Effect of Capsicum and Cinnamon on Cellular Metabolism and Immunoregulation-An *In vitro* Study with Targeted Metabolomics

Ahsan Hameed[1, 2], Edyta Adamska-Patruno[1], Michal Ciborowski[1] , Coral Barbas[2]
[1]Clinical Research Center, Medical University of Bialystok, Poland
[2]The Centre of Metabolomics and Bioanalysis (CEMBIO), CEU-San Pablo University, Spain
[3]Klinika Endokrynologii and Diabetologii, Medical University of Bialystok, Poland

Abstract

Background: Numerous clinical and in vivo studies revealed the antidiabetic and anti-obesity properties of cinnamon and capsicum. However, the antidiabetic/anti-obesity effects of individual said spices at cellular levels are largely unknown and yet to be investigated
Objective: This work specifically aimed to disclose the effects of capsicum and cinnamon polyphenols on the cellular metabolism and immunoregulation of overweight/obese (OW/OB) human peripheral blood mononuclear cells (PBMC) using the targeted metabolomics.
Methodology: Blood was drawn from the OW/OB participants (n = 3 + 3 = 6) to isolate PBMC to be incubated with designated concentration (15 – 4500 µg/mL) of cinnamon and capsicum. Cell culture without the extracts were used as controls. Following the designated time of incubation (48h), the supernatants were isolated, samples were prepared and used for targeted metabolomics using Liquid Chromatography – Triple Quadrupole Mass Spectrometer (LC/QqQ-MS). Data was analyzed using vendor-based software and significantly altered metabolites were selected using multivariate (VIP >1 and *p*-value +/- 0.5) and univariate statistical analysis (corrected *p* <0.05).
Results: Cinnamon and capsicum significantly altered the cellular metabolism as revealed from targeted metabolomics of supernatant from control-to-treated cell cultures. In total 25 significantly varied metabolites were observed in comparison from control-to-treated cell cultures. Most of these significantly varied metabolites are short-chain organic acids, degradation production of amino acids, and intermediate products of glycolysis and TCA cycle. Pathway enrichment analysis showed that capsicum and cinnamon treatments significantly affected nearly 30 pathways in these cell cultures among which citrate cycle (TCA cycle), glyoxylate and dicarboxylate metabolism, pyruvate metabolism, phenylalanine, tyrosine and tryptophan biosynthesis, alanine, aspartate and glutamate metabolism were the most affect pathways.
Conclusion: This study investigated the effects of cinnamon and capsicum on cellular metabolism of PBMC by employing the targeted cell-culture profiling approach. The spices extract greatly affects the metabolic pathways related to glucose and protein degradation.

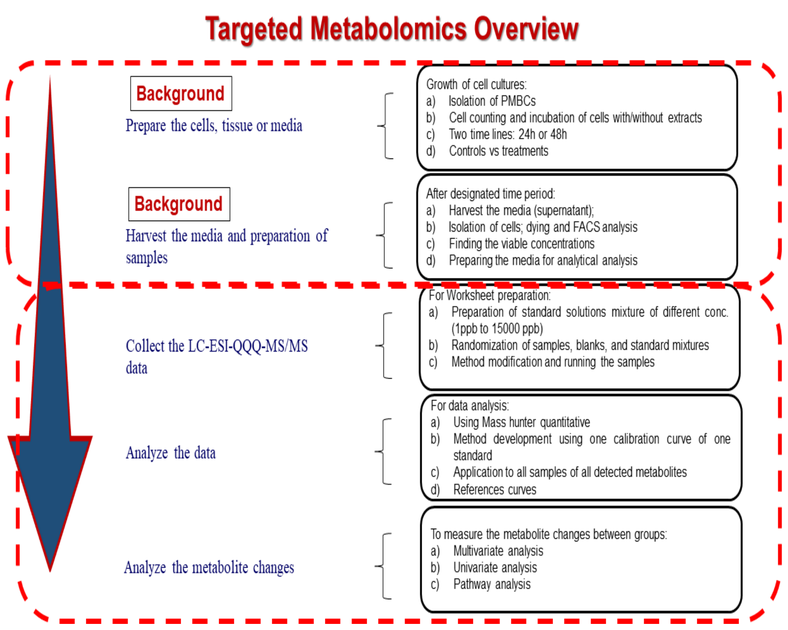
Background

Cinnamon is the main derivative of yellow colorant cinnamon oil, 98% of which composed of a hypoglycemic and hypolipidemic compound called cinnamaldehyde (Bernardo et al., 2015; Zhou et al., 2015; Zhu et al., 2017). This compound is amongst the most studied bioactive molecule in literature with more than 13 diabetic/obese animal models studies with doses range from 20 to 60 mg per body weight (in kg). In mentioned studies, it significantly lowered the level of gluco-lipids, triglycerides, low-density lipoprotein-cholesterol (LDL) and increased insulin sensitivity, blood insulin availability, and high-density lipoprotein-cholesterol (HDL) concentration. Capsicum, also called chili pepper, is consumed routinely as hot spice and mainly composed of capsaicin, capsiate and other analogous bioactive agents such as capsinoids, dihydrocapsiate, and nordihydrocapsiate. With some exceptions, these compounds possess anti-obesity/anti-diabetic properties and could be new target compounds for therapy of those diseases. In short, these clinical in vivo studies shows that the cinnamon and capsicum have become the functional foods of interests due to their natural abundance of variety dynamic bioactive agents which resulted in health endowment and indicated mentioned above health modulating effects. However, their effect at the cellular levels is still not investigated. Therefore, the key objectives of this study will be the investigation of the possible antidiabetic/antiinflammatory/antitumor activities of cinnamon and capsicum nutraceutical on PBMC. We evaluated the therapeutic potential of cinnamon and capsicum nutraceutical by targeted metabolomics using LC/QqQ-MS.

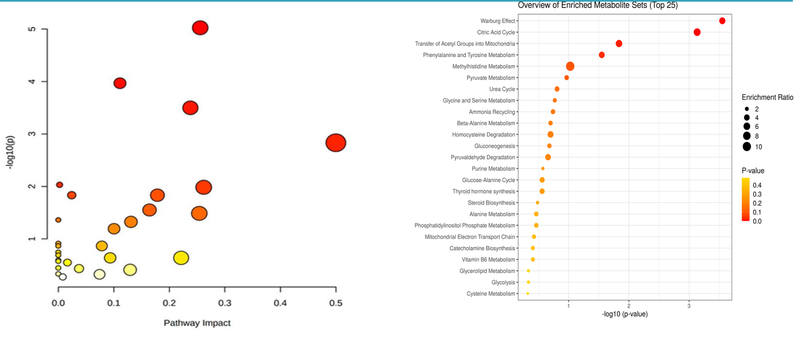
Objective

This work specifically aimed to disclose the effects of capsicum and cinnamon polyphenols on the cellular metabolism and immunoregulation of overweight/obese (OW/OB) (PBMC) using the targeted metabolomics.

Material and Methods



Pathway enrichment analysis



Note: (A) The metabolic pathways significantly matched with the discriminatory metabolites of cohort where pathways are represented by the nodes and (B) Metabolic pathways enrichment analysis of differential metabolites using MetaboAnalyst

Acknowledgement

"This research was conducted within the project which has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 754432 and the Polish Ministry of Science and Higher Education, from financial resources for science in 2018-2023 granted for the implementation of an international co-financed project"

Results

Table 1: List of discriminatory metabolites between controlled and treated cell cultures.

No.	Discriminatory Metabolites	RCP			RP			CIN			Significance 95%	
		%change	P(corr)	VIP	%change	P(corr)	VIP	%change	P(corr)	VIP	p-value	FDR cut-off
1	Uric acid	1702.6106	0.88	2.82	2071.1132	-0.81	2.75	21.6434	-0.52	1.06	0.0046	0.018
2	Quinic acid	4137.0692	0.88	3.05	1117.3759	-0.89	2.54	892.1423	0.96	2.4	0	0.0001
3	Glyceric acid	1628.5755	0.93	1.98	805.6289	-0.7	1.4	101.2907	0.3454	1.0696	0.045	0.1234
4	Citric acid	-92.7158	0.77	1.96	-26.0903	-0.51	2.25	-99.0409	-0.65	1.99	0.1294	0.2358
5	Fumaric Acid	114.2464	0.76	1.61	125.0263	-0.75	1.54	-35.2871	-0.0694	1.2171	0.0027	0.0127
6	L-Malic acid	239.9397	0.59	1.28	217.7006	-0.6	1.22	5.3778	0.1323	0.8686	0.4167	0.5927
7	Myo-inositol	-37.6066	0.57	1.19	-21.8601	-0.3331	0.8452	-42.9709	-0.2259	1.2201	0.5311	0.6945
8	DL-Iso-citiric acid	78.8043	0.7214	1.204	49.4598	-0.52	1.01	-18.6574	0.0075	0.8499	0.5894	0.734
9	L-Cystathionine	-22.4401	0.0509	0.2383	-57.6531	0.33	1.2	-17.8044	-0.0967	0.7877	0.3775	0.5698
10	D-Ribulose 5-phosphate	-49.9218	0.003	0.4194	-104.0101	0.55	1.52	-87.4782	-0.59	1.11	0.0573	0.1327
11	L-Cystine	-5.7498	0.6959	1.1448	20.2315	-0.51	1.08	-40.2375	-0.1041	1.0426	0.8286	0.8452
12	Mevalonic acid	-34.9751	0.5707	0.7671	-27.576	-0.6301	0.7268	-25.8977	-0.0376	1.0939	0.0018	0.0094
13	Mevalonic acid-5-phosphate	-29.2439	0.2623	0.0684	-10.0411	-0.1833	0.0895	-158.9109	0.7	1.47	0.0013	0.0093
14	Taurocholic acid	31.8193	0.1274	0.3929	-7.3843	-0.3079	0.6364	-320.3577	0.86	1.44	0.0018	0.0094
15	Lactic acid	2.5668	-0.4976	0.706	-1.2352	0.3487	0.2917	187.2294	0.9823	1.0914	0.0002	0.0031
16	L-Tyrosine	-10.8948	-0.5005	0.3164	-11.7231	0.6373	0.3518	31.127	0.97	1.5	0.0008	0.0083
17	Allantoin	-37.0867	0.3872	0.5503	-20.0142	-0.5088	0.5239	-98.1475	-0.95	3.01	0.0519	0.126
18	Hypoxanthine	63.5478	0.2364	0.1213	6.316	-0.1057	0.6034	-64.1739	-0.81	1.43	0.0014	0.0093
19	Pyruvic acid	-60.1591	-0.3504	0.9444	12.3072	-0.0876	0.0407	-60.1591	-0.53	1.14	0.0935	0.2043
20	Phenylpyruvic acid	14.9166	0.7551	0.8203	-4.3679	-0.7588	0.7772	62.7658	0.79	1.04	0.7689	0.8003
21	Pyridoxine	-5.8116	0.2442	0.1092	-1.877	-0.1949	0.0105	-11.8363	-0.6938	0.3181	0.0042	0.0177
22	L-Histidine	-9.2906	-0.0618	0.0422	-8.424	0.0048	0.4524	-19.1161	-0.3719	0.5519	0.0063	0.023
23	L-Hydroxyglutaric acid	-39.5482	0.1585	0.1313	-23.5185	-0.666	0.8401	-25.41	-0.0469	1.1422	0.0484	0.1234
24	o-Hydroxy hippuric acid	-0.5584	0.675	0.3919	-1.8521	-0.5006	0.2657	-20.2114	-0.9429	0.4873	0	0
25	Uracil	-1.6908	-0.2855	0.2106	6.6951	-0.0743	0.1423	-8.7477	-0.6653	0.4402	0.0015	0.0093

Note: Positive/negative value of per cent of change means higher/lower change of metabolite level. The *P*(corr)—predictive loading value and VIP value were calculated based on respective PLS-DA models. Variables with VIP >1.0 and absolute *P*(corr) >0.5 were considered significant. RCP = Capsicum (chili pepper); RP = Capsicum (bell pepper); CIN = cinnamon

Table 2: Pathways which were significantly affected by the intervention of selected spices

No.	Pathways	Total	Expected	Hits	Raw p	log10(p)	Holm adjust	FDR	Impact
1	Citrate cycle (TCA cycle)	20	0.32258	5	9.45E-06	5.0247	0.00079363	0.000794	0.25563
2	Glyoxylate and dicarboxylate metabolism	32	0.51613	5	0.000108	3.9679	0.0089368	0.004522	0.11112
3	Pyruvate metabolism	22	0.35484	4	0.000317	3.4986	0.026015	0.008883	0.23794
4	Phenylalanine, tyrosine and tryptophan biosynthesis	4	0.064516	2	0.00147	2.8327	0.11906	0.030867	0.5
5	Alanine, aspartate and glutamate metabolism	28	0.45161	3	0.00931	2.031	0.74482	0.14542	0.0024
6	Phenylalanine metabolism	10	0.16129	2	0.010387	1.9835	0.82057	0.14542	0.2619
7	Glycine, serine and threonine metabolism	33	0.53226	3	0.014702	1.8326	1	0.15437	0.0242
8	Cysteine and methionine metabolism	33	0.53226	3	0.014702	1.8326	1	0.15437	0.17854
9	Tyrosine metabolism	42	0.67742	3	0.028111	1.5511	1	0.26237	0.16435
10	Terpenoid backbone biosynthesis	18	0.29032	2	0.032636	1.4863	1	0.27414	0.25397
11	beta-Alanine metabolism	21	0.33871	2	0.043495	1.3616	1	0.33164	0
12	Pentose phosphate pathway	22	0.35484	2	0.047378	1.3244	1	0.33164	0.13089
13	Glycolysis / Gluconeogenesis	26	0.41935	2	0.0641	1.1931	1	0.41418	0.10044
14	Ascorbate and aldarate metabolism	8	0.12903	1	0.12224	0.91279	1	0.67432	0
15	Taurine and hypotaurine metabolism	8	0.12903	1	0.12224	0.91279	1	0.67432	0
16	Ubiquinone and other terpenoid-quinone biosynthesis	9	0.14516	1	0.13647	0.86496	1	0.67432	0
17	Vitamin B6 metabolism	9	0.14516	1	0.13647	0.86496	1	0.67432	0.07843
18	Aminoacyl-tRNA biosynthesis	48	0.77419	2	0.17988	0.74501	1	0.83945	0
19	Arginine biosynthesis	14	0.22581	1	0.20437	0.68959	1	0.90351	0
20	Glycerolipid metabolism	16	0.25806	1	0.23006	0.63815	1	0.92025	0.09346
21	Histidine metabolism	16	0.25806	1	0.23006	0.63815	1	0.92025	0.22131
22	Pentose and glucuronate interconversions	18	0.29032	1	0.25496	0.59352	1	0.97349	0
23	Pantothenate and CoA biosynthesis	19	0.30645	1	0.26712	0.57329	1	0.97557	0
24	Purine metabolism	65	1.0484	2	0.28238	0.54917	1	0.98833	0.01651
25	Galactose metabolism	27	0.43548	1	0.35776	0.44641	1	1	0
26	Phosphatidylinositol signaling system	28	0.45161	1	0.3683	0.4338	1	1	0.03736
27	Inositol phosphate metabolism	30	0.48387	1	0.38889	0.41017	1	1	0.12939
28	Arginine and proline metabolism	38	0.6129	1	0.46498	0.33256	1	1	0
29	Pyrimidine metabolism	39	0.62903	1	0.47383	0.32438	1	1	0.0743
30	Primary bile acid biosynthesis	46	0.74194	1	0.53194	0.27414	1	1	0.00805