Measurement of Instant Rates of Protein Degradation in the Livers of Intact Mice by the Accumulation of Bestatin-induced Peptides*

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Violeta Botbol and Oscar A. Scornik
From the Department of Biochemistry, Dartmouth Medical School, Hanover, New Hampshire 03756

Bestatin induces the accumulation of di- and tripeptide intermediates in cellular protein breakdown. In liver, a single set of bestatin-sensitive cytosolic peptides are involved in the degradation to amino acids of the major classes of cellular proteins. Accumulation of bestatin-induced peptides, in isolated hepatocytes, is proportional to the rate of protein degradation (Botbol, V., and Scornik, O. A. (1989) J. Biol. Chem. 264, 13504–13509). Injection of 1 mg of bestatin into mice results in detectable amounts of hepatic intermediates in 15 min. We propose to use the accumulation of these peptides as a relative measurement of liver protein degradation. There is at present no other way to determine transient changes in protein breakdown in the tissues of intact animals.

As an example of the applications of this procedure, we present the effects of a single meal on hepatic protein metabolism. Protein synthesis was estimated by the incorporation into liver protein of a massive dose of radioactive leucine (Scornik, O. A. (1974) J. Biol. Chem. 249, 3876–3883) and degradation of long-lived or short-lived proteins by the accumulation of bestatin-induced peptides, labeled in carboxy-C of their Leu or Arg moieties, 1 day or 1 h beforehand. A single meal resulted in an 18% increase in liver protein in 8 h, a 45% increase in the rate of hepatic protein synthesis, and a 3-fold decrease in the rate of breakdown of long-lived proteins. Short-lived proteins were not affected.

To establish the efficiency with which bestatin-induced peptides accumulate in the livers of fasting mice, we compared them with the disappearance, in 1 day, of protein-bound 14C-guanidino-Arg residues, labeled by previous injection of 14C-bicarbonate (Swick, R. W., and Ip, M. M. (1974) J. Biol. Chem. 249, 6836–6841). From this comparison, we estimated that bestatin-induced Leu-labeled intermediates, accumulating in 15 min, represented 39% of the hepatic proteins degraded in that interval. For Arg-labeled intermediates the value was 55%. Correcting for these efficiencies, we estimate that in 4 h a meal decreased the rate of degradation of long-lived Arg-labeled proteins from 2.02 to 0.73%/h. For Leu-labeled proteins the estimated rates were 1.76 and 0.66%/h, respectively.

Although a transient slowdown of liver protein degradation after a single meal had been suggested before, this is the first time that acute changes such as this can be determined directly in intact animals. This novel procedure should be of use to establish the relative importance of nutrients and hormones in the regulation of hepatic protein turnover.

Mammalian liver, because of its high rate of protein turnover and its preferential access to absorbed dietary amino acids in the portal circulation, plays an important role in whole body protein metabolism. Transient changes in protein synthesis and degradation result in the storage of excess amino acids as additional liver protein after a meal, and in the utilization of the stored protein as an endogenous source of amino acids between meals (1, 2). Studies in perfused livers (3) and isolated hepatocytes (4) have established that liver protein turnover is subject to regulation by hormones, notably insulin and glucagon, and the plasma concentration of amino acids. To find out the relative physiological importance of these and other regulatory factors, however, isolated systems are no substitute for experiments in intact animals.

Instant rates of liver protein synthesis can be measured in live animals by a variety of procedures (2), but there has been up to now no reliable way to measure instant rates of protein breakdown. In perfused tissues or in isolated or cultured cells, average rates of protein degradation are usually estimated by the release to the medium of radioactive amino acids from labeled cellular proteins, in the presence of chosing concentrations of unlabeled amino acids. In intact animals this principle cannot be applied because the amino acids produced by protein breakdown are either reincorporated into proteins or catabolized. Average rates of protein degradation in tissues of intact animals have been estimated either as the balance between rates of protein synthesis and net protein changes, or by disappearance of radioactivity from protein labeled with metabolically unstable amino acids (2). These procedures require repeated determination in different groups of animals, over periods long enough to permit measurable changes in protein content and protein radioactivity, usually a day or longer. Transient changes over shorter intervals are usually too small for their accurate estimation with a reasonable number of animals. In this paper, we propose a novel way in which instant rates of liver protein degradation can be estimated in mice.

Work from this laboratory has shown that bestatin, an inhibitor of cytosolic peptidases, induces the accumulation of di- and tripeptide intermediates in the degradation of cellular proteins in liver (5, 6) and other mammalian cells (7–9). In liver, we have presented evidence that a single set of bestatin-sensitive peptidases are involved in the degradation of the major classes of cellular proteins and have suggested that accumulation of bestatin-induced peptides can serve as a relative measure of protein breakdown in intact mice. The conclusion is validated by the simultaneous determination of protein degradation and accumulation of bestatin-induced

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peptides in isolated hepatocytes under different conditions (6). In live mice, measurable amounts of hepatic radioactive peptides accumulate in 15 min (5) and provide a unique opportunity to estimate instant rates of protein breakdown. As an example, we present in this paper diurnal variations in the accumulation of bestatin-induced peptides in the livers of meal-fed mice. We discuss the advantages and limitations of this novel procedure.

**EXPERIMENTAL PROCEDURES**

**Materials**—Bestatin was a generous gift of Dr. Hamao Umezawa, Microbial Chemistry Research Foundation (Tokyo, Japan). L-[1-14C]Leucine, L-[1-14C]ornithine, and 14C-bicarbonate were purchased from Du Pont-New England Nuclear. Cation exchange resin AG-50W-X2 (200–400 mesh) and AG-1W-X2 (200–400 mesh) were from Bio-Rad. All other materials were of reagent grade.

**Animals**—Adult male CD1 mice (30–35 g) were purchased from Charles River Breeding Laboratories. The meal-fed animals were kept, 5 to a cage, with water ad libitum and food was provided only during the first 4 h of darkness (6–10 p.m.). Three days later their weight was determined before and after the meal; only animals that gained 2 or more grams after the 4-h period were used on the fourth day.

**Food Intake and Urinary Nitrogen**—For the measurement of food intake and urinary nitrogen the animals were kept for an additional day in individual metabolic cages. Urine was collected with 50 µl of glacial acetic acid (as a preservative), diluted, and frozen until the assay. Urinary nitrogen (urea + ammonia) was measured by conversion of the urea to ammonia by the action of urease and colorimetric determination of ammonia (Sigma kit #640).

**Liver Protein**—Transient changes in liver protein content after a meal are relatively small. We were concerned that a larger portal blood flow after a meal could result in increased residual blood in the organ and give misleading results. For this reason, total protein was determined in bloodless livers prepared as follows. Mice were killed at the indicated times after the intraperitoneal injection of 14C-bicarbonate (6 µCi). The radioactivity in the guanidino-C of protein-bound arginine was measured in two groups of mice, 24 and 48 h after the injection of 14C-bicarbonate (12). Liver protein was purified from liver homogenates by successive extractions with hot trichloroacetic acid, ethanol:ethyl ether, and acetone, and then fractionated by exhaustive percolation through a small column (5 × 6-mm inner diameter) of AG1 (bicarbonate form) (11). Arginine, because of the high pK of its guanidino group, passed through and was recovered in the eluent. Arginine was converted to urea by treatment with purified liver arginase, and in half of the sample urea was degraded to CO2 and NH3 by further incubation with jack bean urease (12). Urea and radioactivity were determined in both samples, and the specific radioactivity of the 14C-urea (originally the 14C-guanidino group of the arginine) was determined by difference, as described earlier (12).

**Protein Synthesis**—Mice were injected with a massive dose of L-[1-14C]leucine and in their arginine moieties by injection of L-[1-14C]ornithine, which is readily converted to arginine in the liver because of the activity of the urea cycle enzymes. The use of carboxyl-labeled precursors is required for the elimination of 14C label from free amino acids by acid-ninhydrin treatment (7). To study the degradation of short-lived proteins, bestatin (1 mg, intravenously) was injected 1 h after the radioactive precursor. For long-lived proteins, 24 h were allowed to elapse before the injection of bestatin. Measurable peptide radioactivity accumulates in 15 min (5). In our previous work we have presented evidence that: (a) bestatin-induced peptides are intermediates in the degradation of cellular proteins. (b) They comprise di- and tripeptides (c) They result, at least in liver, from the inhibition of a single set of cytosolic peptidases affecting the degradation of cellular, but not extracellular proteins (6). (d) The effects of bestatin are not restricted to the degradation of a particular class of proteins. It induces accumulation of intermediates in the degradation of most hepatic proteins, including short-lived, long-lived, and abnormal proteins (6).

One corollary of these observations is that accumulation of bestatin-induced peptides could serve as a relative measure of liver protein breakdown in intact mice. The conclusion was validated directly in isolated hepatocytes, in which protein degradation and accumulation of peptides could be measured simultaneously. A substantial portion of the peptides (50% with leucine and 90% with arginine) were retained by the cells after a 30-min incubation (6). The reason for suggesting this experimental approach is that there is no other direct way to
measure transient changes of protein degradation in tissues of intact animals (see Introduction). As an example of its application, we now present a study of diurnal variations of hepatic protein turnover in the livers of meal-fed mice.

**Diurnal Variations in Hepatic Protein Content in Meal-fed Mice**—Because feeding is discontinuous in most adult mammals, dietary amino acids must be stored as excess body proteins. The liver plays a major role in this storage. The hepatic storage of proteins is illustrated in Fig. 1. Mice are nocturnal animals; they eat primarily during the first few hours of darkness. If access to food is restricted to 4 h after light is turned off, they learn very quickly (2-3 days) to satisfy their daily food requirements in that period. Restriction of feeding to a well-defined daily meal is not drastically different from their spontaneous feeding pattern, but permits a sharp and controlled delineation of the diurnal cycle (15). In the 4-h period, a 30-g mouse eats approximately 3 g of dry food containing 0.7 g of digestible protein. A large amount of these dietary amino acids is catabolized very rapidly. The digestion, absorption, and catabolism of excess amino acids is completed in a few hours, as shown by the pattern of urea excretion (Fig. 1). It is actually faster than indicated by this parameter because the half-life of \([^{14}\text{C}]\text{urea}\) in the mouse body is approximately 1.5 h (not shown); i.e. urea production peaks sooner than its excretion. The total amount of nitrogen eliminated in the urine during the 10-h period following the start of the meal in this experiment (60 mg) represents 0.38 g of catabolized amino acids, 55% of the ingested protein. Much of the other 0.3 g is probably stored as excess tissue proteins. Liver protein content increases in 8 h from 1.06 ± 0.03 to 1.25 ± 0.05 g/100 g body weight (p < 0.01; Fig. 1), or about 0.06 g for a 30-g mouse. This excess protein is lost between meals when it serves as an endogenous source of amino acids.

The increase in protein synthesis is largely or wholly due to a large proportion of ribosomes in polyribosomes (16). The increase in protein synthesis, however, is insufficient to account for the accumulation of liver protein, from which it has been inferred that protein breakdown must slow down (18, 20). This is confirmed in Table I, where liver protein synthesis was measured by the injection of massive amounts of radioactive leucine. This procedure, previously developed in our laboratory, obviates the need to estimate the specific radioactivity of leucine in the precursor pool. The advantages of this procedure and its validation by comparison with the results of others was discussed in previous publications (2, 10). Liver protein synthesis increased in one experiment from 24.4 to 34.4 mg/h/100 g body weight and in the other from 27.2 to 41.2 mg/h/100 g body weight. The increase translates into the synthesis in 8 h of an excess of 80–112 mg protein/100 g body weight. This is only about one-half of the actual gain in liver protein in this period, 192 mg/100 g of body weight (Fig. 1). The discrepancy between the protein gain and the increase in protein synthesis is even greater when we consider that only half of the newly synthesized proteins represent stable liver components. The other half comprises rapidly turning over or exported proteins (12, 21). In short, the stimulation of protein synthesis is not nearly enough to account for the liver protein gain after the meal; we must focus next on hepatic protein degradation.

**Decreased Accumulation of Bestatin-induced Intermediates after a Meal**—From the data just presented, it follows that the increase in protein synthesis is insufficient to produce by itself the rapid protein gain. A slowdown of protein breakdown

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**Fig. 1. Diurnal variations in urinary N excretion, liver weight, and protein in meal-fed mice. Bars represent average ± S.E.**

**Top panel,** urinary nitrogen excretion. Four mice were placed in individual metabolic cages and trained to eat a single meal between 6-10 p.m. for 3 days. On the fourth day, the amount of food eaten was measured, and the urine was collected in the periods indicated; 2 h before the end of each period the mice received 2 ml of 0.3 M mannitol, to produce osmotic diuresis and thus favor the accurate timing of the urine samples. Average food intake in 4 h in this experiment was 3.10 ± 0.25 g, containing 680 mg of protein or 109 mg of protein/N. Total urinary N excretion in the 10 h following the start of the meal was 60 mg, or 55% of the ingested protein N. **Middle and bottom panels,** liver weight and protein. These measurements were from separate experiments in which groups of 5 (weight) or 8 mice each (protein) were killed at the indicated times. For further details, see "Experimental Procedures." To better visualize the diurnal nature of these changes, although the actual measurements are those represented by the vertical bars, the plot is repeated over a period of 2 days.

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**Table 1**

| Time after start of meal | Liver protein radioactivity | Liver protein synthesis |
|--------------------------|-----------------------------|-------------------------|
| h dpn/100 g body weight  | mg/h/100 g body weight      |
|--------------------------|-----------------------------|
| 0                        | 30,856 ± 1,112 (8)          | 24.4 (100%)             |
| 4                        | 43,492 ± 1,898 (8)          | 34.4 (141%)             |
| Experiment 1             |                             |                         |
| 0                        | 34,316 ± 1,871 (6)          | 27.2 (100%)             |
| 4                        | 52,000 ± 1,833 (6)          | 41.2 (151%)             |
| Experiment 2             |                             |                         |

**Note:** Table I shows the results of experiments on the accumulation of liver protein after a single meal, in which the results are compared to those from a separate experiment, not shown. Two separate experiments are shown. Values are average ± S.E. (number of mice).
must be also occurring. Accurate estimation of protein degradation by balance is, however, difficult because protein gain in 8 h is calculated as a small difference between two large numbers: (1.25 ± 0.05) − (1.06 ± 0.03) = 0.19 ± 0.08 g/100 g of body weight. Reliable measurement of changes over shorter periods would be virtually impossible. Also, because of the transient nature of the process, one cannot confirm the suggested decrease in hepatic protein breakdown by measuring the disappearance of radioactive protein. With an average rate of degradation of stable liver proteins of 30%/day (2, 12), the loss of radioactive proteins in 8 h would be in the order of 10%, not large enough for accurate estimations with a reasonable number of animals.

It is in transient situations, such as the effect of a single meal on hepatic protein metabolism, that the accumulation of bestatin-induced peptides proves most useful. After the meal, the accumulation of either arginine- or leucine-labeled peptides decreases by a factor of three (Table II, Experiments 1 and 2). Note that the radioactivity in bestatin-induced peptides, as a fraction of the corresponding protein radioactivity, is larger with arginine than with leucine. This is consistent with our previous observation, in isolated hepatocytes, that arginine-labeled peptides are retained more effectively within the cells (6).

One source of concern is that after the meal, bestatin may be less efficient in promoting the accumulation of peptides. This could occur because of changes in the extent of mitochondrial trapping of peptides (6), their leakage from the hepatocytes (6), the concentration of peptides in the cytosol, the activity of the peptidases, or their sensitivity to the inhibitor. To test possible differences in the efficiency of bestatin, we measured the accumulation of intermediates in the degradation of short-lived proteins, which should not be affected by the meal. In cultured cells and isolated hepatocytes, conditions that result in faster degradation of long-lived proteins, such as serum or amino acid starvation, do not affect the rate of breakdown of short-lived proteins (22–24). Both classes of proteins are probably degraded by different mechanisms. Short-lived proteins may be predominantly broken down by the cytosolic ubiquitin-dependent system (25–28). Stimulated degradation of long-lived proteins appears to depend on autophagy. This role of autophagy is particularly well documented in liver in the case of fasting (20, 29–33), glucagon, or amino acid deficiency (34–39). There is also evidence of autophagy during stimulated protein degradation in cultured cells (40–42). Because of this background information, we expected that the meal would not affect the breakdown of short-lived hepatic proteins. In animals in which liver proteins were labeled by injection of carboxyl-labeled ornithine 1 h before the experiment, the meal had indeed little effect on the accumulation of bestatin-induced peptides (Table II, Experiment 4). The experiment indicates that the livers are equally sensitive to bestatin in both conditions. Therefore, the effect of the meal on the accumulation of bestatin-induced peptides with long-lived proteins must reflect a real decrease in the rate of breakdown of these proteins.

Another source of concern is that in the intact mouse, part of the peptides could derive their radioactivity from long-lived proteins indirectly, through reincorporation of the label into short-lived proteins. In experiments with isolated cells, the distinction between long- and short-lived proteins is unambiguous, as long as it is properly measured in the presence of unlabeled amino acids in the medium, which prevents the

| Condition | Relative peptide radioactivity | 10³ peptide cpm/protein cpm |
|-----------|--------------------------------|-----------------------------|
| Experiment 1: long-lived, l-[1-¹⁴C]ornithine | | |
| Before meal | No bestatin | 0.54 ± 0.03 (2) | 3.32 ± 0.13 (2) | 2.78 (100%) |
| 4 h after meal starts | Bestatin (15 min) | 0.36 ± 0.02 (2) | 1.36 ± 0.18 (2) | 1.00 (36%) |
| 8 h after meal starts | | 0.48 ± 0.02 (2) | 1.46 ± 0.20 (2) | 0.95 (34%) |
| 16 h after meal starts | | 0.43 ± 0.02 (2) | 1.85 ± 0.13 (2) | 1.42 (51%) |
| Experiment 2: long-lived, l-[1-¹⁴C]leucine | | |
| Before meal | No bestatin | 0.60 ± 0.04 (2) | 2.32 ± 0.30 (2) | 1.72 (100%) |
| 4 h after meal starts | Bestatin (15 min) | 0.55 ± 0.12 (2) | 1.19 ± 0.24 (2) | 0.64 (37%) |
| Experiment 3: short-lived, l-[1-¹⁴C]ornithine | | |
| No food 7 a.m. to 7 p.m. | No bestatin | 0.66 ± 0.07 (4) | 2.06 ± 0.26 (5) | 12.4 (100%) |
| Same + leucine | | 2.08 ± 0.14 (5) | | |
| Experiment 4: short-lived, t-[1-¹⁴C]ornithine | | |
| Before meal | No bestatin | 3.9 ± 0.5 (3) | 16.3 ± 0.6 (5) | 12.4 (100%) |
| 4 h after meal starts | Bestatin (15 min) | 5.7 ± 0.8 (3) | 16.9 ± 1.4 (4) | 11.2 (90%) |
reincorporation into short-lived proteins of the radioactive amino acids produced by degradation of long-lived proteins. It could be argued that in the intact mouse significant reincorporation occurs, and that enough label accumulates in short-lived proteins to be a source of radioactive intermediates. If that were the case, the effect of a meal could be due, not to a decrease in protein breakdown, but to isotopic dilution by the large amounts of unlabeled leucine or arginine reaching the liver during absorption of digested food. If such unlabeled amino acids chase the labeled ones in the liver, and prevent their reincorporation, and if a large portion of the radioactive peptides derive directly from newly synthesized short-lived proteins, a decrease of peptide radioactivity could be conceivable even in the absence of slower protein breakdown. Although this possibility seemed remote, we judged it was important to eliminate it by the following experiment (Table 11)

The radioactivity in the guanidino-C of protein-bound arginine was measured in two groups of mice, 24 and 48 h after injection of 14C-bicarbonate. The leucine and ornithine-labeled animals (parts B and C) were used to determine the accumulation of bestatin-induced peptides, as in Table II, except that 36 h were allowed to elapse between the injection of the label and that of bestatin, to coincide with the midpoint of the following determination. The radioactivity in the guanidino-C of protein-bound arginine (part A) was measured in triplicate in a pool of livers from two groups of mice, killed 24 and 48 h after the injection of 14C-bicarbonate (see "Experimental Procedures"). All values are averages ± S.E. (number of animals in the group). The apparent $K_d$ for the degradation of guanidino-C-protein, between 24 and 48 h after injection of the label, was calculated assuming an exponential disappearance during that interval (12). In parts B and C, peptides were purified from liver homogenates and their radioactivity determined after acid hydrolysis and ninhydrin treatment as described before and expressed as a ratio between peptide and protein radioactivity (5). Average total liver protein radioactivity in B was 459 ± 19 × 10^3 cpm; and in C, 267 ± 6 × 10^3 cpm. For comparison, the apparent $K_d$ for bestatin-induced peptides accumulating in 15 min was recalculated for 1 day as explained in the text.
Bestatin-induced Peptides as Measure of Liver Protein Degradation

h, or 12.9%/day. This value represents 39% of the protein degradation calculated by the procedure of Swick and Ip (11). The experiment was also repeated under the same conditions with livers labeled in the carboxy-C of Arg moieties by the injection of L-[1,14C]ornithine 36 h beforehand (Table III, Experiment C). The arginine-labeled peptides represented 0.186% of the hepatic protein radioactivity, or 55% of the true protein degradation. As with the experiments in Table II discussed before, the more efficient accumulation of arginine-labeled intermediates is consistent with our previous observation in isolated hepatocytes, that these peptides are better retained within these cells (6).

With the efficiency of accumulation of bestatin-induced peptides thus known, it is possible to estimate absolute rates of breakdown. For instance, using the value of 55% for Arg-peptides (Table III), if we now return to Experiment 1 in Table II, the rate of degradation of long-lived proteins (defined in this experiment as the radioactive proteins remaining in the liver 1 day after injection of the precursor) was, before the meal, 0.278 × (60 min/15 min) × (100/55) = 2.02%/h. Because we know, from the study of short-lived proteins (Experiment 4, Table II) that food intake has little or no effect on the sensitivity of the liver to bestatin, we can calculate in the same way that this rate dropped to 0.73, 0.69, and 1.03%/h, 4, 8, and 16 h after the beginning of the meal. With the value of 39% for Leu-labeled intermediates (Table III), in Experiment 2 of Table II, degradation dropped 4 h after the meal from 1.76 to 0.86%/h.

The comparison with the procedure of Swick and Ip (11) can be in principle applied to any other condition in which unchanging degradation of liver proteins can be studied for at least 1 day. To ascertain that bestatin efficiency is not affected by acute changes, such as food intake, comparison of bestatin-induced intermediates in the degradation of short-lived proteins can be used, as already discussed in the previous section. As with all experiments with pulse-labeled proteins, including the procedure of Swick and Ip (11) and measurements in isolated or cultured cells, the apparent $K_d$ will necessarily be affected by the length of the chase. A complete description of the breakdown of radioactive cellular proteins can only be attained either by continuous administration of the precursor, until all classes of proteins are uniformly labeled; or by extensive analysis of the apparent rates obtained after progressively longer chase intervals (1). We should stress, however, that our major interest here is not to account for the increase in liver protein content after a meal by a detailed balance of protein synthesis and degradation. This can be done more accurately during periods of sustained liver growth, including the refeeding of protein-depleted animals (21). Where this novel procedure should be irreproachable is in establishing, in intact animals, the relative importance of nutrients and hormones in the transient changes in protein metabolism after a meal. For this, the slower degradation of long-lived proteins is most important; these are the proteins that are most clearly affected in perfused livers (3) and isolated hepatocytes (4).

Present Limitations of the Procedure—As a way to estimate transient changes in protein breakdown, the accumulation of bestatin-induced peptides has at present some limitations. (a) Compared with cultured cells, hepatocytes are very sensitive to bestatin (see Fig. 3 in Ref 6). Other cell types may require larger concentrations, which may be difficult to achieve in intact animals. The maximum solubility of bestatin in aqueous media is 5 mg/ml. Because it is undesirable to inject excessive volumes of fluid to animals, solubility may become a limitation in studies with other tissues. (b) Bestatin is at present expensive and this limits its use to small animals. Its chemical structure is, however, relatively simple and it can be synthesized (43); we thus expect that cost represents only a transient problem. None of these limitations constitute an impediment for the application of the procedure to studies in the livers of small animals. We expect it will also be useful for studies in other tissues.

Conclusions—From the experiments presented above we conclude that 4 h after a meal, storage of excess liver protein results from a 45% increase in protein synthesis and a 65% decrease in the degradation of long-lived proteins. A decrease in liver protein breakdown after a meal was suggested before because the rate of hepatic protein synthesis did not increase enough (18, 20), and based on electron microscopic evidence of decreased hepatic autophagy (20, 31). Slower protein degradation was found in perfused livers derived from mice refed after a short period of starvation (44). A sustained decrease in protein degradation has been documented in livers after recovery from protein depletion, both by balance between protein synthesis and accumulation and by the disappearance of radioactive liver proteins labeled by injection of 14C-bicarbonate (21). This is, however, the first time that transient changes in hepatic protein breakdown can be demonstrated directly in intact animals. From studies with perfused livers (3) and isolated hepatocytes (4), we know some of the parameters that could be responsible for the effects. After a meal, higher levels of circulating amino acids and insulin, and lower concentrations of glucagon could all contribute to stimulation of net protein accumulation in body tissues (3, 45). Measurement of bestatin-induced peptides will now permit the establishment of the relative importance of these or other variables in liver of intact animals. The procedure may also prove useful for studies in other tissues.

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Bestatin-induced Peptides as Measure of Liver Protein Degradation

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