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Metabolism of 2-hydroxy-4-(methylthio)butanoate (HMTBA) in lactating dairy cows

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ABSTRACT

The objectives of the current study were to determine the fate and contribution to Met kinetics of 2-hydroxy-4-(methylthio)butanoate (HMTBA) at the whole-body, splanchnic, and mammary levels. Four multicatheterized cows (31.3 kg of milk/d; 17.7 kg of DMI/d) were used in a crossover design, with two 1-wk periods, to determine the metabolic fate of HMTBA and its effect on Met metabolism. Over the last 2 d of each period, cows were infused, via a jugular vein, with saline or HMTBA (Alimet, Novus International Inc., St. Louis, Mo) at the rate of 36 g/d. During the last 8 h, the HMTBA infusion was substituted by equimolar [1-^{13}C] HMTBA (8.79 mmol/h) and L[methyl-$^{2}H_3$]Met (1.31 mmol/h) was infused in all cows. During the last 5 h, hourly samples (n = 6) were collected to determine plasma flows plus the isotopic enrichments (IE) and concentrations of HMTBA (^{13}C) and Met (both ^{13}C and $^{2}H_3$) in plasma from an artery plus portal, hepatic, and mammary veins. The IE of [^{13}C] and [$^{2}H_3$] Met were also determined in milk protein taken over the last 1 h of infusion in HMTBA-infused cows. The infused HMTBA increased whole-body plasma flux of Met by 6.5 mmol/h (from 17.9 to 24.4 mmol/h). Based on enrichments of ^{13}C-labeled Met, 3.8 mmol/h of Met flow through plasma was derived directly from HMTBA. These 2 estimates accounted for between 43 to 74% of the HMTBA dose infused, contributing to increased whole-body Met availability. Although the portal-drained viscera, liver, and mammary gland (MG) extracted 11, 37, and 3.4%, respectively, of the infused HMTBA, tissue net Met fluxes were either unchanged (portal-drained viscera, MG) or even reduced (liver: −7.9 vs. −2.4 ± 0.6 mmol/h). Therefore, net postsplanchnic supply of Met decreased from 7.0 to 2.9 mmol/h between control and HMTBA-infused cows, compared with needs for milk protein secretion of 7.6 and 8.1 mmol/h, respectively. The HMTBA provided directly 15% of the Met required for milk protein secretion, with 0.2 mmol/h synthesized within the MG, whereas 1.1 mmol/h originated from Met produced in other tissues and transported to the MG through blood circulation. Most of the remainder needed by the MG arose from unlabeled Met released from protein breakdown in extra-splanchnic tissues and that was not reused to support intracellular protein synthesis, as this function was performed by Met synthesized from HMTBA in situ. Absorbed HMTBA, therefore, both produces and spares Met for use by the MG.
Key words: analog, methionine, splanchnic, mammary

INTRODUCTION

The hydroxy analog of methionine, 2-hydroxy-4-(methylthio)butanoate (**HMTBA**), has long been proposed as a means to provide Met and increase milk and protein yields of dairy cows fed rations limited in Met (Polan et al., 1970). The analog HMTBA is more resistant to rumen microbial degradation than L-Met (Belasco, 1972), but estimations of postrumen availability of HMTBA vary greatly. In dairy cows, between 5% (Noftsger et al., 2005) and 50% (Koenig et al., 1999, 2002) of the ingested dose was reported to flow postrumen. Direct measures of net portal absorption of HMTBA averaged 13% of the ingested dose (Lapierre et al., 2007), although this is an underestimate that does not account for any conversion to Met or metabolism by gut tissues (McCollum et al., 2000; Lobley et al., 2006b). In practice, the effectiveness of HMTBA in dairy cow husbandry depends not only on the amount absorbed across the gastrointestinal tract but also on the metabolic conversion to Met by body tissues. Most previous studies have focused on the former aspect and few data are available on the fate of HMTBA absorbed by ruminants.

The HMTBA feed supplement is a racemic mixture of D- and L-isomers that can both be converted to 2-oxo-

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4-methylthiobutanoate (also produced by deamination of D-Met) followed by amination to L-Met. The oxidase for L-HMTBA exists predominantly in the peroxisomes within liver and kidney, whereas the dehydrogenase for D-HMTBA is a mitochondrial enzyme found in most tissues (Dibner and Knight, 1984; McCollum et al., 2000; Dibner, 2003). Despite this wide distribution of tissue enzyme activities, most of the studies conducted in chickens have focused their attention at studying the liver as the site of both removal (Wang et al., 2001) and metabolism of HMTBA (Dibner and Ivey, 1992). However, recent studies in ruminants have shown that conversion of HMTBA to L-Met does occur across a range of tissues both in vitro (McCollum et al., 2000) and in vivo (Lobley et al., 2006a). For example, for sheep infused with labeled HMTBA, all tissues studied synthesized Met, but to different extents, with the highest rates for liver and kidney. Interestingly, most of the newly synthesized Met exported into the circulation was derived from renal metabolism (Lobley et al., 2006a,b). Although the ovine liver extracted 25% of the absorbed HMTBA, the net hepatic flux of Met decreased with HMTBA supplementation. This was despite increases in both plasma concentrations and whole-body flux for Met, indicating both production and release of Met derived from HMTBA in peripheral (nonhepatic) tissues (Wester et al., 2006). In dairy cows, the liver also extracted a considerable proportion (34%) of absorbed HMTBA (Lapierre et al., 2007), but no evaluation of the concomitant effect on Met kinetics was conducted. Indeed, there are no reports on the metabolic fate of HMTBA in dairy cows, where the demand for Met is exacerbated to sustain milk protein yield.

Therefore, the objectives of the current study were to determine the fate of HMTBA at the whole-body level and across both splanchnic and mammary tissues and the contribution to Met kinetics across these organs.

MATERIALS AND METHODS

Animals and Treatments

Four cows, averaging 650 ± 92 (SD) kg and 131 ± 12 DIM at the beginning of the study, were used in a randomized crossover design with two 1-wk experimental periods. Cows had been implanted, at least 4 mo before the start of the project, with catheters into the portal vein, one hepatic vein, and the caudal aorta via a mesenteric artery for blood sampling and in 2 distal mesenteric veins for para-amino hippuric acid (***p*AH**) infusion to determine splanchnic blood flow (Huntington et al., 1989). Cows were fed a fixed amount of TMR every 2 h in equal meals plus 1 kg of hay/d (Tables 1 and 2). The diet was balanced to provide sufficient crude, degradable and nondegradable protein, energy, and MP, but with Met contributing only 1.83% of MP and, therefore, estimated as deficient (NRC, 2001; Table 1). The cows were kept in a tie-stall barn and were milked twice per day, at 12-h intervals. During the last 2 d of each experimental period, one jugular vein of each cow was infused with either saline (control) or unlabeled HMTBA (1.5 g/h; Alimet, Novus International Inc., St. Louis, MO; 88% = 8.79 mmol/h). During the last 8 h of infusion, the jugular vein of each cow was infused with L[methyl-$^{2}H_3$]Met (1.31 mmol/h) and over the same period when cows received unlabeled HMTBA, this was substituted by [1-^{13}C]HMTBA (also at 8.79 mmol/h). In order to determine the concentrations and the isotopic enrichment (**IE**) of HMTBA (^{13}C-HMTBA), Met (^{13}C and $^{2}H_3$-Met), and [^{13}C]bicarbonate in the arterial, portal, hepatic, and mammary plasma, hourly blood samples were simultaneously collected from arterial, portal, hepatic, and mammary sources from 3 to 8 h of the infusion of labeled Met. Mammary blood samples were collected by venipuncture. In addition, the cows were milked 7 and 8 h after the initiation of the labeled Met infusion, with each milking conducted following oxytocin injection. Milk was sampled at this last milking for determination of the enrichment of Met in CN in HMTBA-infused cows. During the sampling period, *p*AH (14.4 g/h preceded by a priming dose of 2 g) was infused into the mesenteric vein of cows, beginning at least 40 min before the first blood sample. Prior to the initiation of the infusions of labeled material, blood samples were collected from all of the vessels to determine the natural abundance of HMTBA, Met, and CO_2.

Immediately after collection, 3-mL samples of blood, collected in airtight syringes, were analyzed for partial pressure of CO_2 (pCO_2) and pH with a blood gas analyzer (model IL 1306, Instrumentation Laboratory, Lexington, MA). Larger blood samples (10 mL) were collected in heparinized syringes and two 1-mL blood portions were injected into evacuated vacutainers containing 1 mL of frozen lactic acid for measurement of CO_2 enrichment (Read et al., 1984) to determine HMTBA (plus Met) oxidation across the tissues. A small portion of blood was immediately used to determine hematocrit by the microcentrifuge method. The remainder of the blood was immediately placed on ice and centrifuged (15 min, 1,800 × *g* at 4°C) within 30 min of collection to yield plasma. For analysis of Met, Phe, and Tyr concentrations, 1 g of fresh plasma was added to 0.2 g of an internal standard of AA labeled with stable isotopes. The internal standard solution was prepared with labeled AA diluted in water with the following concentrations: DL-[1-^{13}C]Met (86 μ*M*); L-[1-^{13}C]Phe (247 μ*M*); and L-[^{15}N]Tyr (245 μ*M*).