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Original Research

Whey protein hydrolysate and branched-chain amino acids downregulate inflammation-related genes in vascular endothelial cells

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ABSTRACT

A recent review of clinical studies reports that dairy products may improve inflammation, a key etiologic cardiovascular disease risk factor. Yet the impact of dairy proteins on inflammatory markers is controversial and could be mediated by a differential impact of whey proteins and caseins. In this study, we hypothesized that whey proteins may have a greater anti-inflammatory effect than caseins. A model of human umbilical vein endothelial cells, with or without TNF-α stimulation, was used to investigate the effect of several dairy protein compounds on inflammation. Specifically, the impact of whey proteins either isolate or hydrolysate, caseins, and their amino acids on expression of *TNF*, *VCAM-1*, *SOD2*, and *eNOS* was examined. After a 24-hour incubation period, whey protein hydrolysate, leucine, isoleucine, and valine attenuated the TNF-α–induced endothelial inflammation by normalizing *TNF* and *eNOS* gene expression. This effect was not observed in unstimulated cells. Oppositely, caseins, a whey protein/casein mixture (1:4 w/w), and glutamine aggravated the TNF-α–induced *TNF* and *SOD2* gene expression. Yet caseins and whey protein/casein mixture decreased *VCAM-1* expression in both unstimulated and stimulated human umbilical vein endothelial cells. Measurement of TNF-α in cell supernatants by immunoassay substantiates gene expression data without reaching statistical significance. Taken together, this study showed that whey proteins and their major amino acids normalize TNF-α–induced proinflammatory gene expression in endothelial cells.

Abbreviations: ACE, angiotensin-converting enzyme; BCAA, branched-chain amino acid; CVD, cardiovascular diseases; ELISA, enzyme-linked immunosorbent assay; eNOS, endothelial nitric oxide synthase; HUVECs, human umbilical vein endothelial cells; IL, interleukin; NF-κB, nuclear factor κB; NO, nitric oxide; RT-PCR, reverse transcriptase polymerase chain reaction; SOD, superoxide dismutase; TNF-α, tumor necrosis factor–alpha; VCAM-1, vascular cell adhesion molecule–1; T2D, type 2 diabetes; WPCN, whey protein and casein mixture; WPH, whey protein hydrolysate; WPI, whey protein isolate.

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1. Introduction

Recent epidemiological studies reported that adequate dairy intake may lower incidence of type 2 diabetes (T2D) and cardiovascular diseases (CVD) [1,2], yet the underlying mechanisms remain unclear. The health benefits of dairy foods could be mediated by an improvement of low-grade systemic inflammation, which is considered as a key etiologic factor in the development and progression of T2D and CVD [3,4]. A systematic review of human clinical trials reported a beneficial impact of dairy products on inflammation in subjects with metabolic disorders, whereas the impact in healthy subjects was either beneficial or neutral [5].

The mixed results reported for dairy products in human studies may be also attributed to a differential effect of whey proteins, caseins, and amino acids on inflammation [6]. Bovine milk contains around 30 g/L of dairy proteins, including 80% of caseins and 20% of whey proteins [7]. Dairy protein hydrolysates contain bioactive peptides with anti-inflammatory properties [6,8,9] but also amino acids. Amino acid profile differs between caseins and whey proteins [10]; caseins contain a high proportion of glutamine and proline residues and minor proportions of arginine, whereas whey proteins contain branched-chain amino acid (BCAA; leucine, isoleucine, and valine) residues. An acute clinical study reported decreased postprandial mRNA levels of inflammatory markers following ingestion of milk or yogurt [11]. These results suggest that dairy nutrients can regulate the transcriptome, as it has already been demonstrated in mice [9]. Yet little is known about the impact of dairy proteins and amino acids on gene regulation.

Low-grade systemic inflammation in obese and/or T2D individuals has been linked to endothelial dysfunction and atherosclerosis development [3]. Endothelial dysfunction is characterized by a decreased nitric oxide (NO) availability and hence a reduced endothelial nitric oxide synthase (eNOS) activity. The decreased NO availability enhances macrophage infiltration through adhesion molecules such as vascular cellular adhesion molecule (VCAM)–1 and the release of proinflammatory cytokines, including interleukins (ILs) and tumor necrosis factor (TNF)-α [12]. Furthermore, antioxidant enzymes such as superoxide dismutases (SOD) can neutralize reactive oxygen species, which have been linked to endothelial dysfunction [13]. Endothelial cells are active participants in inflammation.

The unique protein and amino acid composition of dairy products may regulate cytokine gene expression and production. Yet mechanistic studies that compared several dairy protein compounds are scarce [14]. In this study, we hypothesize that whey proteins and their major amino acids, BCAAs, have a greater anti-inflammatory potential than caseins. To test this hypothesis, a model of endothelial cells was used to compare the effects of several dairy protein compounds, including whey protein, caseins, and amino acids, on inflammatory gene expression. We first investigated dairy protein compounds in healthy endothelial cells. We then incubated dairy protein compounds in cells stimulated with TNF-α to induce inflammation, as it was suggested that dairy intake exerts anti-inflammatory effects in individuals with metabolic disorders.

2. Methods and materials

2.1. Materials

Nonhydrolyzed Whey Protein Isolate 90 (WPI) was from Milk Specialties Global (Fond du Lac, WI, USA) and Lacprodan DI-3095 whey protein hydrolysate (WPH) from Arla Food Ingredients (Viby J, Denmark). Minimum protein content of WPI and WPH was, respectively, 90% and 82%. Caseins from bovine milk (minimum protein content 87%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). L-Leucine, L-isoleucine, L-valine, L-glutamine, L-proline, and L-arginine were purchased from MP Biomedicals LLC (Solon, OH, USA). Human umbilical vein endothelial cells (HUVECs), as well as the EGM Bullet kit (endothelial growth medium) and EBM (endothelial basal serum-free medium), were from Lonza (Walkerville, MD, USA). Recombinant human TNF-α and enzyme-linked immunosorbent assay (ELISA) kit for TNF-α determination were from R&D Systems (Minneapolis, MN, USA). The TRI Reagent was supplied by Molecular Research Center Inc. (Cincinnati, OH, USA). High-Capacity cDNA Archive Kit, TaqMan gene expression assays, and Taqman Fast Advanced Master Mix were obtained from Applied Biosystems (Foster City, CA, USA).

2.2. Cell culture

HUVECs were grown in EGM containing 5% fetal bovine serum and maintained at 37°C in 5% CO_2 in a humidified atmosphere. Medium was changed every 2 days until cells reached 80% to 90% confluence. Cell viability was determined by performing trypan blue staining. Cells between passages 2 and 5 were used to carry out all experiments.

2.3. Experimental protocol

HUVECs (2.5×10^5 cells/mL) were seeded into 6-well plates for 48 hours in EGM. Subsequently, cells were incubated for 24 hours in serum-free medium supplemented with WPI (0.5 or 5 mg/mL), WPH (0.5 or 5 mg/mL), caseins (1 mg/mL), a mixture of WPI and caseins (WPCN, 0.25/1 mg/mL), leucine (0.2 or 2 mmol/L), isoleucine (0.2 or 2 mmol/L), valine (0.2 or 20 mmol/L), glutamine (0.2 or 20 mmol/L), proline (0.2 or 20 mmol/L), or arginine (0.2 or 20 mmol/L). These physiological concentrations were selected according to previous reports [14], plasma amino acid values after ingestion of dairy proteins [15], and solubility. For WPCN, the ratio 1:4 (*w*/*w*) was selected to represent the proportions of whey proteins and caseins in milk [7]. Serum-free medium (EBM) was used to dilute the WPI, WPH, and amino acids. This medium was also used as control for these treatments. Sodium hydroxide (1 mol/L) was used to prepare the casein stock solution (50 mg/mL). Caseins, WPCN treatments, and their appropriate control contained the same quantity of sodium hydroxide. In another experiment, TNF-α (2 ng/mL) was added for the 24-hour treatment period to investigate the effect of a low-grade inflammation. Each experiment was carried out in triplicate.