Short Communication: Net Uptake of Nonesterified Long Chain Fatty Acids by the Perfused Caudate Lobe of the Caprine Liver

D. G. Mashek and R. R. Grummer¹

Department of Dairy Science
University of Wisconsin, Madison 53706

ABSTRACT

The objective was to determine whether net uptake of various nonesterified long chain fatty acids differs in the caprine liver. Caudate lobes were isolated from four mature goats and perfused (1 ml/min $\times$ g wet tissue) with buffer containing 0.3 mM of each palmitic, stearic, oleic, linoleic, linolenic, eicosapentaenoic, and docosahexaenoic acids. The amount of fatty acid in the perfusate decreased over time for all fatty acids with the exception of stearic acid. There was no net uptake of stearic acid, which was significantly different from all other fatty acids examined, with the exception of oleic acid. Net hepatic uptake of oleic acid was numerically, but not significantly lower than palmitic, linoleic, linolenic, eicosapentaenoic, and docosahexaenoic acids. It was concluded that net uptake of fatty acids was similar for all fatty acids tested with the exception of stearic acid.

(**Key words:** fatty acids, hepatic uptake)

The ruminant liver extracts between 12 to 25% of plasma NEFA during each pass through the liver under in vivo conditions (Emery et al., 1992). Uptake of NEFA is the predominant route by which fatty acids are supplied to the liver (Emery et al., 1992). However, few studies have measured hepatic uptake of individual long-chain fatty acids in ruminant animals. Thompson and Darling (1975) and Thompson et al. (1975) studied hepatic net uptake of palmitic, stearic, and oleic acids in sheep. These studies showed no differences between palmitic and oleic acid net uptake by the liver. However, hepatic net uptake of stearic acid was lower than palmitic and oleic acids when expressed as a percentage of the concentration reaching the liver. Hepatic net uptake of other long-chain fatty acids is unknown in rumi-

nants. Therefore, the objective of the current study was to compare hepatic net uptake of various nonesterified long-chain fatty acids in the perfused caprine liver.

Four crossbred male goats between the ages of 4 and 10 mo were used. The goats were anesthetized with 1.5 g of sodium thiopental (Abbott Laboratories, North Chicago, IL) and the caudate lobe of the liver (39 ± 7.9 g; mean $\pm$ SD) was removed. After removal of the caudate lobe, the goats were euthanized with 10 ml of Beuthanasia-D Special (Schering-Plough, Union, NJ). The isolated caudate lobe was immediately flushed with 120 ml of Krebs-Ringer buffer and transported to a warm room maintained at 37°C. A catheter was inserted into the caudate lobe and buffer was perfused at a rate of 1 ml/min $\times$ g wet tissue. Buffer was oxygenated by continuous flow through a Hospal dialysis membrane (Gambro Renal Products, Lakewood, CO). After 2 min, the Krebs-Ringer buffer was replaced with 250 ml of Krebs-Ringer buffer containing 0.3 mM each of palmitic, stearic, oleic, linoleic, linolenic, eicosapentaenoic, and docosahexaenoic acids bound to albumin in a 4:1 fatty acid:albumin ratio. We chose 0.3 mM of each fatty acid because many fatty acids approach or exceed that concentration in serum near parturition (Rukkamsuk et al., 2000). Having all the fatty acids at 0.3 mM, as opposed to different physiological concentrations of each fatty acid, allows for direct comparisons of fatty acids without the confounding effects of concentration. A 10-ml sample of the media was taken before the start of the perfusion and at 10-min intervals for the next 60 min.

Lipid was extracted from 4 ml of perfusion media according to Dole (1956) following the addition of butylated hydroxytoluene (0.01%) to each tube. Extracts were resuspended in 50 μ l of chloroform:methanol (2:1) and 25 μ l were spotted onto Adsorbosil Plus 1 silica gel plates (Alltech Associates Inc., Deerfield, IL), which were developed with hexane:diethyl ether:glacial acetic acid (90:10:1). Plates were dried under nitrogen and spots corresponding to fatty acids were visualized with 5% Rhodamine G in ethanol. The silica gels were extracted twice with chloroform:methanol (2:1), and the lipids were dried under nitrogen. Methyl esters were formed according to Sukhija and Palmquist (1988) and

Received June 28, 2002.

Accepted November 28, 2002.

Corresponding author: R. R. Grummer; e-mail: rgrummer@wisc.edu

¹Partially funded by: Bioproducts Inc., Church and Dwight, Diamond V, Degussa, Kemin Industries, Land O'Lakes/Farmland Feed, Archer Daniels Midland, Pioneer Hybrids and Zinpro.

were analyzed by gas chromatography. Margaric acid (C17:0) was used as an internal standard for all samples.

Raw data were recorded as the amount of individual fatty acids in the perfusate. To account for differences in perfusate loss due to sampling, the total amount of each fatty acid in the perfusate was multiplied by the amount of preperfusion perfusate (250 ml) divided by the amount of perfusate remaining at the time of the individual sample. Data were analyzed using the mixed procedure of SAS (SAS, 1999). Individual slopes for the amount of fatty acid in the perfusate (dependent variable) over time were generated for each goat using a model that included fatty acid and time within fatty acid. The original model tested for linear and quadratic effects; however, the quadratic term was removed from the model due to lack of significance ($P > 0.25$). The slopes representing changes in the amount of fatty acid in the perfusate over time for each goat were then compared using a model containing the fixed effects of fatty acid and the random effects of goat. Treatment differences were tested using the Tukey-Kramer adjustment. To generate least-squares means for the amount of fatty acid in the perfusate over time, a separate model containing fixed effects of fatty acid, time within fatty acid, and the random effect of goat was used. When analyzing the above data, we included fatty acid in the model even though each fatty acid was not applied to a separate lobe. Therefore, an assumption in the analysis is that the presence of an individual fatty acid does not influence the uptake of another fatty acid. The experiment was designed and analyzed in this way because using individual caudate lobes for each fatty acid would be inefficient. Additionally, a mixture of fatty acids in the perfusate is more representative of physiological conditions than if the perfusate only contained one fatty acid. Positive slopes for the amount of fatty acid in the perfusate over time indicate net release and negative slopes indicate net uptake of fatty acids by the liver. Data are presented as an amount of the time zero (preperfusion) sample.

Preperfusion fatty acid concentrations were 296, 292, 298, 281, 284, 288, and 295 μM for palmitic, stearic, oleic, linoleic, linolenic, eicosapentaenoic and docosahexaenoic acid, respectively. The slopes for relative amounts of individual fatty acids contained in the perfusate over time are shown in Table 1 and Figure 1. All fatty acids, with the exception of stearic and oleic acids, had significant ($P < 0.05$) negative slopes thus indicating net uptake of fatty acids. Stearic acid had a positive, nonsignificant slope ($P > 0.15$), which was significantly different ($P < 0.05$) from all other fatty acids tested, with the exception of oleic acid. Oleic acid had a negative slope (i.e., net uptake), but the slope only tended

Table 1. Slopes representing the net hepatic uptake for individual fatty acids. Slopes were obtained by regressing the amount of fatty acid in the perfusate over time relative to the time zero sample.

Fatty acid	Slope	SEM
Palmitic	-.61 ^b	0.18
Stearic	.21 ^a	0.18
Oleic	-.33 ^{ab}	0.18
Linoleic	-.61 ^b	0.18
Linolenic	-.62 ^b	0.18
Eicosapentaenoic	-.63 ^b	0.18
Docosahexaenoic	-.67 ^b	0.18

^{ab}Slopes with unlike superscripts differ ($P < 0.05$).

to be different from zero ($P < 0.15$). The slope of oleic acid was not different ($P > 0.15$) from that of palmitic, linoleic, linolenic, eicosapentaenoic, and docosahexaenoic acids.

Data from the present study support the *in vivo* data of Thompson and Darling (1975) and Thompson et al. (1975), which showed low or negative stearic acid net hepatic uptake and similar net hepatic uptakes of palmitic and oleic acids in ruminants. Studies in rodent and human hepatocytes have shown decreased (Emmison and Agius, 1988; Bruce and Salter, 1996) or similar (Kvilekval et al., 1994; Dokko et al., 1998) stearic acid net uptake by the liver compared with other long-chain fatty acids. However, the mechanisms regulating stearic acid uptake or release have not been elucidated. Fatty acid transport into cells is thought to involve both facilitative and passive processes (Abumrad et al., 1998). In either process, the amount of fatty acid dissociated from albumin is thought to be a determinant for uptake into the cell (Stremmel et al., 2001). Recently, Demant et al. (2002) showed that stearic acid had the

Figure 1. Relative concentrations of individual NEFA in the perfusate over time. Data are expressed as the amount of fatty acid contained in the perfusate relative to the time zero sample (preperfusion).

lowest rate constant for dissociation from bovine serum albumin compared to palmitic, oleic, linoleic, and linolenic acids. Therefore, the binding affinity of fatty acids to albumin may be an important factor regulating their uptake.

Transport through the cell membrane is also thought to be a rate-limiting process in fatty acid uptake (Hamilton, 1998). The rates of desorption of fatty acids from phospholipid bilayers decreased with increasing chain length and increased with addition of each double bond (Zhang et al., 1996). Thus, it could be speculated that a low rate of removal from the cell membrane into the cytosol may impede stearic acid uptake, whereas uptake of the shorter or more unsaturated chain fatty acids are less hindered.

In summary, changes in the amounts of fatty acid in the perfusate over time were similar among most long-chain fatty acids tested with the exception of stearic acid. The reason for different behavior of stearic acid is unknown, but it may be related to the hydrophobic nature of the acid.

REFERENCES

- Abumrad, N., C. Harmon, and A. Ibrahim. 1998. Membrane transport of long-chain fatty acids: evidence for a facilitated process. *J. Lipid Res.* 39:2309–2318.
- Bruce, J. S., and A. M. Salter. 1996. Metabolic fate of oleic, palmitic, and stearic acid in cultured hamster hepatocytes. *Biochem. J.* 316:847–852.
- Demant, E. J. F., G. V. Richier, and A. M. Kleinfeld. 2002. Stopped-flow kinetic analysis of long-chain fatty acid dissociation from bovine serum albumin. *Biochem. J.* 363:809–815.
- Dokko, R. C., Cho, B. H. S., and B. H. Chung. 1998. Cellular uptake of stearic, oleic, linoleic, and linolenic acid and their effects on synthesis and secretion of lipids in Hep-G2 cells. *Int. J. Biochem. Cell Biol.* 30:65–76.
- Dole, V. P. 1956. A relationship between non-esterified fatty acids and glucose in plasma and the metabolism of glucose. *J. Clin. Invest.* 35:150–154.
- Emery, R. S., J. S. Liesman, and T. H. Herdt. 1992. Metabolism of long chain fatty acids by ruminant liver. *J. Nutr.* 122:832–837.
- Emmison, N., and L. Agius. 1988. Fatty acid uptake and metabolism to ketone bodies and triacylglycerol in rat and human hepatocyte cultures is dependent on chain-length and degree of saturation. *FEBS Lett.* 236:83–88.
- Hamilton, J. A. 1998. Fatty acid transport: Difficult or easy? *J. Lipid Res.* 39:467–481.
- Kvilekval, K., J. Lin, W. Cheng, and N. Abumrad. 1994. Fatty acids as determinants of triglyceride and cholesteryl ester synthesis by isolated hepatocytes: Kinetics as a function of various fatty acids. *J. Lipid Res.* 35:1786–1794.
- Rukkwamsuk, T., M. J. H. Geelen, T. A. M. Kruip, and T. Wensing. 2000. Interrelation of fatty acid composition in adipose tissue, serum, and liver of dairy cows during the development of fatty liver postpartum. *J. Dairy Sci.* 83:532–539.
- SAS. 1999. SAS/STAT User's Guide (Version 8.1), SAS Inst. Inc., Cary, NC.
- Stremmel, W., J. Pohl, A. Ring, and T. Herrmann. 2001. A new concept of cellular uptake and intracellular trafficking of long-chain fatty acids. *Lipids* 36:981–989.
- Sukhija, P. S., and D. L. Palmquist. 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. *J. Agric. Food Chem.* 36:1202–1206.
- Thompson, G. E., and K. F. Darling. 1975. The hepatic uptake of individual free fatty acids in sheep during noradrenaline infusion. *Res. Vet. Sci.* 18:325–327.
- Thompson, G. E., J. W. Gardner, and A. W. Bell. 1975. The oxygen consumption, fatty acid and glycerol uptake of the liver in fed and fasted sheep during cold exposure. *Q. J. Exp. Phys.* 60:107–121.
- Zhang, F., F. Kamp, and J. A. Hamilton. 1996. Dissociation of long and very long chain fatty acids from phospholipid bilayers. *Biochemistry* 35:16055–16060.