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

Marine S. Da Silva, Cyril Bigo, Olivier Barbier, Iwona Rudkowska

Endocrinology and Nephrology, CHU de Québec Research Center and the Department of Kinesiology, Faculty of Medicine, Université Laval, Québec, Québec, Canada G1V 4G2
Laboratory of Molecular Pharmacology, CHU de Québec Research Center and the Faculty of Pharmacy, Université Laval, Québec, Québec, Canada G1V 4G2

ARTICLE INFO

Article history:
Received 25 August 2016
Revised 18 January 2017
Accepted 19 January 2017

Keywords:
 Branched-chain amino acid
 Casein
 Endothelial cells
 Tumor necrosis factor-alpha
 Whey protein

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.

© 2017 Elsevier Inc. All rights reserved.

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.

* Corresponding author at: Endocrinology and Nephrology, CHU de Québec Research Center, CHUL-T4-55B - 2705, boul. Laurier, Québec, Québec, Canada G1V 4G2. Tel.: +1 418 525 4444x46380.

E-mail addresses: marine-da-silva.1@ulaval.ca (M.S. Da Silva), cyril.bigo@crchudequebec.ulaval.ca (C. Bigo), olivier.barbier@crchudequebec.ulaval.ca (O. Barbier), iwona.rudkowska@crchudequebec.ulaval.ca (I. Rudkowska).

http://dx.doi.org/10.1016/j.nutres.2017.01.005
0271-5317/© 2017 Elsevier Inc. All rights reserved.
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

Non-hydrolyzed 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% CO2 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.
2.4. **RNA extraction and cDNA synthesis**

Total RNA was isolated from control or treated cells, according to the TRI Reagent acid:phenol protocol, as specified by the supplier. After spectrophotometric quantification and verification of the total RNA quality, cDNA was generated from 1 μg of total RNA and stored in aliquots at −20°C until analyses.

2.5. **Quantitative real-time reverse transcriptase polymerase chain reaction**

Real-time polymerase chain reaction (PCR) was used to measure mRNA expression levels of SOD2 (Hs00167309_m1), eNOS (Hs01574659_m1), TNF (Hs00174128_m1), and VCAM-1 (Hs01003372_m1) using TaqMan gene expression assays. The real-time PCRs were performed using a Viia7 Real-Time PCR System instrument from Applied Biosystems. For each reaction, the final volume of 10 μL was comprised of 5 μL of Taqman Fast Advanced Master Mix, 0.5 μL of TaqMan Gene Expression assay, and 0.5 μL of cDNA. Conditions for real-time PCR were 95°C for 20 seconds, 95°C for 1 second, and 60°C for 20 seconds for 40 cycles. Threshold cycle (Ct) values were analyzed using the comparative Ct (ΔΔCt) method [16]. The amount of target gene (2−ΔΔCt), normalized to β-actin gene expression (Hs99999903_m1), is represented as the fold change in mRNA expression relative to the appropriate control, set at 1.

2.6. **Enzyme-linked immunosorbent assay**

HUVECs were treated as above, and supernatants were collected, centrifuged at 1500 rpm for 10 minutes at 4°C, and stored at −80°C. The concentration of TNF-α in cell supernatants was determined by ELISA, following the manufacturer’s instructions.

2.7. **Statistical analyses**

Unless otherwise stated, all data are presented as means ± SEM of 3 separate experiments, with 3 technical replicates in each experiment (n = 9). Gene expression data were log-transformed to achieve normality. A multifactorial analysis of variance was used to assess the effect of treatment, concentration, and TNF-α stimulation, followed by Tukey test for multiple data comparison. Differences between control without TNF-α control and control with TNF-α were evaluated by Student t test. Data were analyzed with SAS statistical software, version 9.4 (SAS Institute Inc, Cary, NC, USA). Statistical significance was set at P < .05.

Heatmaps representing log(2)-ratio change values in treatments compared with control of TNF, VCAM-1, SOD2, and eNOS genes were constructed using the online graphing tool Plotly (https://plot.ly/plot).

### 3. Results

#### 3.1. **Effects of TNF-α on endothelial inflammatory gene expression**

Under control conditions, stimulation of HUVECs with 2 ng/mL TNF-α for 24 hours caused an upregulation of TNF, VCAM-1, and SOD2 and a downregulation of eNOS relative gene expression (Table).

#### 3.2. **Effects of dairy proteins on endothelial inflammatory gene expression**

The effect of dairy proteins on TNF, VCAM-1, SOD2, and eNOS gene expression in unstimulated and TNF-α-stimulated HUVECs is presented in Fig. 1.

Significantly different effects of whey proteins on TNF and VCAM-1 gene expression upon TNF-α stimulation were observed. In HUVECs not stimulated with TNF-α, treatment with WPI, which are pure whey proteins, decreased significantly TNF expression by 44% and 62% at 0.5 and 5 mg/mL, respectively. WPI at 5 mg/mL also decreased VCAM-1 expression by 43%. The hydrolyzed form of WPI (WPH) had no significant effect on TNF and VCAM-1 gene expression in unstimulated cells. Oppositely, in TNF-α-stimulated cells, WPI at 0.5 mg/mL increased TNF expression (2.3 ± 0.3-fold), whereas the higher dose of WPI (5 mg/mL) had no effect. Moreover, WPH at 0.5 mg/mL significantly reduced the expression of TNF and VCAM-1 genes by 38% and 50%, respectively. Taken together, these results suggest that both whey protein form (WPI or WPH) and TNF-α stimulation influence the inflammatory gene expression in HUVECs. Specifically, WPI decreased TNF and VCAM-1 gene expression in unstimulated cells, whereas WPH lowered expression of these genes in TNF-α-stimulated cells.

Caseins and a mixture of caseins and WPI (WPCN) lowered VCAM-1 expression in a similar fashion in both unstimulated and TNF-α-stimulated HUVECs. Yet WPCN also caused a 2-fold increase of TNF expression. Moreover, TNF expression was increased in HUVECs cotreated with caseins and TNF-α.

Dairy proteins also impact SOD2 expression. WPI (5 mg/mL) decreased SOD2 expression by 65% and 57% in unstimulated and TNF-α-stimulated cells, respectively. Caseins and WPCN slightly upregulated SOD2 expression in HUVECs in TNF-α-stimulated cells. The expression of eNOS was not influenced by dairy protein treatments in both unstimulated and TNF-α-stimulated cells.

#### 3.3. **Effects of amino acids on endothelial inflammatory gene expression**

The effect of BCAA on TNF, VCAM-1, SOD2, and eNOS gene expression in unstimulated and TNF-α-stimulated HUVECs is

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mean fold expression</th>
<th>SEM</th>
<th>P value (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF</td>
<td>32.1</td>
<td>2.0</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>16.1</td>
<td>2.2</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>SOD2</td>
<td>10.3</td>
<td>0.9</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>eNOS</td>
<td>0.67</td>
<td>0.03</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

\(^a\) P value: vehicle control with TNF-α control vs vehicle control without TNF-α (Student t test, n = 9 for each group).
presented in Fig. 2. In unstimulated HUVECs, leucine (2 mmol/L) significantly lowered TNF expression, whereas valine (20 mmol/L) caused a slight upregulation of VCAM-1 and SOD2. Isoleucine did not influence gene expression of TNF, VCAM-1, eNOS, and SOD2. TNF-α stimulation significantly modified the effect of each BCAA on TNF and eNOS gene expression ($P < .0001$). Leucine, isoleucine, and valine attenuated the TNF-α–induced inflammatory response by decreasing TNF expression and increasing eNOS expression in a dose-dependent manner (Fig. 2). Leucine and isoleucine did not influence VCAM-1 and SOD2 expression, whereas valine at 20 mmol/L increased VCAM-1 expression and decreased SOD2 expression.

The effect of glutamine, proline, and arginine in unstimulated and TNF-α–stimulated HUVECs is presented in Fig. 3. In unstimulated HUVECs, glutamine at higher dosage (20 mmol/L) raised expression of TNF, VCAM-1, and SOD2, whereas eNOS expression was not influenced. In TNF-α–stimulated cells, glutamine increased TNF and VCAM-1 gene expression, although in a lesser extent than in unstimulated cells ($P < .001$, compared with glutamine in unstimulated cells), and had no effect on SOD2 and eNOS gene expression. Proline raised expression of TNF and VCAM-1 at higher dosages (20 mmol/L) without affecting eNOS and SOD2 expression in unstimulated cells. In TNF-α–stimulated cells, proline did not influence the expression of all the genes measured. Arginine significantly decreased SOD2 gene expression and had no effect on TNF, VCAM-1, and eNOS gene expression in both unstimulated and TNF-α–stimulated cells.

In sum, BCAA attenuated the TNF-α–induced inflammatory response in HUVECs. Oppositely, glutamine amplified the effect of TNF-α by increasing TNF, VCAM-1, and SOD2 gene expression. Proline increased inflammatory gene expression in unstimulated cells, whereas arginine decreased SOD2 expression. Heatmaps that summarize the effect of all dairy protein compounds on TNF, VCAM-1, SOD2, and eNOS gene expression in unstimulated and TNF-α–stimulated HUVECs are presented in Fig. 4.

3.4. Effects of dairy proteins and amino acids on TNF-α excretion

The effect of dairy protein compounds on TNF-α excretion in TNF-α–stimulated HUVECs is shown in Fig. 5. Similarly to gene expression data, WPH, leucine, and valine decreased, whereas WPI increased, TNF-α excretion, although it did not reach statistical significance. All the other treatments, including caseins and WPCN, did not influence TNF-α excretion. TNF-α was not detected in unstimulated HUVECs.

4. Discussion

The results of this study demonstrate that physiological concentrations of dairy proteins and amino acids have differential effects on the expression of genes associated with inflammatory response in human endothelial cells.
Whey protein hydrolysate and BCAA have anti-inflammatory properties by attenuating TNF-α–induced gene expression. Oppositely, caseins, the WPCN mixture, and glutamine have mixed effects on inflammatory gene expression. To our knowledge, this is the first in vitro study that compares the effect of caseins, whey proteins, and amino acids on endothelial gene expression.

In this study, whey protein hydrolysate showed an anti-inflammatory potential by lowering both TNF and VCAM-1 expression. Similarly, a study reported that whey proteins had an anti-inflammatory effect by decreasing IL-8 production in Caco-2 intestinal cells [17]. The beneficial effect of whey proteins may be mediated their major amino acids, namely, BCAAs. In this work, BCAAs attenuated the TNF-α–induced inflammatory response in HUVECs. Supplementation with BCAAs, especially leucine, lowered inflammatory gene expression in the adipose tissue and the liver, together with a decreased macrophage infiltration in obese mice [9,18-20]. Therefore, these data support our observations. Our results also indicate that whey proteins and BCAAs mostly exerted...
their beneficial effect in the TNF-α-stimulated HUVECs. TNF-α-stimulated HUVECs might be considered as a representative model for disease state, as it has already been proposed [21]. Therefore, dairy protein compounds may exert their benefits in individuals with a more deteriorated metabolic profile rather than in healthy individuals. This suggestion is supported by human clinical studies, as a recent review concluded that dairy products have a greater anti-inflammatory effect in individuals with metabolic disorders than in healthy individuals [5]. Future intervention studies should investigate the effect of dairy proteins according to the health status of individuals.

Caseins and WPCN reduce VCAM-1 expression but increase TNF expression especially in TNF-α-stimulated cells. Moreover, the major amino acid in caseins, glutamine, has proinflammatory effects in both unstimulated and stimulated cells. Yet caseins, WPCN, and glutamine do not increase TNF-α excretion. Indeed, caseins were often used as the control

Fig. 3 – Expression of TNF, VCAM-1, SOD2, and eNOS in unstimulated and TNF-α-stimulated HUVECs after exposure to glutamine, proline, or arginine for 24 hours. Gene expression was measured by RT-PCR (n = 9 for each group). Each value represents the amount of mRNA relative to the appropriate control normalized for β-actin, which is arbitrarily set at 1. Values are means ± SEM. *Significantly different compared with control without TNF-α (P < .05). #Significantly different compared with control with TNF-α (P < .05).
diet to investigate inflammation in animal studies [22]. Nevertheless, casein-derived tripeptides were reported to have blood pressure-lowering effects via the inhibition of angiotensin-converting enzyme (ACE) [23,24]. ACE inhibition had anti-inflammatory effects by decreasing the release of cytokines and increasing NO production in the endothelium [8,25]. Thus, the protective effect of caseins against CVD could be mediated by ACE-inhibiting peptides. Accordingly, Marcone et al [26] reported that casein-derived peptides reduced adhesion molecules and IL-8 in TNF-α-stimulated human aortic endothelial cells. Another study showed that a casein hydrolysate decreased TNF, IL-1, IL-8, and IL-10 gene expression in porcine colonic explants [27]. Studies also demonstrate that glutamine decreased circulating inflammatory markers (IL-6,

---

**Fig. 4** - Heatmaps representing the effect of dairy proteins and amino acids on mRNA levels of TNF, VCAM-1, SOD2, and eNOS compared with control in (A) unstimulated and (B) TNF-α-stimulated HUVECs.

---

**Fig. 5** - Effect of (A) dairy proteins and (B) amino acids for 24 hours on TNF-α excretion in TNF-α-stimulated HUVECs. HUVECs were incubated for 24 hours with TNF-α (2 ng/mL) and WPI or WPH at 2 concentrations (0.5 or 5 mg/mL), caseins (CN, 1 mg/mL), or WPCN (1:4) or amino acids (0-20 mmol/L). TNF-α excretion in cell supernatants was measured by ELISA. Each value represents the amount of TNF-α compared with the control (basal medium + TNF-α), set at 1 (dash line). Values are means ± SD.
IL-1α, MIP-1α, GM-CSF, MIP-2, IFNγ, and E-selectin) in a rodent model of endothelial dysfunction [28]. Yet glutamine had no effect on plasma TNF-α, VCAM-1, and ICAM-1 as well as MCP-1, CCL5, IL-1α, IP-10, and IL-10 concentrations [28]. Taken together, these data may suggest mixed effects of caseins and glutamine on inflammatory markers. This clearly warrants further research.

Results show no impact of dairy proteins and amino acids on eNOS expression, except for BCAAs that increase eNOS expression in inflamed HUVECs. On the contrary, dairy proteins and amino acids influence SOD2 expression independently of the TNF-α stimulation. Specifically, caseins, WPCN, glutamine, and valine increase SOD2 expression, whereas WPI and arginine decrease its expression. SOD2 deficiency has been associated to an accelerated atherosclerosis development [13], whereas SOD activity was diminished in severely obese individuals [29]. Transgenic mice overexpressing eNOS were protected against atherosclerosis development [30], and diet-induced obese mice overexpressing SOD2 showed a reduction in oxidative stress damage [31]. Therefore, increasing eNOS and SOD2 gene expression is beneficial against inflammation induced by oxidative stress. Furthermore, caseins did not influence eNOS expression. Oppositely, a study reported that casein hydrolysates increased the release of NO in human aortic endothelial cells, suggesting an increased activity of eNOS [25]. In sum, caseins, WPCN, glutamine, and BCAAs upregulate certain antioxidant genes in endothelial cells.

This work suggests that the composition of dairy products in terms of proteins as well as amino acids may impact differentially inflammatory markers. Studies have shown that dairy protein hydrolysates may decrease inflammatory response by inhibiting nuclear factor κB (NF-κB) pathway [26,32,33]. Moreover, glutamine, leucine, and proline can inhibit the NF-κB pathway [34]. NF-κB is a transcription factor activated by proinflammatory signals, such as TNF-α, to regulate the transcription of numerous inflammatory genes. Genes regulated by NF-κB include TNF, VCAM-1, and SOD2 [35], which were the genes mostly impacted by dairy protein compounds in this work. Therefore, it could be suggested that whey protein hydrolysate and BCAAs attenuated TNF-α-induced inflammatory gene expression through a mechanism involving the NF-κB pathway. Oppositely, whey protein isolate, caseins, and WPCN are whole proteins and may involve other pathways. For instance, autophagy impacts mitochondrial function and oxidative stress, and this pathway can be activated by proteins [36].

A major strength of this work is that the influence of both dairy proteins and amino acids was examined. As dairy proteins are broken down into amino acids upon digestion, investigating both proteins and amino acids is a considerable asset. Potential limitations should be considered. First, only TNF and VCAM-1 genes were selected because they were the most frequently investigated in cell studies; hence, it allows us to compare our results with others. Other proinflammatory genes should be examined. Second, different observations might have been made if HUVECs were incubated with dairy protein compounds for 6 or 12 hours instead of 24 hours. Nevertheless, a 24-hour incubation was performed to mimic a chronic exposure to dairy proteins and TNF-α. Finally, these results have to be validated in vivo. This work establishes the need for in-depth investigation regarding changes in whole transcriptome and cytokine profiles to ascertain the effects of different dairy proteins in animal or human studies.

In conclusion, this comparative study shows that whey protein and their major amino acids BCAAs may have a protective role against inflammation in endothelial cells. Caseins and their major amino acid, glutamine, globally exerted proinflammatory effects, yet caseins may increase antioxidant genes. In addition, results suggest that particular dairy protein compounds may exert their beneficial action, even at low dosages, according to the inflammatory status. Overall, the distinct properties of dairy protein nutrients, especially whey protein hydrolysate and BCAAs, support the preventive potential of dairy-based functional foods for vascular health.

Acknowledgment

The authors do not declare any conflict of interest. The authors would like to thank Patricia Savard from the Institute of Nutrition and Functional Foods (INAF, Université Laval, Québec, Québec, Canada) who generously provided whey protein powders. MSDS received scholarships from the Centre de recherche en endocrinologie moléculaire et oncologique et génomique humaine, the Department of Kinesiology of Université Laval, and the CHU de Québec Foundation. IR holds a Junior 1 Research Scholar from the Fonds de Recherche du Québec-Santé. This work was supported by the Natural Sciences and Engineering Research Council of Canada.

References


