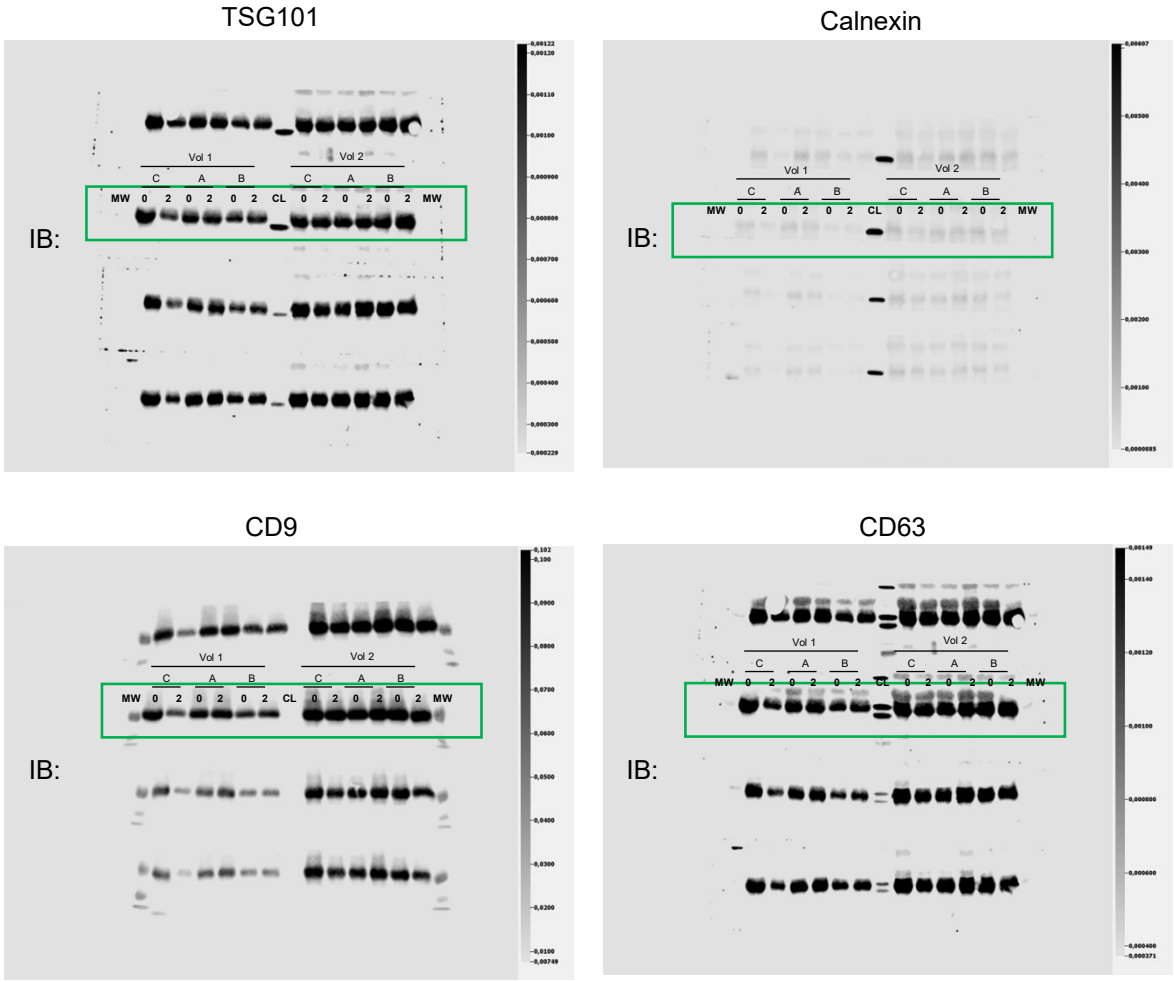


Fig. S5. Original Western blots from Supplementary Figure S4B. The green square represents the cropped band for each protein for the final figure. CL: cell lysate; MW: molecular weight marker; Vol1: volume 1; Vol2: volume 2; IB: immunoblot.

Table S1.- Hypercaloric breakfast (900 Kcal) provided to volunteers in the clinical trial on the postprandial effect of a cocoa-carob blend.

Food	***g or mL***	***Kcal***	***Fats (g)***	***SFA* (g)***	***Carbohydrates (g)***	***Sugars (g)***	***Proteins (g)***
Full-cream milk	250	157	9	6.3	11.5	11.5	7.5
Pineapple juice	200	106	0.19	0	23.63	18.8	1
Honey	20	66	0	0	20	19	0
Croissants (4 units)	140	560	33.6	16.8	56.4	14.4	8.4
Cocoa-carob blend	10	21	-	-	1.1	0.87	1.8

*SFA, saturated fatty acids

Table S2.- Glucagon-like peptide-1 (GLP-1) levels and the corresponding increases and area under the curve (AUC) of participants in the clinical trial on postprandial state effect of a cocoa-carob blend (CCB) in subjects with type 2 diabetes treated with metformin (cross-over design, *n=20*). Group Ovw: patients with overweight patients (BMI≤30, *n=8*). Group Obe: patients with obesity (BMI>30, *n=12*). Letters C, B and A refer to the three different interventions carried out: C) hypercaloric breakfast (control), B) hypercaloric breakfast with CCB intake (10g) the night before, and A) hypercaloric breakfast with CCB (10g). All data are expressed in mU/mL except the time values which are expressed in minutes (min). Data represent means ± SEM. No significant differences were found.

Parameter	**Complete group, *n=20***			**Group Ovw, *n=8***			**Group Obe, *n=12***		
	C	***B***	***A***	***C***	***B***	***A***	***C***	***B***	***A***
Time (min)									
0	32.6 ± 3.5	34.7 ± 4.9	37.3 ± 3.6	25.3 ± 3.9	28.4 ± 6.6	32.1 ± 5.8	37.5 ± 4.9	38.9 ± 6.9	40.7 ± 4.4
60	61.9 ± 5.4	62.2 ± 5.0	64.3 ± 5.9	56.6 ± 7.6	58.2 ± 7.7	59.1 ± 9.7	65.4 ± 7.4	64.9 ± 6.8	67.8 ± 7.5
120	46. ± 3.9	49.6 ± 3.7	49.0 ± 3.9	39.6 ± 3.8	50.6 ± 6.1	45.6 ± 3.3	51.4 ± 5.8	49.0 ± 4.9	51.3 ± 6.1
AUC (mg/dL/min)									
0-60	2,880 ± 246	2,973 ± 298	3,075 ± 264	2,495 ± 336	2,630 ± 384	2,754 ± 415	3,136 ± 334	3,201 ± 425	3,290 ± 342
0-120	4,862 ± 421	5,160 ± 477	5,279 ± 427	3,931 ± 432	4,789 ± 649	4,715 ± 531	5,482 ± 586	5,407 ± 680	5,654 ± 611
60-120	3,360 ± 297	3,416 ± 237	3,496 ± 289	2,913 ± 327	3,288 ± 387	3,191 ± 407	3,657 ± 434	3,501 ± 310	3,699 ± 402

Table S3.- Cytokine LPS-induced levels of participants in the clinical trial on postprandial state effect of a cocoa-carob blend (CCB) in subjects with type 2 diabetes treated with metformin (cross-over design). Letters C, B and A refer to the three different interventions carried out: C)

hypercaloric breakfast (control), B) hypercaloric breakfast with the CCB intake (10g) the night before, and A) hypercaloric breakfast with CCB (10g). All data are expressed in pg/mL except the time values which are expressed in minutes (min). Data represent means ± SEM. For TNF-*α* (*n=14*) and IL-6 (*n=14*), concentration values are divided by 10, and area under the curve (AUC) values are divided by 1,000.

Interventions									
	C	***B***	***A***	***C***	***B***	***A***	***C***	***B***	***A***
	TNF-*α*			**IL-6**			**IL-10**		
Time (min)									
0	119 ± 18[a]	100 ± 10[a]	118 ± 17[a]	1,447 ± 163[a]	1,226 ± 156[a]	1,392 ± 208[a]	13 ± 8[a]	13 ± 1[a]	22 ± 4[a]
120	114 ± 18[a]	99 ± 14[a]	125 ± 12[a]	1,112 ± 144[a]	1,083 ± 178[a]	1,086 ± 176[a]	10 ± 1[a]	10 ± 2[a]	15 ± 2[a]
240	146 ± 27[a]	1470± 24[a]	159 ± 24[a]	1,356 ± 185	1,308 ± 214[a]	1,230 ± 176[a]	12 ± 1[a]	11 ± 1[a]	22 ± 5[a]
Maximum value	158 ± 27	147 ± 22	162 ± 23	1,598 ± 176	1,549 ± 215	1,568 ± 204	13 ± 1	14 ± 1	28 ± 6
T *max* (min)	200 ± 20	164 ± 30	200 ± 24	78 ± 30	104 ± 33	78 ± 30	111 ± 35	71 ± 31	131 ± 35
AUC (pg/mL/min)									
0-120	143 ± 21[a]	122± 14[a]	148 ± 17[a]	1,571 ± 175[a]	1,421 ± 193[a]	1,511 ± 213[a]	1,433 ± 110[a]	1,400 ± 95[a]	2,248 ± 314[a]
0-240	322 ± 49[b]	292 ± 38[b]	334 ± 49[b]	3,398 ± 379[b]	3,071 ± 382[b]	3,170 ± 430[b]	2,995 ± 201[b]	2,878 ± 226[b]	5,284 ± 925[b]

T *max*: Time to reach the maximum concentration. [a,b] Different letters in the same column indicate significant differences across time (ANOVA with Tukey post-hoc test, $p<0.05$).