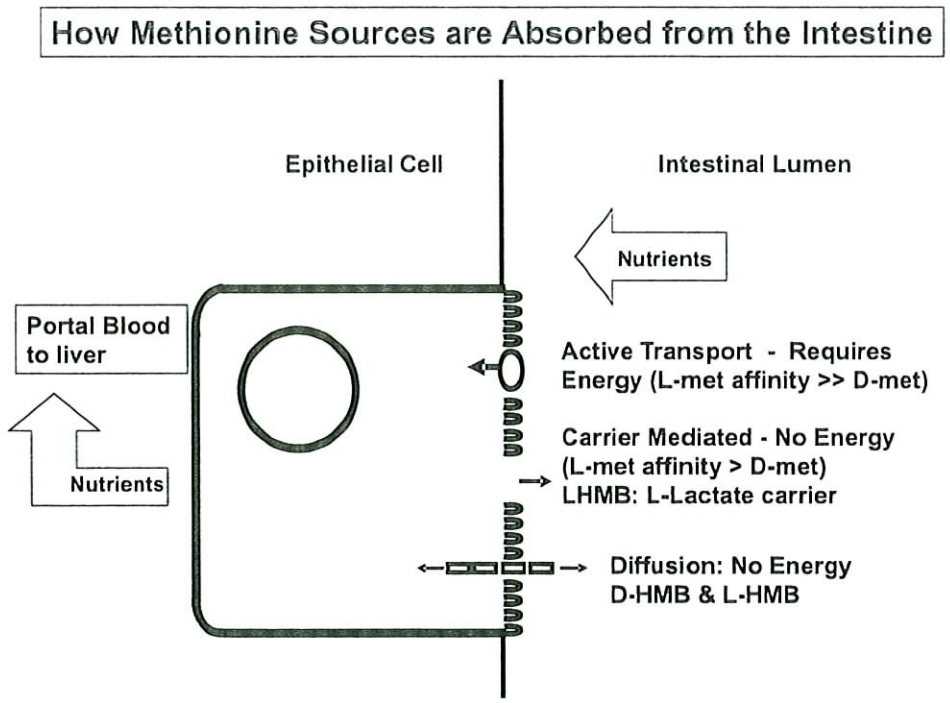
Figure 3 Schematic of the various transport systems in the gut and their participation in methionine source uptake. L-Met and D-met compete for the same carrier although D-met is much lower affinity. There is a minor component of HMB uptake that uses an energy independent carrier system that also transports L-lactate. Most of the uptake of HMB occurs in the upper gut, where low pH allows the undissociated acid to diffuse across cell membranes.




Thus, HMB absorption is very complex. The transport mechanism will not be the same along the entire length of the gut. For these reasons, the model system chosen is a particularly important consideration. To compare rates of absorption of HMB and methionine, the model system must resemble the foregut with its low pH and continuous blood flow. A concentration gradient must be maintained to permit continuing diffusion. This is only an issue *in vitro*, because blood flow maintains the concentration gradient *in vivo*. It is an important consideration, however, in the interpretation of *in vitro* models of nutrient absorption such as enterocyte or membrane vesicle uptake studies. These can be used to study mechanism of absorption, but the results should not be extrapolated to relative rates of absorption *in vivo*.

To look at comparative rates of absorption, the most straightforward data interpretation is restricted to *in vivo* measurements where a diffusion gradient can be maintained by blood flow. Therefore, in order to determine the rate and site of maximum absorption, appearance of $^{14}$C-HMB was measured following its injection into ligated segments of duodenum, jejunum and ileum of living birds. Of the three segments tested, duodenum was the most effective in the absorption of HMB. Overall rate of appearance in the blood stream of the animal was very similar for HMB and L-met (Knight and Dibner, 1984).

Similar absorption rates for HMB and L-met or DL-met in the intact animal have been reported by others. The data reported by Saroka and Combs (1983) from which they concluded that there was no difference in the rates of absorption between HMB and L-met, were obtained using a crop intubation in a live bird and then measuring the rate of

appearance in the blood stream. Similar data were obtained using intact or caecectomized cockerels (Han *et al.*, 1990) from which the authors conclude that the intestinal absorption of DL-HMB is highly efficient. Finally, Esteve-Garcia and Austic (1993) also studied the absorption and excretion of radiolabeled DL-HMB in broilers, and concluded that performance of birds fed Alimet at normal levels of supplementation would not be limited by inefficiencies in intestinal absorption or losses due to renal excretion. In contrast, the presence of dietary synthetic supplemental methionine appears to down-regulate the transporters required for both methionine and lysine absorption (Soriano-Garcia *et al.*, 1999).

## Use of HMB by liver cells

These studies were designed to study the use of HMB as a source of L-met for protein synthesis. Most nutrients are initially metabolised in the liver and since an important use of supplemental methionine is in the polypeptide chains of protein, a direct experimental approach was to examine HMB as a substrate for protein synthesis in chick liver cells. An additional goal in these studies was to compare protein synthesis rates in the presence of limiting amounts of HMB and DL-met. The liver cell studies were designed to compare the two sources on a cellular level to avoid some of the complexities inherent in growth studies, particularly those comparing amino acid sources. As discussed by Combs *et al.* (1968), feeding studies involving low levels of an essential amino acid, especially methionine, are often confounded by differences in feed intake at equimolar levels of supplementation. Performance in such studies cannot be used to assign a metabolic efficiency level to the amino acid source because intake differences affect all of the dietary nutrients, not just the one under study. Metabolic efficiency comparisons are only valid when intake is the same or when intake differences are included in the statistical model (Combs *et al.*, 1968; Baker, 1984). Studies in which intake was equalized have demonstrated molar equivalence of HMB and DL-met in crystalline amino acid diets (Knight *et al.*, 1991).

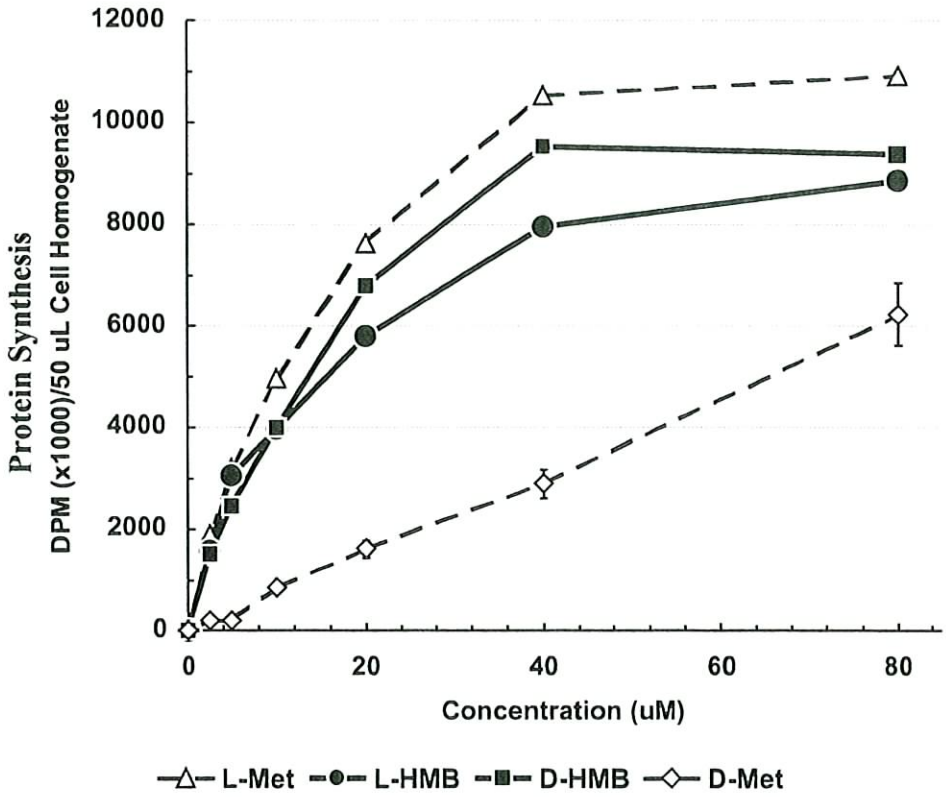
Two methods were used to study the use of HMB and DL-met by liver cell cultures. First, radiolabeled HMB or DL-met was used to study directly the incorporation of these molecules into protein synthesized by the cells in culture. Second, radiolabeled leucine was used as an indicator to study the ability of the sources to support protein synthesis. The first method had the advantage of being a direct measure of incorporation and the second method had the advantage of permitting the study of separate isomers of DL-met and DL-HMB, which could not be obtained in radiolabeled form.

The primary liver cell culture system was used to document a complete time course and dose response of the two sources. DL-met and HMB provided equal amounts of L-met in support of protein synthesis (Dibner, 1983). In addition, other time-course studies showed that incorporation was linear over the first 24-hour period. There were no significant differences in incorporation at any time point tested. Equivalent incorporation of HMB and DL-met was also observed in similar cultures of porcine hepatocytes (Knight *et al.*, 1998).

In addition to the work described above, the hepatocyte culture system was used to study protein synthesis with an indirect label, $^{14}$C-leucine. This permitted the study of material that could not be obtained in radiolabeled form. In order to test the reproducibility of data obtained using the direct label, the DL racemic mixtures of HMB and DL-met were tested in the indirect system (Dibner, 1985). This confirmed that the sources supported equal amounts of protein synthesis when leucine was used as the probe. *Figure 4* is an illustration of a study in which the separate isomers of HMB were compared with D and L

methionine in this indirect assay. It is very interesting that, although the racemic mixtures were equivalent, the separate isomers were quite different. Pure L-met, which is the end product of the conversion of both of the supplemental methionine sources is very well used, the two HMB isomers are intermediate and very close to one another, while D-met gives the lowest level of incorporation in chick liver cells. This means that, in the earlier assays of the racemic mixtures these differences between the D- and L-isomers of methionine effectively cancelled each other out, which could lead to the assumption that D-met is used better, in fact, than it really is. Similar differences between D-met and L-met protein incorporation have also been observed in the intact chick (Vazquez-Anon *et al.*, 2000).

**Figure 4 Dose related comparison of the four individual isomers using $^{14}$C-leucine incorporation as the indicator of protein synthesis. Incorporation of leucine was highest in cells fed L-met (△). Cells fed either the L (●) or D (■) isomer of HMB showed intermediate incorporation, while cells fed D-met (◇) showed significantly less protein synthesis.**




## Direct use of HMB by intestine and skeletal muscle

Although the D-HADH tissue distribution indicated that many tissues have the capability to oxidize D-HMB to KMB, it did not prove that these tissues convert D-HMB to L-met without the intervention of hepatic enzymes. In order to test this hypothesis, a short-term tissue culture method was developed that could test the ability of isolated tissues to