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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 release[1]. The use of anti-inflammatory agents to reduce this inflammation may help to prevent or alleviate these conditions[2]. 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 1μg/ml insulin, 0·25μM dexamethasone, 0·5 mM 3-isobutyl-1-methyxanthine (IBMX) and 2μM 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 Chemotactic 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.

1. Calder PC, Ahluwalia N, Brouns F, *et al.* (2011) *Br J Nutr* **106**, Suppl 3:S5–78.
2. Esser N, Paquot N, Scheen AJ (2015) *Expert Opin Investig Drugs* **24**, Suppl 3:283–307.