Mechanisms of Ageing and Development 162 (2017) 1–8

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev

# Consumption of protein-enriched milk has minor effects on inflammation in older adults—A 12-week double-blind randomized controlled trial

Gyrd O. Gjevestad[a,b], Inger Ottestad[a], Anne Sofie Biong[b], Per Ole Iversen[a,c], Kjetil Retterstøl[a,d], Truls Raastad[e], Bjørn S. Skålhegg[a], Stine M. Ulven[a], Kirsten B. Holven[a,f,*]

[a] Department of Nutrition, Institute of Basic Medical Sciences, P.O. Box 1046, Blindern, 0317 University of Oslo, Norway
[b] TINE SA, Centre for Research and Development, P.O. Box 7, Kalbakken, 0902 Oslo, Norway
[c] Department of Hematology, Oslo University Hospital, P.O. Box 4950 Nydalen, 0424 Oslo, Norway
[d] Lipid Clinic, Oslo University Hospital, P.O. Box 4950 Nydalen, 0424 Oslo, Norway
[e] Department of Physical Performance, Norwegian School of Sport Sciences, P.B. 4104 U.S., 0806 Oslo, Norway
[f] National Advisory Unit on Familial Hypercholesterolemia, Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, P.O. Box 4950 Nydalen, 0424 Oslo, Norway

ARTICLE INFO

*Article history:*
Received 16 September 2016
Received in revised form 18 January 2017
Accepted 28 January 2017
Available online 3 February 2017

*Keywords:*
Milk protein
Older adults
High-protein
Inflammation
Peripheral blood mononuclear cells
mRNA

ABSTRACT

*Introduction:* Aging is associated with increased levels of circulating inflammatory markers and reduced muscle mass and strength.
*Objective:* We investigated whether intake of protein-enriched milk for 12 weeks would influence markers of inflammation among adults ≥70 years of age with reduced physical strength.
*Methods:* In a double-blind randomized controlled intervention study, subjects were randomly allocated into two groups, receiving a protein-enriched milk (2 × 20 g protein/d, n = 14, mean (±SD) age 76.9 ± 4.9 yrs) or an isocaloric carbohydrate drink (n = 17, age 77.7 ± 4.8 yrs) for 12 weeks. We measured serum and mRNA expression levels of inflammatory markers in PBMCs.
*Results:* Significant differences in the mRNA expression of *nuclear receptor subfamily, group H, member 3* (*NR1H3,* encoding the LXRα transcription factor) and *interferon gamma* (*INFG)* were observed between groups. The mRNA level of *TNFRSF1A* was significantly reduced, while the mRNA level of *dipeptidyl-peptidase 4* (*DPP4)* was significantly increased, in the control group. The serum level of TNFα increased significantly in the control group, while sTNFRSF1A increased significantly in both groups, but with no significant differences between groups.
*Conclusion:* Consumption of a low-fat, protein-enriched milk for 12 weeks had minor effects on inflammatory related markers in older adults compared to an isocaloric carbohydrate drink.


*Abbreviations:* B2M, beta-2-microglobulin; BMI, body mass index; CCL, chemokine (C-C motif) ligand; cDNA, complimentary deoxyribonucleic acid; CXCL16, chemokine (C-X-C motif) ligand 16; DPP4, dipeptidyl-peptidase 4; DXA, dual energy X-ray absorptiometry; g, gravity; Gadd45, growth arrest and DNA-damage-inducible, alpha; hs-CRP, high sensitive C-reactive protein; IFNG, interferon gamma; IGF1, insulin-like growth factor 1; IL, interleukin; IL1RN/IL1Ra, interleukin 1 receptor antagonist; liver X receptor alpha, LXRα; mRNA, messenger RNA; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; NR1H3, nuclear receptor subfamily, group H, member 3; PBMC, peripheral blood mononuclear cells; PDK4, pyruvate dehydrogenase kinase, isozyme 4; qPCR, quantitative polymerase chain reaction; rpm, rounds per minute; Runx2, runt-related transcription factor 2; sTNFRSF1A, soluble tumor necrosis factor receptor 1; TBP, TATA box binding protein; TLDA, TaqMan Low-Density array; TLR, toll-like receptor; TNFα, tumor necrosis factor alpha; TNFSF11, tumor necrosis factor ligand superfamily member 11; TNFRSF1A, tumor necrosis factor receptor superfamily, member 1A; TNFRSF11A, tumor necrosis factor receptor superfamily, member 11A.

* Corresponding author at: Department of Nutrition, Institute of Basic Medical Sciences, P.O. Box 1046, Blindern, 0317 University of Oslo, Norway.
*E-mail addresses:* g.o.gjevestad@medisin.uio.no (G.O. Gjevestad), inger.ottestad@medisin.uio.no (I. Ottestad), anne.sofie.biong@tine.no (A.S. Biong), poiversen@hotmail.com (P.O. Iversen), kjetil.retterstol@medisin.uio.no (K. Retterstøl), truls.raastad@nih.no (T. Raastad), b.s.skalhegg@medisin.uio.no (B.S. Skålhegg), smulven@medisin.uio.no (S.M. Ulven), k.b.holven@medisin.uio.no (K.B. Holven).

http://dx.doi.org/10.1016/j.mad.2017.01.011
0047-6374/

## 1. Introduction

The risk of developing chronic diseases is increased in older adults (Chung et al., 2011; Calder et al., 2011; Singh and Newman, 2011; Simon et al., 2015; Guarner and Rubio-Ruiz, 2015), and an elevated level of circulating inflammatory markers are often observed (Singh and Newman, 2011; Franceschi, 2007). Growing evidence indicates that inflammatory markers, such as high-sensitive C-reactive protein (hs-CRP), interleukin 6 (IL6), and tumor necrosis factor alpha (TNFα), are associated with loss of muscle mass and muscle strength (Legrand et al., 2013; Schaap et al., 2009; Visser et al., 2002; Cesari et al., 2004; Marzetti et al., 2014; Aleman et al., 2011; Bartali et al., 2012; Taaffe et al., 2000; Vina et al., 2016), possibly contributing to the development of sarcopenia (Bartali et al., 2012; Beyer et al., 2012; Laviano et al., 2014).

Dietary strategies to prevent the onset of chronic, low-grade inflammation are therefore suggested to improve several health outcomes and to prolong longevity among elderly (Singh and Newman, 2011; Franceschi, 2007; Kritchevsky et al., 2005; Panickar and Jewell, 2015). Foods, including fruits and vegetables, fish, whole grains and some vitamins, are shown to exert anti-inflammatory effects (Calder et al., 2011). The effects of dairy products on inflammatory markers are less clear (Draganidis et al., 2016). Some epidemiological evidence indicates that low-fat dairy products are inversely associated with the level of inflammatory markers among healthy adults (Panagiotakos et al., 2010; Azadbakht et al., 2005; Esmaillzadeh and Azadbakht, 2010), but the data are inconclusive (Rashidi Pour Fard et al., 2015). In randomized controlled trials a reduction in the circulating levels of TNFα, IL6 and chemokine (C-C motif) ligand 2 (CCL2) have been shown after consuming dairy products in obese subjects (van Meijl and Mensink, 2010; Zemel et al., 2010), but the data are not conclusive (Van Loan et al., 2011; Wennersberg et al., 2009). A reduction of the same inflammatory markers have been observed in subjects with the metabolic syndrome (Stancliffe et al., 2011), but not in healthy adults (Drouin-Chartier et al., 2015; Tricon et al., 2006), nor in adult subjects with an elevated level of hs-CRP (Labonte et al., 2014) after consuming dairy products.

High protein diets have increased in popularity, and these are widely used in combination with weight reduction (Hu, 2005) and in sports nutrition (Goisser et al., 2015) to preserve muscle mass and promote muscle strength. Few have examined the possible effects of high protein diets on risk factors for chronic diseases (Rashidi Pour Fard et al., 2015; Wolfe, 1995; Clifton and Keogh, 2007; Schwingshackl and Hoffmann, 2013), among them level of inflammatory markers (Santesso et al., 2012; Teunissen-Beekman et al., 2015). No negative effects on inflammatory markers are observed (Santesso et al., 2012; Teunissen-Beekman et al., 2015), but long-term clinical studies are scarce (Santesso et al., 2012; Teunissen-Beekman et al., 2015; Ziegler and Sidani, 2011).

In a double-blind randomized controlled intervention study, we investigated whether an increased daily intake of a low-fat, protein-enriched milk could alter markers of inflammation in peripheral mononuclear blood cells (PBMCs) and in serum among community dwelling elderly men and women above the age of 70 years with reduced muscle strength and functional performance.

## 2. Materials and methods

### 2.1. Study population and study design

The present study is part of a research project were men and women (≥70 years) living at home were recruited to a 12 week double-blind, randomized controlled intervention trial, conducted from August 2014 to September 2015 at Oslo and Akershus University College of Applied Sciences, Norway. The primary aim of this study was to investigate the effect of increased intake of protein-enriched milk on muscle mass and physical strength. A detailed description of participant recruitment, enrollment, selection criteria, and compliance are given elsewhere (Ottestad et al., 2017). In brief, 2820 subjects were invited to participate in the study. 438 subjects met to screening of which 388 were excluded. Thus, 50 older subjects, with either reduced hand grip strength (<20 kg in women and <30 kg in men), gait speed <1 m/s, timed step stair test ≥8.4 s or timed five times sit to stand test >12.5 s, and otherwise weight stable and apparently healthy, were randomized. Among the exclusion criteria were a Mini-Mental State Examination score <24, a Mini Nutritional Assessment score <17 and high intakes of dairy products (≥4 dl/day of milk, cultured milk and/or yoghurt). In total, 36 subjects completed the study. The intervention group received a protein-enriched milk (n = 17, 2 × 0.4 L/d; 2 × 20 g protein/d), whereas the control group received an isocaloric carbohydrate drink (n = 19, 2 × 0.4 L/d), for 12 weeks. The subjects consumed the test drinks together with breakfast and the evening meal, and they were encouraged to maintain their habitual diet and physical activity level throughout the study period.

All subjects provided written informed consent, and we conducted the study according to the Declaration of Helsinki. We received approval for all procedures involving human subjects by the Regional Committees for Medical and Health Research Ethics, Health Region South East, Norway. The study was registered at Clinicaltrials.gov (ID no. NCT02218333).

### 2.2. Study products

TINE SA (Oslo, Norway) produced and provided the protein-enriched milk and the isocaloric control drink. The protein-enriched milk contained on average 5.0% protein, 4.6% carbohydrates, <0.1% fat and provided approximately 167 kJ (39 kcal)/100 g. The control drink was prepared from carbohydrates (sugar, xantan gum and Maltosweet™[a]). To give the control drink a milky appearance the producer added titandioksid (E171). Both drinks contained approximately 178 mg/100 g of calcium.

### 2.3. Blood sampling and sample preparation

Venous blood samples were collected after an overnight fast (≥12 h) in BD Vacutainer® CPT™ cell preparation tubes with sodium heparin (Becton Dickenson, NJ, USA) and in silica gel tubes (Becton Dickenson Vacutainer Systems, Plymouth, UK) at baseline and after 12 weeks. Within two hours of blood collection PBMCs were collected by density gradient centrifugation of the blood samples (1636*g*) for 25 min at room temperature (RT). The cells were washed twice (300*g*, 10 min at RT) in phosphate-buffered saline (PBS) without calcium chloride and magnesium chloride. After the last washing step, excess PBS was discarded. The pellet was dissolved in the remaining liquid and transferred to an Eppendorf tube, centrifuged (13,000*g*, 3 min at 4 °C) and frozen at −80 °C until further analysis. Serum samples were centrifuged (1500*g*, 15 min at RT) after being left on the bench top for at least 30 min. Serum sample for the determination of cytokines were frozen at −80 °C until further analysis. Serum samples for the determination of hs-CRP, and EDTA-blood for the differential blood count, were sent to an accredited laboratory (Fürst Laboratories, Oslo, Norway) for further analysis.

### 2.4. Isolation of RNA

mRNA was isolated from thawed PBMCs using QiaCube from QIAGEN GmbH (Germany) in accordance with the protocol RNeasy