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Short communication: Effects of *trans* fatty acids on markers of inflammation in bovine mammary epithelial cells

P. Rezamand and M. A. McGuire[1]
Department of Animal and Veterinary Science, University of Idaho, Moscow 83844-2330

ABSTRACT

Trans fatty acids (tFA) contribute to inflammation. The objective was to investigate the effects of tFA on mRNA expression of proinflammatory markers in cultured bovine mammary epithelial cells (MAC-T cell line). Bovine mammary epithelial cells were grown in Dulbecco's Modified Eagle Medium containing 10% fetal bovine serum. Cells were then subcultured in a medium lacking fetal bovine serum, to which incremental concentrations (up to 90 μ*M*) of elaidic acid (*trans*-9 C18:1) or linoleidic acid (*trans*-9, *trans*-12 C18:2) were added. Bovine serum albumin (fatty acid-free) solutions were added and cells were collected at specific time points over 48 h. Then, RNA was extracted and converted to complementary DNA for quantitative real-time PCR analysis of proinflammatory gene expression. Presence of elaidic acid caused increases in mRNA expression of interleukin (IL)-1β (3.4-fold; dose-independently over a 6-h period) and intercellular adhesion molecule (ICAM)-1 (up to 1.4-fold) relative to that for cells treated with no tFA, whereas expression of IL-6 and IL-8 was reduced 0.75- and 0.85-fold, respectively. Presence of linoleidic acid reduced mRNA expression of IL-6 and IL-8 relative to that for control (0.95- and 0.87-fold, respectively). *Trans* mono- and dienoic fatty acids upregulated mRNA expression of IL-1β and ICAM-1, whereas expression of IL-6 and IL-8 was downregulated in MAC-T cells. Since these genes are ultimately involved in inflammation, elaidic or linoleidic acid, either directly fed or formed in the rumen during biohydrogenation, may alter the risk for mastitis in vivo.
Key words: *trans* fatty acid, inflammation, bovine, mammary

Inflammation affects metabolism and is a common contributing factor in many patho-physiological conditions such as atherosclerosis, insulin resistance, and mastitis (Mozaffarian, 2006). Over the past 20 yr, a great deal of information has been generated concerning the relationship among dietary *trans* fatty acids (**tFA**), immune function, and inflammatory response. *Trans* fatty acids are unsaturated fatty acids containing one or more double bonds in the *trans* configuration. Among these fatty acids (**FA**), vaccenic acid (*trans* 11 C18:1) is a major tFA found in the rumen as a result of microbial biohydrogenation of unsaturated fatty acids (Wolff et al., 1998). Partial hydrogenation of unsaturated fatty acids can result in production of elaidic acid (*trans*-9 C18:1) to produce more solid fat. Because a *trans* configuration for a double bond resembles that of saturated FA, substitution of a FA with a *cis* double bond can modify cell membrane fluidity, eicosanoid production, and production of a wide range of immune and inflammatory mediators. Greater dietary tFA intake was associated with increases in markers of systemic inflammation via elevated production of proinflammatory cytokines (Mozaffarian et al., 2004a,b; Mozaffarian, 2006). For instance, in patients with known heart disease, positive correlations were observed between erythrocyte membrane tFA content, a marker of dietary tFA intake, and several biomarkers of inflammation including IL-1β, IL-6, IL-10, and tumor necrosis factor (**TNF**) system activity including TNF and soluble TNF receptors 1 and 2 (Mozaffarian et al., 2004b). Increased TNF system activity was observed when dietary tFA intake of healthy individuals increased (Mozaffarian et al., 2004a). The positive association between increased tFA and inflammation may be specific for the tFA isomer consumed. Several studies cited by Mozaffarian (2006) demonstrated an association between increased intake of *trans* C18:1 and *trans* C18:2 and elevated systemic inflammatory mediators. Findings on the effects of tFA on markers of immune function and inflammation are equivocal. For instance, when human subjects were fed diets enriched in *trans*-11 and *trans*-12 C18:1, no significant increases in proinflammatory markers (e.g., TNF-α, IL-6, and IL-8) or immune functions (e.g., phagocytosis) were observed compared with subjects fed a diet without tFA (Kuhnt et al., 2007). Nutritional studies in dairy cattle to examine a causal role of increased tFA intake on inflammatory responses are limited. Rodriguez-

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[1]Corresponding author: mmcguire@uidaho.edu

Sallaberry et al. (2007) did not detect an alteration in plasma concentrations of TNF-α or IL-4 for periparturient cows fed a tFA-enriched diet compared with cows fed a saturated fat-enriched diet. The hypothesis was that gene expression of selected pro-inflammatory markers in bovine mammary epithelial cells (**MAC-T**) would be upregulated by increased tFA. Therefore, the objective was to determine the effect of *trans* monoenoic (elaidic acid: *trans*-9 C18:1) and dienoic (linoleidic acid: *trans*-9 *trans*-12 C18:2) FA on the mRNA expression of select inflammatory markers in an in vitro model using a MAC-T cell line.

The MAC-T cells were provided as a generous gift from the laboratory of Gregory Bohach (University of Idaho, Moscow) and grown in Dulbecco's Modified Eagle Medium (**DMEM**; Gibco/Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum (Gibco/Invitrogen), insulin (10 μg/L; Sigma-Aldrich, St. Louis, MO), and hydrocortisone (48 μg/L; Sigma-Aldrich). Near-confluent (~80–85%) MAC-T cells were seeded onto untreated 100-mm plastic dishes at 1×10^6 cells/dish overnight. Incremental concentrations of elaidic or linoleidic acid (Nu-Chek Prep, Elysian, MN) were prepared by adding a 20 m*M* tFA solution in ethanol to a 0.5 m*M* solution of fatty acid-free BSA at 33 parts to 1 part (MP Biomedicals, Solon, OH). This solution was vortexed vigorously and added to DMEM (without fetal bovine serum) culture medium in appropriate concentrations for experiments. Concentrations tested were based upon the relation of eladic acid to vaccenic acid (*trans*-11 18:1) output in milk fat (Mosley and McGuire, 2007) and analysis of elaidic acid concentrations in various plasma lipid pools of lactating cows (Tyburczy et al., 2008). Triplicate dishes were used for each FA concentration and time point. Cells were then washed 3 times with Hanks' Balanced Salt Solution (**HBSS**; Sigma-Aldrich) and cultured with treatment medium. Control medium contained an equimolar amount of BSA with no tFA. Cells were incubated at 37°C and 5% CO_2 for 0.75, 1.5, 3, 4.5, and 6 h (short) or 24 and 48 h (long), and then washed with HBSS. Total RNA was extracted (Qiagen Mini Plus kit with gDNA eliminator column; Qiagen, Valencia, CA) from each culture dish and cDNA was generated using the High Capacity Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Gene expression of IL-1β, intercellular adhesion molecule (**ICAM**)-1, IL-6, and IL-8 was measured in triplicate using a 7500 Fast real-time PCR system (Applied Biosystems) using custom designed TaqMan MGB probes (Applied Biosystems) described previously (Aitken et al., 2009). Glyceraldehyde 3-phosphate dehydrogenase was used as a housekeeping gene in all samples. Primers (forward: GCTACACTGAGGACCAGGTT; reverse: AGCATCGAAGGTAGAAGAGT-

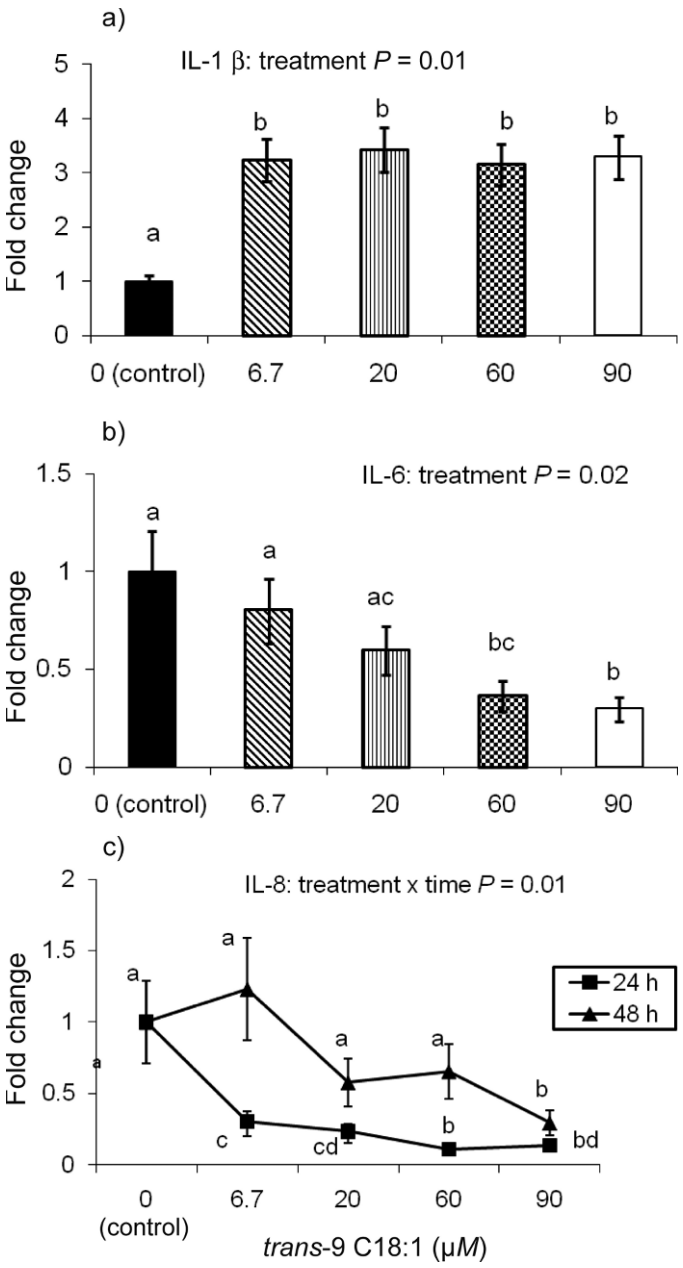

Figure 1. Gene expression (fold change) of inflammatory markers in mammary epithelial (MAC-T) cells exposed to incremental concentrations (0 to 90 μ*M*) of elaidic acid (*trans*-9 C18:1). Original Δ cycle threshold (Ct) values (Ct values normalized with GAPDH as housekeeping gene) were used for statistical analysis. a) An effect of treatment ($P = 0.01$) was observed over 6 h on expression of IL-1β mRNA. B) Although no significant effect of treatment was observed over 6 h on expression of IL-6 mRNA, an effect of treatment ($P = 0.02$) was observed over 48 h. c) Although no significant effect of treatment was observed over 6 h on expression of IL-8 mRNA, an effect of treatment by time ($P = 0.01$) was observed over 48 h. Means ± SEM are illustrated in fold change ($2^{-\Delta\Delta Ct}$ method) relative to control (no *trans* fatty acids). Columns (Figures 1a and 1b) or points on either the 24- or 48-h line relative to control (Figure 1c) that do not share a similar letter differ ($P \leq 0.05$).