J. Dairy Sci. 101:1–9
https://doi.org/10.3168/jds.2017-13550

Milk from cows fed a diet with a high forage:concentrate ratio improves inflammatory state, oxidative stress, and mitochondrial function in rats

Gina Cavaliere,* Giovanna Trinchese,* Nadia Musco,† Federico Infascelli,† Chiara De Filippo,* Vincenzo Mastellone,† Valeria Maria Morittu,‡ Pietro Lombardi,† Raffaella Tudisco,† Micaela Grossi,† Vincenzo Monda,§ Monica I. Cutrignelli,† Antonietta Messina,§ Serena Calabrò,† Heleena B. Moni,* Luigi Stradella,# Giovanni Messina,‖ Marcellino Monda,§ Marianna Crispino,* and Maria Pina Mollica*[1]
*Department of Biology, and
†Department of Veterinary Medicine and Animal Production, University of Naples Federico II, 80134 Napoli, Italy
‡Department of Health Science, University Magna Graecia of Catanzaro, 88100 Catanzaro, Italy
§Department of Experimental Medicine, and
#Department of Gynecology, Obstetric and Reproductive Science, University of Campania "Luigi Vanvitelli," 81100 Caserta, Italy
‖Department of Clinical and Experimental Medicine, University of Foggia, 71122 Foggia, Italy

ABSTRACT

Excessive energy intake may evoke complex biochemical processes characterized by inflammation, oxidative stress, and impairment of mitochondrial function that represent the main factors underlying noncommunicable diseases. Because cow milk is widely used for human nutrition and in food industry processing, the nutritional quality of milk is of special interest with respect to human health. In our study, we analyzed milk produced by dairy cows fed a diet characterized by a high forage:concentrate ratio (high forage milk, HFM). In view of the low n-6:n-3 ratio and high content of conjugated linoleic acid of HFM, we studied the effects of this milk on lipid metabolism, inflammation, mitochondrial function, and oxidative stress in a rat model. To this end, we supplemented for 4 wk the diet of male Wistar rats with HFM and with an isocaloric amount (82 kJ, 22 mL/d) of milk obtained from cows fed a diet with low forage:concentrate ratio, and analyzed the metabolic parameters of the animals. Our results indicate that HFM may positively affect lipid metabolism, leptin:adiponectin ratio, inflammation, mitochondrial function, and oxidative stress, providing the first evidence of the beneficial effects of HFM on rat metabolism.
Key words: n-6:n-3 ratio, inflammatory status, redox status, mitochondrial function

INTRODUCTION

Recently, awareness of the importance of diet to human health has increased. Excessive energy intake, and particularly the excess or inadequate processing of fat in the body, may lead to activation of complex biochemical processes such as inflammation, oxidative stress, and impairment of mitochondrial function (Hernández-Aguilera et al., 2013). These processes are the main factors that underlie aging and noncommunicable diseases, the main types of which are cardiovascular and chronic respiratory diseases, cancers, and diabetes. Indeed, an unhealthy diet often leads to obesity and metabolic disturbances, which have become a serious public health issue worldwide. In this regard, it bears emphasizing that understanding the cellular and molecular mechanisms underlying these metabolic diseases is a crucial step in their prevention and treatment.

Mitochondria, the primary cellular energy-generating system, are known to synthesize key molecules during inflammation and oxidation and thereby serve as the main source of free radicals. Therefore, it is no surprise that mitochondrial dysfunctions are associated with inflammation and other energy-dependent disturbances where cellular oxidative damage is caused by the generation of reactive oxygen species (**ROS**) exceeding the natural antioxidant activity (Chan, 2006). A growing body of evidence has suggested that a low-grade, chronic inflammatory state may be linked to obesity and its comorbidities, as well as to noncommunicable diseases (Hernández-Aguilera et al., 2013). It is important to underline that the metabolic changes induced by inflammation include alterations in mitochondrial function. Therefore, mitochondrial dysfunction can be both the cause and consequence of inflammatory processes and elicit metabolic adaptations that might be either protective or become progressively detrimental (Currais, 2015).

Various nutritional components are known to modulate the inflammatory state, mitochondrial function,

Received July 23, 2017.
Accepted November 13, 2017.
[1]Corresponding author: mariapia.mollica@unina.it

and ROS production, thus influencing metabolic homeostasis. To prevent or limit metabolic disorders, special attention should be paid to the choice of appropriate nutritional strategies. The fatty acid profile—in particular, the content of the essential fatty acids n-3 and n-6—is considered an important parameter to determine the nutritional value of food (Daley et al., 2010). These 2 classes of essential fatty acids, not interconvertible, are metabolically and functionally distinct and often have different physiological functions (i.e., pro- and anti-inflammatory activity for n-6 and n-3, respectively). In particular, a low n-6:n-3 ratio, ranging from 2 to 4, is considered optimal for human health (Simopoulos, 2002). Recent studies have shown that diet is the decisive factor determining the fatty acid profile of cow milk (Sterk et al., 2011); for instance, a high forage:concentrate ratio (**F:C**) results in a milk with low n-6:n-3 ratio.

The conjugated linoleic acids are a group of healthy fatty acids. They are positional and geometric isomers derived from octadecadienoic acid, whose content is high in milk fat, and the CLA have been suggested to have immunomodulating, anticarcinogenic, and anti-atherosclerosis properties (Dilzer and Park, 2012). The major isomer of CLA, *cis*-9,*trans*-11 (rumenic acid), represents up to 80% of total CLA in food. Ruminant CLA comes from 2 sources: (1) rumen biohydrogenation and (2) endogenous synthesis in the mammary gland and adipose tissue by the activity of stearoyl-CoA desaturase on *trans*-11 18:1, the biohydrogenation intermediate of several 18-carbon UFA (Shingfield et al., 2010). The CLA level in the milk from different ruminant species is significantly increased when animals are fed with fresh forage (Jahreis et al., 1997; Kelly et al., 1998; Griinari and Bauman, 1999; Tudisco et al., 2010, 2012, 2014).

Based on this data, some Italian breeders are feeding dairy cows with a high F:C (70:30), which is different from that used in intensive farms (which range from 55:45 to 35:65). By feeding animals a diet with high F:C, milk with a low n-6:n-3 ratio and high CLA level was obtained to satisfy consumer demand for healthy foods (Rubino, 2014).

Several studies have indicated that administration of CLA and n-3 fatty acids to rats improves fatty acid oxidation and decreases inflammation and oxidative stress through the modulation of mitochondrial function (Lionetti et al., 2014; Mollica et al., 2014; Cavaliere et al., 2016). We hypothesized that milk from cows fed a high-forage diet (hereafter, high forage milk, **HFM**), by modulating mitochondrial function, would ameliorate the inflammatory state and oxidative stress in consumers. To test this hypothesis, we evaluated, in a rat model, the effects of HFM administration on energy balance, lipid metabolism, and anti-inflammatory and antioxidant defenses, compared with those rats fed isoenergetic amounts of milk obtained from cows fed with a diet with a low F:C ratio (low forage milk, **LFM**).

MATERIALS AND METHODS

Cow Feeding

Milk was obtained from a farm located in a hilly area of central Italy (Segni, Rome, Italy; 13°0′E, 41°41′N, 668 m above sea level). The farm produced 2 types of commercial milk (LFM and HFM) from Italian Friesian cows (~40 animals for each type of milk) fed 2 different diets (lower or higher F:C ratio, respectively). Ingredients, F:C ratio, chemical composition, and nutritive value of the 2 cow diets as well as feed intake are reported in Table 1.

Rat Handling and Feeding

Male Wistar rats (Charles River, Calco, Lecco, Italy) were individually caged in a temperature-controlled room and exposed to a daily 12-h light/12-h dark cycle with free access to chow and drinking water. Young animals (60 d old; about 350 g of BW) were used; one group (n = 7) was killed at the beginning of the study to establish baseline measurements. The remaining rats, fed with a standard diet, were divided

Table 1. Diets fed to cows[1]

Item	LFM	HFM
Diet ingredients (kg as fed)		
Corn silage	20.0	—
Mixed hay[2]	—	7.0
Alfalfa hay	5.0	8.5
Wheat bran	1.6	1.0
Corn meal	3.5	3.0
Triticale	1.5	1.0
Fava bean	—	2.0
Sunflower panel	1.6	—
Soybean meal	1.8	—
Forage:concentrate ratio, DM basis	55:45	70:30
Intake (kg of DM)	18.9	19.3
Chemical composition (g/kg of DM)		
CP	150.0	130.0
Crude fat	29.1	18.8
NDF	386.0	491.0
ADF	266.0	403.0
ADL	79.2	103.0
Starch	138.0	96.4
Ash	63.6	78.9
NE_L (MJ/kg)	6.3	5.7

[1]LFM and HFM = diets fed to cows to produce low and high forage milk that was later fed to rats in an animal model.

[2]*Vicia sativa*, *Avena sativa*, *Lolium multiflorum*, *Trifolium alexandrinum*, and *Trifolium squarrosum*.