

## SUPPLEMENTARY MATERIAL

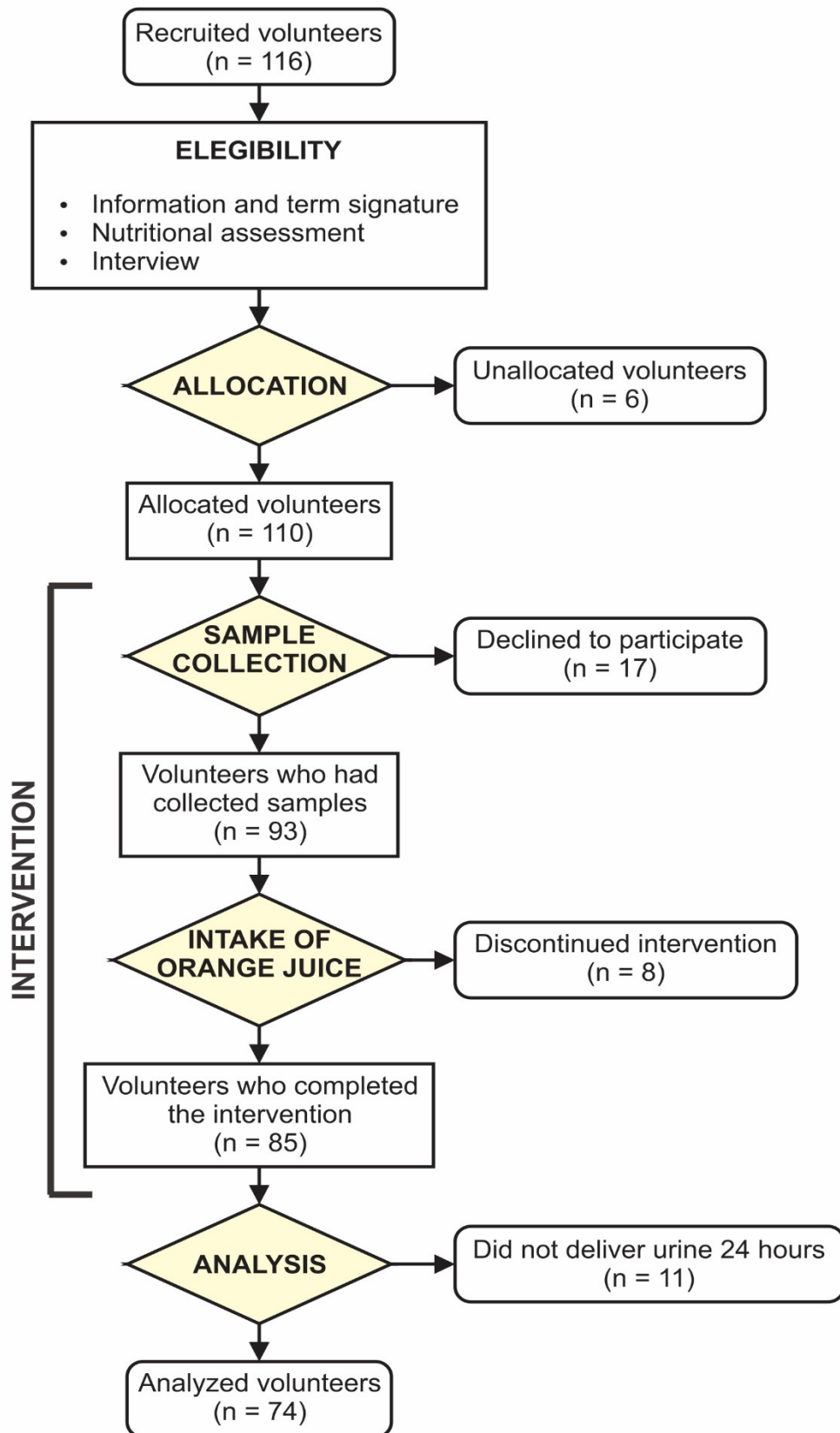
**Blood pressure and bod fat % reduction is mainly related to flavanone phase II conjugates and minor extension by phenolic acids after long term intake of orange juice**

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**Figure S1.** The flowchart of the clinical study.

**Table S1.** Energy and macronutrient consumption before (T0) and after 60 days (T60) of intervention with daily consumption of 500 mL of orange juice

Variable	T0	T60	<i>p</i> -value
Energy (kcal)	<b>1699.00 ± 744.65<sup>b</sup></b>	<b>1868.92 ± 854.47<sup>a</sup></b>	<b>0.0041</b>
Carbohydrates (g.100g <sup>-1</sup> )	<b>203.00 ± 11.27<sup>b</sup></b>	<b>251.66 ± 117.16<sup>a</sup></b>	<b>0.0001</b>
Proteins (g.100g <sup>-1</sup> )	92.00 ± 53.75	88.71 ± 57.72	0.8112
Lipids (g.100g <sup>-1</sup> )	55.00 ± 30.37	55.78 ± 32.17	0.5939
Dietary fiber (g.100g <sup>-1</sup> )	12.00 ± 7.10	12.38 ± 7.78	0.6662

Results expressed as mean ± standard deviation (n = 74). *P*-value calculated using Wilcoxon Test. Values in bold letters and different superscript letters indicate statistical significance ( $p < 0.05$ ) between T0 and T60.

**Table S2.** Mass spectra data of flavanone phase II conjugates in 24-hour urine by LC-qTOF-MS/MS after the consumption of single dose (500 mL) of orange juice

Phase II conjugates	Peak	RT (min)	[M-H] <sup>-</sup> (m/z)		MS/MS (m/z)
			Theor	Expl	
Hesperetin-diglucuronide isomer 1	1	8.5	653.1348	653.1382	477.1073/301.0762
Eriodictyol-glucuronyl-sulfate	2	10.5	543.0439	543.0425	463.874/ 367.0142/ 287.0542
Naringenin-glucuronyl-sulfate	3	10.5	527.0490	527.0431	351.1048/271.0734
Hesperetin-glucosyl-sulfate	4	11.0	543.0803	543.0820	381.0270/301.0714
Hesperetin diglucuronide isomer 2	5	11.2	653.1348	653.1381	477.1053/301.0740
Hesperetin glucuronyl-sulfate	6	11.5	557.0595	557.0592	477.1056/301.0723
Naringenin- glucuronide isomer 1	7	12.0	447.0921	447.0917	271.0616
Naringenin 7-glucuronide*	8	14.5	447.0921	447.0919	271.0602
Hesperetin glucuronide isomer	9	15.0	477.1027	477.1056	301.0714
Eriodictyol sulfate isomer	10	15.2	367.0118	367.0100	287.0542
Hesperetin 7-glucuronide*	11	15.9	477.1027	477.1073	301.0756
Hesperetin sulfate isomer1	12	16.0	381.0274	381.0280	301.0705
Hesperetin 3'-glucuronide*	13	16.5	477.1027	477.1034	301.0700
Eriodictyol sulfate isomer 2	14	16.6	367.0118	367.0110	287.0553
Hesperetin sulfate isomer2	15	17.5	381.0274	381.0278	301.0722
RT: Retention time.			*Compound identity	confirmed	with standard.

**Table S3.** Mass spectra of phenolic acids detected in 24-hour urine by LC-qTOF-MS/MS after the consumption of single dose (500 mL) of orange juice

Phenolic acids	Peak	RT (min)	[M-H] <sup>-</sup> (m/z)		MS/MS (m/z)
			theor	expl.	
Benzene-triol isomer 1	1	2.2	125.0233	125.0217	*
(Dihydroxyphenyl) propanoic	2	2.6	181.0495	181.0477	137.0202
Benzene-triol isomer 1	3	2.6	125.0233	125.0228	*
Methoxy hydroxymandelic acid Hydroxy (hydroxy-methoxyphenyl) acetic acid	4	2.8	197.0444	197.0439	137.0224
Dihydroxyphenylacetic acid isomer 1	5	3.3	167.0338	167.0359	*
Hydroxyphenylacetic acid-sulfate isomer 1	6	3.4	230.9957	230.9959	151.0386
Hydroxybenzoic acid-sulfate isomer 1	7	3.5	232.9750	232.9760	153.0187
Hydroxybenzoic acid-sulfate isomer 2	8	4.1	232.9750	232.9754	*
3,4-Dihydroxybenzoic acid	9	4.1	153.0182	153.0189	**
(Hydroxyphenyl)propanoic acid-sulfate isomer 1	10	4.1	261.0063	261.0062	181.0483
Hydroxybenzoic acid	11	4.1	137.0233	137.0236	*
(Hydroxyphenyl) propanoic acid-glucuronide isomer 1	12	4.2	357.0816	357.0812	181.049
Benzoic acid-sulfate	13	4.2	216.9801	216.9808	137.0235
Benzene-diol	14	4.4	109.0284	109.0281	*
Hydroxyhippuric acid isomer 1	15	4.4	194.0447	194.0452	*
Hydroxyphenylacetic acid-glucuronide isomer 1	16	4.5	327.0710	27.0709	151.0403
Dihydroxyphenylacetic acid isomer 2	17	4.6	167.0338	167.0334	*
3-Hydroxy (hydroxy-methoxyphenyl) propanoic acid isomer 1	18	4.7	211.0600	211.0601	*
-Methoxycinnamic acid-glucuronide isomer 1	19	4.8	369.0816	369.0812	193.0495
(Hydroxyphenyl) propanoic acid-sulfate isomer 2	20	4.9	261.0063	261.0050	181.0456
Hydroxyphenylacetic acid-sulfate isomer 2	21	5.0	230.9957	230.9965	151.0387
4'-hydroxyphenylacetic acid	22	5.0	151.0389	151.0365	**
Caffeic acid-glucuronide	23	5.0	355.0660	355.0649	179.0341
Hydroxyhippuric acid isomer 2	24	5.0	194.0447	194.0440	*
(Hydroxyphenyl) propanoic acid-glucuronide isomer 2	25	5.3	357.0816	357.0812	*
Hippuric acid-glucuronide	26	5.3	370.0757	370.0746	194.0462
(Hydroxyphenyl) propanoic acid-sulfate isomer 3	27	5.4	261.0063	261.0047	181.047
Hydroxy(hydroxyphenyl)propanoic acid	28	5.5	181.0495	181.0496	119.0492
Hydroxyphenylacetic acid-sulfate isomer 3	29	5.5	230.9957	230.9961	151.0391
Caffeic acid sulfate isomer 1	30	5.8	258.9907	258.9917	179.035
Methoxycinnamic acid-glucuronide isomer 2	31	5.8	369.0816	369.0817	193.0477
Hydroxy (hydroxy-methoxyphenyl) propanoic acid isomer 2	32	5.8	211.0600	211.0602	*

**Table S3. (Cont)**

Phenolic acids	Peak	RT (min)	[M-H] <sup>-</sup> (m/z)		MS/MS (m/z)
			theor	expl.	
(Hydroxyphenyl) propanoic acid-glucuronide isomer 3	33	6.0	357.0816	357.0820	181.0457
Hydroxyphenylacetic acid-sulfate isomer 4	34	6.0	230.9957	230.9963	151.0379
(Hydroxyphenyl) propanoic acid-sulfate isomer 4	35	6.1	261.0063	261.0052	181.047
Caffeic acid-sulfate isomer 2	36	6.2	258.9907	258.9911	*
Hydroxyphenylacetic acid-glucuronide isomer 2	37	6.2	327.0710	327.0742	151.0401
(Hydroxyphenyl) propanoic acid-glucuronide isomer 4	38	6.4	357.0816	357.0819	181.0497
(Methoxyphenyl)propanoic acid-sulfate isomer 1	39	6.5	275.0220	275.0211	195.0648
Coumaric acid-sulfate isomer 1	40	6.7	242.9958	242.9983	163.0358
Hydroxycinnamic acid	41	6.7	163.0390	163.0394	*
Hippuric acid	42	6.7	178.0498	178.0495	77.0387**
(Methoxyphenyl)propanoic acid-glucuronide isomer 1	43	6.8	371.0973	371.0967	195.0688
Caffeic acid-sulfate isomer 3	44	6.8	258.9907	258.9935	179.0359
Hydroxy-methoxycinnamic acid isomer 1	45	7.0	193.0495	193.0499	134.037
(Hydroxyphenyl) propanoic acid-glucuronide isomer 5	46	7.0	357.0816	357.0834	181.0512
-Methoxycinnamic acid-sulfate 1	47	7.1	273.0063	273.0063	193.0497
(Hydroxyphenyl)propanoic acid isomer 1	48	7.1	165.0546	165.0534	*
(Phenyl)propionic acid sulfate isomer 1	49	7.1	245.0114	245.0130	165.0562
Hydroxyphenylacetic acid-glucuronide isomer 3	50	7.2	327.0710	327.0742	151.041
Hydroxyphenylacetic acid-sulfate isomer 5	51	7.2	230.9957	230.9984	151.0395
(Phenyl)propanoic acid-glucuronide isomer 1	52	7.3	341.0867	341.0866	165.0561
(Methoxyphenyl)propanoic acid-sulfate isomer 2	53	7.4	275.0220	275.0218	195.0661
Methoxycinnamic acid-glucuronide isomer 3	54	7.5	369.0816	369.0838	193.0482
Coumaric acid sulfate isomer 2	55	7.6	242.9958	242.9963	163.0397
(Hydroxyphenyl) propanoic acid-glucuronide isomer 6	56	7.7	357.0816	357.0841	181.0509
3'-hydroxyphenylacetic acid	57	7.7	151.0389	151.0392	**
Methoxycinnamic acid-glucuronide isomer 4	58	7.8	369.0816	369.0816	193.0492
-Methoxycinnamic acid-sulfate 2	59	7.9	273.0063	273.0078	193.0486
(Methoxyphenyl)propanoic acid-glucuronide isomer 2	60	7.9	371.0973	371.0967	195.0645
(Phenyl)propanoic acid-glucuronide isomer 2	61	8.0	341.0867	341.0869	165.0544
(Methoxyphenyl)propanoic acid-sulfate isomer 3	62	8.1	275.0220	275.0233	195.00684
(Methoxyphenyl)propanoic acid-glucuronide isomer 3	63	8.8	371.0973	371.0962	195.0644

**Table S3. (Cont)**

Phenolic acids	Peak	RT (min)	[M-H] <sup>-</sup> (m/z)		MS/MS (m/z)
			theor	expl.	
(Hydroxyphenyl)propanoic acid isomer 2	64	8.9	165.0546	165.0549	*
(Phenyl)propionic acid sulfate isomer 2	65	9.0	245.0114	245.0111	165.055
-Methoxycinnamic acid-sulfate 3	66	9.2	273.0063	273.0075	193.0494
Coumaric acid-glucuronide isomer 1	67	9.5	339.0710	339.0725	163.0368
(Phenyl)propanoic acid-glucuronide isomer 3	68	9.5	341.0867	341.0876	165.0599
Hydroxyhippuric acid isomer 3	69	9.5	194.0447	194.0447	*
(Methoxyphenyl)propanoic acid-glucuronide isomer 4	70	9.8	371.0973	371.0993	195.0674
Hydroxy (hydroxy-methoxyphenyl) propanoic acid isomer 3	71	11.3	211.0600	211.0601	*
3-(4'-hydroxyphenyl)propanoic acid	72	10.0	165.0546	165.0561	**
Phenylpropanoic acid	73	10.6	149.0597	149.0594	*
Methoxycinnamic acid-glucuronide isomer 5	74	11.5	369.0816	369.0812	193.0499
(Hydroxyphenyl)propanoic acid isomer 3	75	11.5	165.0546	165.0530	*
Coumaric acid-glucuronide isomer 2	76	11.7	339.0710	339.0731	163.0408
(Methoxyphenyl)propanoic acid-sulfate isomer 4	77	12.0	275.0220	275.0237	195.0597
Hydroxy-methoxycinnamic acid isomer 2	78	15.4	193.0495	193.0496	*

RT: Retention time. \*No fragmentation. \*\*Compound identity confirmed with standard.

**Table S4.** Ratio between total excretion of phenolic acids and phase II conjugates (Acids/Phase II)

Low ratio		Intermediate ratio		High ratio	
Volunteer ID	ratio	Volunteer ID	ratio	Volunteer ID	ratio
81	0.04	82	3.08	98	9.96
55	0.06	11	3.22	47	9.70
66	0.14	107	3.23	19	10.23
31	0.25	38	3.48	76	10.25
52	0.62	41	3.49	16	10.38
78	0.71	113	3.98	69	10.75
50	1.24	22	4.00	62	11.13
27	1.26	110	4.22	97	13.00
53	1.27	64	4.38	95	13.15
14	1.45	106	4.41	54	13.72
61	1.46	23	4.82	75	17.27
79	1.54	17	4.83	88	17.86
51	1.55	40	4.85	85	21.06
1	1.70	37	4.85	80	21.37
13	1.72	116	5.02	91	21.54
111	1.73	90	5.09	39	25.36
72	1.96	102	5.18	96	29.05
15	2.17	73	5.36	109	32.01
63	2.56	46	5.42	93	36.08
3	2.70	5	5.83	84	90.41



83	2.73	36	5.88
28	2.83	21	5.89
114	2.83	105	6.04
		65	6.15
		20	6.65
		86	7.08
		57	7.17
		30	7.29
		18	7.97
		99	8.11
		10	8.71

Total N = 74 subjects. **Table S5.** Anthropometric variables and biochemical parameters of the volunteers, according to sex, before (T0) and after 30 (T30) and 60 days (T60) of orange juice consumption

	Men				Women			
	T0	T30	T60	<i>p-value</i>	T0	T30	T60	<i>p-value</i>
Weight (kg)	73.04 ± 11.10	72.83 ± 7.69	73.42 ± 11.15	0.1753	61.12 ± 7.81	65.36 ± 5.85	61.37 ± 7.61	0.2021
Body fat (%)	20.15 ± 5.60	21.41 ± 4.20	19.44 ± 5.51	0.2339	31.98 ± 5.94	29.29 ± 9.58	31.21 ± 6.41	0.0500
SBP (mmHg)	122.45 ± 9.13	120.20 ± 11.71	120.60 ± 9.87	0.5359	111.93 ± 11.20	111.00 ± 10.26	108.60 ± 10.88	0.0613
DBP (mmHg)	70.07 ± 7.09	69.31 ± 5.51	68.56 ± 7.41	0.1744	67.95 ± 6.87	67.80 ± 7.02	66.03 ± 6.40	0.1740
Glucose (mg.dL <sup>-1</sup> )	92.07 ± 5.31	91.22 ± 4.29	92.75 ± 5.93	0.4608	89.39 ± 6.36	91.43 ± 6.40	91.01 ± 5.09	0.0500
Insulin (mU.L <sup>-1</sup> )	10.96 ± 3.92	*	12.44 ± 5.50	0.0549	10.49 ± 3.57	*	11.48 ± 4.03	0.0527
HOMA-IR	2.48 ± 0.88	*	2.68 ± 0.90	0.0689	2.31 ± 0.79	*	2.41 ± 0.48	0.0566
Cholesterol (mg.dL <sup>-1</sup> )								
LDL	90.60 ± 31.86	90.65 ± 26.27	90.11 ± 31.64	0.5857	83.61 ± 25.28	83.48 ± 21.25	80.00 ± 22.15	0.1077
HDL	<b>52.21 ± 11.33<sup>a</sup></b>	<b>51.71 ± 7.09<sup>ab</sup></b>	<b>48.81 ± 12.24<sup>b</sup></b>	<b>&lt;0.0001</b>	<b>67.02 ± 15.61<sup>a</sup></b>	<b>64.76 ± 17.62<sup>ab</sup></b>	<b>60.63 ± 15.35<sup>b</sup></b>	<b>0.0106</b>

Total	160.56 ± 32.08	159.52 ± 30.02	157.90 ± 33.19	0.2206	167.79 ± 30.15	165.20 ± 21.82	165.30 ± 27.16	0.3188
Triglycerides (mg.dL <sup>-1</sup> )	89.87 ± 46.21	87.33 ± 36.82	87.97 ± 41.99	0.8920	86.13 ± 38.67	85.95 ± 19.75	78.42 ± 39.35	0.2916
AST (U.L <sup>-1</sup> )	22.59 ± 11.18	19.13 ± 8.41	19.74 ± 7.30	0.0897	15.93 ± 5.29	15.77 ± 3.69	15.34 ± 4.71	0.1122
ALT (U.L <sup>-1</sup> )	23.62 ± 13.27	19.39 ± 7.19	19.61 ± 8.33	0.1182	13.74 ± 6.92	13.56 ± 4.30	13.07 ± 7.01	0.0548
Creatinine (mg.dL <sup>-1</sup> )	0.94 ± 0.10	0.96 ± 0.10	0.95 ± 0.12	0.8190	0.73 ± 0.09	0.73 ± 0.08	0.76 ± 0.11	0.0569

AST: Aspartate transaminase. ALT: Alanine transaminase. LDL: Low-density lipoprotein. HDL: High-density lipoprotein. HOMA-IR: Homeostatic model assessment-Insulin resistance. SBP: Systolic Blood Pressure. DBP: Diastolic Blood Pressure. *P*-value calculated using Friedman Test. Values in bold letters and different superscript letters indicate statistical significance ( $p < 0.05$ ) among T0, T30, and T60 to each sex (mean ± standard deviation). \*Insulin was not evaluated at T30.

**Table S6.** Anthropometric variables and biochemical parameters of the volunteers, according to BMI classification, before (T0) and after 30 (T30) and 60 days (T60) of orange juice consumption

	Eutrophic				Overweight			
	T0	T30	T60	<i>p-value</i>	T0	T30	T60	<i>p-value</i>
Weight (kg)	63.07 ± 8.25	65.09 ± 8.29	63.42 ± 8.63	0.2941	75.41 ± 10.35	70.66 ± 7.58	75.31 ± 9.44	0.9693
Body fat (%)	24.82 ± 8.32	24.56 ± 6.63	24.09 ± 7.79	0.6060	29.67 ± 8.40	26.77 ± 7.01	29.00 ± 9.01	0.3831
SBP (mmHg)	115.98 ± 11.93	112.80 ± 15.58	112.60 ± 12.62	0.0500	119.90 ± 9.00	117.01 ± 15.11	117.00 ± 11.00	0.5674
DBP (mmHg)	68.58 ± 7.28	68.42 ± 7.45	66.42 ± 6.41	0.1022	69.95 ± 5.71	67.66 ± 6.47	68.86 ± 8.23	0.2405
Glucose (mg.dL <sup>-1</sup> )	89.90 ± 5.88	90.96 ± 4.68	91.53 ± 5.61	0.8028	91.59 ± 5.96	90.88 ± 6.56	93.76 ± 6.41	0.0652
Insulin (mU.L <sup>-1</sup> )	10.53 ± 3.91	*	11.27 ± 3.37	0.4330	11.12 ± 3.17	*	11.78 ± 3.76	0.4120
HOMA-IR	2.33 ± 0.85	*	2.65 ± 1.09	0.0650	2.51 ± 0.75	*	2.86 ± 1.15	0.1799
Cholesterol (mg.dL <sup>-1</sup> )								
LDL	84.92 ± 28.82	87.20 ± 28.80	88.15 ± 26.14	0.1922	96.68 ± 30.32	93.91 ± 10.29	93.05 ± 29.43	0.7014
HDL	<b>61.65 ± 15.92<sup>a</sup></b>	<b>58.28 ± 20.86<sup>ab</sup></b>	<b>55.42 ± 15.52<sup>b</sup></b>	<b>&lt;0.0001</b>	54.81 ± 13.33	53.50 ± 16.25	53.52 ± 14.09	0.0518
Total	161.87 ± 31.15	160.40 ± 26.73	159.50 ± 30.89	0.1583	171.27 ± 30.71	169.06 ± 24.69	168.00 ± 28.20	0.5396
Triglycerides (mg.dL <sup>-1</sup> )	83.95 ± 39.46	83.25 ± 37.91	79.60 ± 39.52	0.7303	99.36 ± 48.47	95.56 ± 33.38	91.81 ± 43.16	0.5562
AST (U.L <sup>-1</sup> )	17.54 ± 5.75	17.03 ± 4.40	17.45 ± 6.69	0.3612	19.80 ± 9.20	17.05 ± 5.67	16.30 ± 4.04	0.0595
ALT (U.L <sup>-1</sup> )	16.86 ± 9.90	16.09 ± 7.48	16.15 ± 9.35	0.0683	22.30 ± 11.67	18.00 ± 6.49	17.56 ± 8.13	0.0591
Creatinine (mg.dL <sup>-1</sup> )	0.83 ± 0.14	0.84 ± 0.12	0.85 ± 0.15	0.2739	0.86 ± 0.15	0.87 ± 0.14	0.87 ± 0.14	0.2613

AST: Aspartate transaminase. ALT: Alanine transaminase. LDL: Low-density lipoprotein. HDL: High-density lipoprotein. HOMA-IR: Homeostatic model assessment-Insulin resistance. SBP: Systolic Blood Pressure. DBP: Diastolic Blood Pressure. *P*-value calculated using Friedman Test. Values in bold letters and different superscript letters indicate statistical significance ( $p < 0.05$ ) among T0, T30, and T60 to each BMI class (mean ± standard deviation). \*Insulin was not evaluated at T30.