

The effect of a caseinate hydrolysate on cytokine release and RNA expression in TNF- α stressed 3T3-L1 adipocytes

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Obesity and diabetes are linked to chronic inflammation and elevated pro-inflammatory cytokine release¹. The use of anti-inflammatory agents to reduce this inflammation may help to prevent or alleviate these conditions². Bioactive peptides with various physiological functions, including anti-inflammatory activity, have been isolated from bovine casein following hydrolysis by plant, mammalian and microbial-derived proteinases. The objective of this study was to determine the effect of intact caseinate, a caseinate hydrolysate and their simulated gastrointestinal digests (SGID) on cytokine production and RNA expression in Tumour Necrosis Factor- α (TNF- α) stressed adipocytes.

A 5 kDa permeate of a Flavourzyme[®] hydrolysate of sodium caseinate was generated at laboratory scale. The unhydrolysed caseinate (UH) and the 5 kDa permeate of the hydrolysate (H) were subjected to SGID. Pre-adipocytes (3T3-L1) were differentiated to adipocytes over three weeks in media containing 1 μ g/ml insulin, 0.25 μ M dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX) and 2 μ M rosiglitazone. After differentiation, adipocytes were pre-treated with test samples at 0.05 % (w/v) for 24 hours, following which stress was induced using TNF- α for 24 hours. The production of pro-inflammatory cytokines; Interleukin (IL)-6 and Monocyte Chemoattractant Protein-1 (MCP-1) along with the anti-inflammatory adipokine: adiponectin, were then measured using ELISA. The RNA expression of IL-6 was also measured using quantitative RT-PCR. Data was expressed as a percentage of the TNF- α stressed control.

	Adiponectin Cytokine production (% Control)		MCP-1 Cytokine production (% Control)		Interleukin-6 Cytokine production (% Control)		Interleukin-6 RNA expression (% Control)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	100.0	1.0	100.0	8.0	100.0	0.9	100.0	35.8
UH	96.4	2.4	116.8	8.0	267.8*	16.1	1706.9*	84.9
UH _{SGID}	98.6	2.8	115.1	4.9	260.0*	5.9	1987.7*	19.5
H	95.9	0.2	99.9	7.7	108.9	14.8	44.7	28.6
H _{SGID}	98.5	3.3	108.9	7.4	142.2*	10.4	68.1	57.5

UH: Unhydrolysed Casein, H: Hydrolysate. Values are means of two independent experiments. Statistical analysis was by ANOVA followed by Dunnett’s test. * $P < 0.05$

IL-6 production and RNA expression were significantly ($P < 0.05$) increased by UH and UH_{SGID} compared to control. The hydrolysate (H) did not significantly change IL-6 production. H_{SGID} produced a significant increase in IL-6 production although RNA expression was not affected. None of the samples produced significant changes in MCP-1 or adiponectin production. In conclusion, UH and UH_{SGID} appear to have a pro-inflammatory effect on cytokine release in adipocytes. Enzymatic hydrolysis of caseinate using Flavourzyme[®] appears to decrease this effect.

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2. Esser N, Paquot N, Scheen AJ (2015) *Expert Opin Investig Drugs* **24**, Suppl 3:283–307.