Effect of Insulin on Protein Synthesis

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SUMMARY

The mechanism by which insulin controls protein metabolism is not fully understood. Insulin stimulates protein synthesis; it also enhances transport of some amino acids, but the latter action does not appear to be sufficient explanation of the increase in synthesis. The various actions seem to be independent of effects on glucose metabolism. In diabetic muscle there are fewer than normal polysomes, and insulin rapidly enhances attachment of monomers to messenger-RNA. Insulin also increases the effectiveness of cell sap in catalyzing protein synthesis by ribosomal systems. The way in which the hormone may affect either initiation or peptide synthesis is not known.

Experiments are reported bearing on whether availability of amino acids could be a mechanism by which effects of insulin are mediated. Activity of liver and muscle soluble fractions declines on fasting and, for the latter tissue, possibly also on a low protein diet. Sap from fasting animals allows a much smaller response of isolated ribosomes to added amino acids. Availability of glutamate in amino acid mixtures may be of special importance. However, insulin can influence the activity of the sap fraction of diaphragm muscle during incubation without the presence of amino acids in the medium. Understanding of what mechanisms are involved will depend on resolution of the critical sap factors. DIABETES *21* (Suppl. 2):447-52, 1972.

Interest in the control by insulin of protein synthesis has grown in recent years. The wasting effect of diabetes has, however, been known since classical times, and the ability of insulin to stem nitrogen loss[1] and to reverse the excess of venous over arterial plasma amino-nitrogen of diabetic subjects[2] was quickly recognized following isolation of the hormone. By 1931 it could be concluded[3] that "an increase in peripheral synthesis of protein from blood and tissue amino acids might accompany the hypoaminoacidaemia produced by insulin." In 1933 Luck and Morse[4] stated, "More and more we have come to believe that the effect (of insulin to induce hypoaminoacidaemia) is independent of effects on carbohydrate metabolism."

Effects of insulin on isolated tissues

The more modern part of the story starts with the finding that insulin added in vitro to isolated tissues stimulates the incorporation of labeled amino acids into the protein of the preparations.[5,6]

Developments of this observation and the present position are summarized in table 1. The stimulation of incorporation into protein is seen with all the usual amino acids and is not dependent on a simultaneous enhancement of uptake of glucose by the tissue.[7,8] Insulin also stimulates accumulation of a number of amino acids but, at least as seen with isolated systems, only a minority show a clear increase (table 2). This is true both for amino acids which enter peptide linkage and for unutilizable ones.

TABLE 1

INSULIN ENHANCES:
Incorporation of amino acids into protein of muscle (Effects not dependent on stimulation of glucose uptake)
Transport of some but not all amino acids into muscle cell (Enhances incorporation of amino acids synthesized intracellularly)
INSULIN SUPPRESSES:
Rate of protein catabolism in perfused liver and isolated adipose tissue (No similar observation yet available for muscle)
IN DIABETES, LIVER AND MUSCLE EXHIBIT:
Decreased ratio of polysomes to monoribosomes (Ratio rapidly enhanced by injection of insulin)
Decreased capacity of cell sap and pH 5 fraction to support amino acid incorporation (Enhanced by insulin treatment)

A fuller discussion (including bibliography) of these actions can be found in references 46 and 47 which also discuss the role of insulin in regulating, in liver and adipose tissue, the level of several enzymes at important control points in metabolism.

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TABLE 2
Accumulation of amino acids by muscle

Uptake enhanced by insulin	
Alanine(?)	Aminoisobutyric acid
Asparagine	Cycloleucine
Glutamine	Ethionine
Glycine	Isovaline
Histidine(?)	Sarcosine
Methionine	
Proline	
(Hydroxyproline)	
Serine	
Threonine(?)	
Uptake not enhanced by insulin	
Alanine(?)	β-Alanine
Arginine	γ-Aminobutyric acid
Aspartic acid	α, γ-Diaminobutyric acid
Cystine	α-Methyltyrosine
Glutamic acid	Norleucine
Histidine(?)	Ornithine
Isoleucine	α-Aminobicyclo-2,2,1-heptene-2-carboxylic acid
Leucine	
Lysine	
Phenylalanine	
Threonine(?)	
Tryptophan	
Tyrosine	
Valine	

For a more detailed bibliography, see references 47 and 48. The placing of asparagine and glutamine has not been previously reported.

It is claimed that uptake of several amino acids, listed in table 2 as unresponsive, responds in rabbit muscle.[9] Experience with guinea pig and mouse muscle* and with chicken embryo heart,[10] however, suggests that the responses are substantially the same as shown in table 2. Insulin also enhances the incorporation into protein of amino acids synthesized in the cell,[11] an observation taken to mean that the effect on protein synthesis is not dependent on a stimulation of transport of amino acids into the tissue, although this may be helpful.

With perfused liver and isolated adipose tissue, insulin appears to suppress catabolism of protein.[12,13] A similar phenomenon may eventually be shown for muscle. Unfortunately, at present we know very little of the mechanism by which protein catabolism occurs and, therefore, how it is controlled, but there is reason to believe that its rate relates inversely with the rate of synthesis.[14]

Control of protein synthesis

The rate of protein synthesis in a tissue will obviously be affected by (a) the total number of ribosomes present, (b) the proportion of ribosomes in polysomes, and (c) regulation of the rate of movement of ribosomes along the messenger. Although in diabetes the RNA content of several tissues declines, (b) and (c) will be the more important in short-term regulation.

Several tissues from diabetic animals, most notably skeletal muscle, show a decreased rate of protein synthesis and are characterized by having a smaller proportion of their ribosomes contained in polysomes.[15] Administration of insulin rapidly leads to an increase in the proportion of ribosomes in polysomes—even when RNA synthesis is not occurring. Thus insulin promotes initiation, the process of attachment of ribosomes to messenger-RNA. How this is affected by insulin is not known, but it seems unlikely that any intrinsic difference exists between ribosomes from normal and from diabetic tissues.[16-18]

The extent of regulation in vivo of the rate of movement of ribosomes once initiated along the messenger is less clear. For hepatic cells in culture, it is claimed that the rate of protein synthesis is accountable solely in terms of the amount of RNA present and the proportion of ribosomes in polysomes.[19] Determination of the position in vivo of the growing peptide chain between the donor and acceptor sites on the ribosome suggests no limitation of the availability of aminoacyl-tRNA, GTP or transfer enzymes, either normally or in diabetes.[20] In differentiated tissues, however, available evidence suggests additional regulators, since rates of protein synthesis can vary greatly independently of changes in (a) and (b).[21-23]

In cell-free systems, consisting of mixtures of ribosomes and cell sap or pH 5 enzymes, the origin of the soluble fraction affects how much amino acid incorporation takes place. Thus, pH 5 fraction from liver and muscle of diabetic animals supports less protein synthesis than that from normal animals and is raised on treatment with insulin.[14,24,25] Fasting also reduces its efficacy, possibly because of the reduction in the level of circulating insulin.[25]

Special role of amino acids

The problem which arises with induction of diabetes and administration of insulin to the intact animal is to know the extent to which change in polysome formation or activity of the pH 5 fraction is the result of the increased uptake of glucose and amino acid that is bound to occur simultaneously or is directly related to the availability of insulin. This is particularly so since the decline in the number of polysomes in liver and heart muscle in diabetes appears to be slow.[26,27] Although insulin apparently does not promote uptake

* Unpublished observations. It is possible that with mouse diaphragm leucine is responsive, but negative results were obtained with valine, phenylalanine, arginine and lysine.

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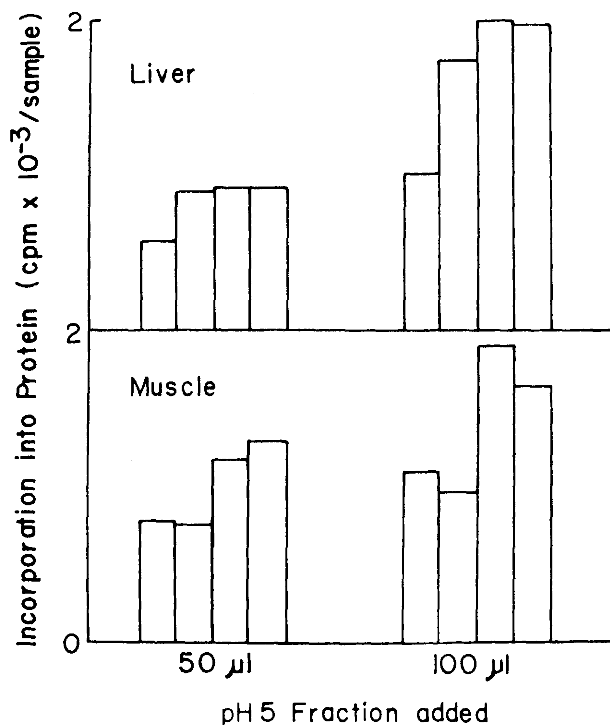

FIG. 1. The effect of different diets on the comparative activity of pH 5 fraction of liver and muscle in promotion of leucine incorporation by hepatic ribosomes. Ribosomes from liver and sap and pH 5 fraction from liver and muscle were prepared and the incubations carried out as previously described.[25] The incubations were always carried out at several concentrations of sap and pH 5 fraction to ensure that their concentrations were not saturating. (It should be noted that a system in which activity is proportional to sap added may still, at all sap concentrations, show strict proportionality to the quantity of ribosomes added; protein synthesis is hardly a first or even second order process.) In each group of blocks there are four dietary treatments (for two days): from left to right, (1) starvation, (2) low protein diet (mainly starch and lard), (3) the same supplemented to 20 per cent with casein, and (4) standard diet.

of all amino acids (table 2), an obvious possibility is that increased uptake of certain amino acids might be of critical importance and have a role in enhancing initiation and sap activity. Whether this is so remains to be determined. It would relate effects of the hormone on amino acid transport and protein synthesis and would be consistent with a membrane site of action of insulin. Certainly with cells in culture, glucose and amino acids (seemingly both rather than specifically one) promote polysome formation.[28,29] Incubation of liver slices with high concentrations of amino acids increases the potency of the sap when added to ribosomes,[30] and addition of mixtures of amino acids to hepatic cell-free systems appears to enhance polysome formation and incorporating capacity.[31]

The situation in vivo can be compared with that in ascites cells and liver slices by asking whether protein starvation of rats, as opposed to total starvation, reduces the activity of the sap fraction to support incorporation by ribosomes. Under the conditions used, the answer to this question is that hepatic sap activity does not decline when a normal diet is replaced for two days by a diet very low in protein but equal in caloric content (figure 1). The situation with skeletal muscle is less clear, and there seems to be some indication of declining activity under the same conditions on the protein-free diet. This decline becomes more pronounced by the third day. A distinction between the behavior of liver and muscle would be consistent with the proportionally greater decline in rate of protein synthesis in the latter under conditions of nitrogen shortage[32] and the loss of amino acids under fasting conditions.[33] Thus, there is evidence that availability of amino acids in vivo may control cell factors involved in translation.

An additional observation to come out of these experiments concerns the effect that addition of a complete amino acid mixture has on the rate of leucine incorporation. Isolated ribosomes usually incorporate a labeled amino acid quite well when only a single amino acid is added, possibly because of other amino acids already in the system attached to activating enzymes and transfer-RNA and because the rate of protein synthesis is usually low. Addition of a complete mixture of amino acids increases the rate of incorporation, but apparently to a variable degree depending on the nutritional state of the donor of the soluble fraction. Thus, starvation not only reduces the activity of the pH 5 fraction when a single labeled amino acid is added, but it also strikingly diminishes the degree of stimulation seen on addition of a complete amino acid mixture (figure 2). In fact, at the lowest levels of fasted fractions added, there was no response at all. There are, of course, several possible explanations. The effects, however, seem unlikely to be directly related to availability of either activating enzymes or transfer-RNA, but could result from lack of some factor (e.g. promoting initiation; see reference 31) present in minimal amounts in the pH 5 fraction whose level is subject to variation. The observations were unaffected by whether the ribosomes came from fed or fasted animals. The results of figures 1 and 2 thus suggest that a "translation factor" is to be found, but that after breakup of the cell it is in the soluble fraction rather than on the ribosomes.[15] Such a factor may be inducible by amino acid supply.

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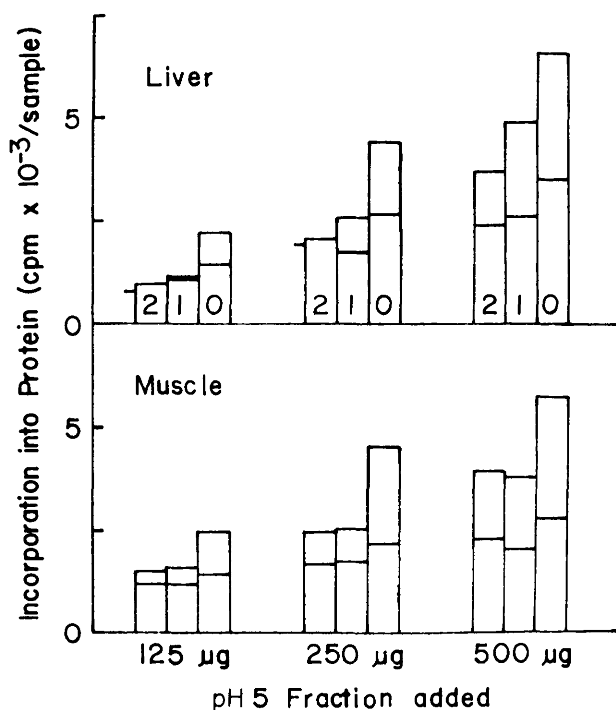

FIG. 2. The ability of pH 5 fraction from fed and fasted rats to stimulate incorporation of leucine into protein in the presence and absence of nineteen other amino acids. Conditions of preparation and incubation of fractions was as described previously.[25] The pH 5 precipitate prepared from liver was redissolved in a volume equal to that of the supernatant from which it was prepared, that from muscle in a quarter volume. The amino acids were added each to a final concentration of 50 μM. The numbers 2, 1 and 0 in the columns indicate the days food had been withheld. The total size of the columns indicates the incorporation in the presence of the mixture of amino acids. Incorporation in the absence of the amino acid mixture is represented by the bar in the columns.

In the search for amino acids that might be of crucial importance in controlling polysome profiles and incorporation rates, past studies have tended to concentrate on the role of the essential amino acids, tryptophan in particular.[31] Lack of an amino acid is bound to cause cessation of peptide elongation when its codon occurs, but tryptophan less than most because of its low frequency. *A priori* it could be argued that if any amino acid is to have a special importance greater than its individual requirement, one subject to synthesis would have advantages. It is possible that glutamate is such an amino acid. In my experience, it is the only amino acid whose omission from an otherwise complete mixture of amino acids seems to impair the rate of leucine incorporation (table 3). The explanation of this is not known. Levels of the dicarboxylic amino acids might well be expected to change under different

TABLE 3
Effect of glutamate on incorporation of leucine by hepatic ribosomes

Amino acid mixture added	Incorporation of leucine (c.p.m./tube)
Only labeled amino acid	3933
Complete mixture	5972
Mixture minus glutamate	4336
Same mixture with glutamate added	5797

Preparation of ribosomes and pH 5 fraction was as described in reference 25. Incorporation (in 0.5 ml.) took place with about 200 μg. ribosomes, 150 μg. pH 5 fraction protein and other constituents as before. The complete amino acid mixture was twenty amino acids, each at a final concentration of 50 μM.

metabolic conditions, but glutamate, which is present in large quantities in most tissues, is not one of the amino acids showing response to insulin (table 2 and reference 33) nor one of those shown to be especially important in controlling polysome aggregation.[31,34] It does, however, appear to be of particular importance in the nitrogen metabolism of muscle.[35]

Insulin and protein synthesis

From the foregoing, it is clear that amino acids, under appropriate circumstances, can affect the rate of protein synthesis, but the various observations do not suggest in what way their effects relate, if at all, to the action of insulin. One of the striking features of the stimulation by the hormone of amino acid incorporation in isolated tissues is its independence of a requirement for glucose or added amino acids. Although it is possible that even in the normal animal administration of insulin may enhance polysome formation,[36] attempts to measure labeling of aminoacyl-transfer-RNA in perfused heart suggest that the enhanced incorporation is probably already determined by the time the amino acid has become attached to transfer-RNA.[37] How this comes about is not clear, but a possible explanation is that it results from the fact that sap isolated from muscle after incubation with insulin shows greater activity in promoting incorporation by hepatic ribosomes than sap from tissue similarly incubated without insulin (table 4). This would seem to be an observation of some interest for two reasons: First, it should be possible to isolate from the sap the specific factor or factors involved and to see whether they are similar to those changing in the liver slices incubated with high amino acid levels,[30] and secondly, this observation suggests that pro-

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TABLE 4

Capacity of cell sap from diaphragms incubated with insulin to support incorporation by hepatic ribosomes

Sap from diaphragm incubated:	Incorporation of leucine (c.p.m./tube)
Without insulin	610
With insulin (0.1 unit/ml.)	770

Diaphragms were incubated in Krebs-Ringer bicarbonate buffer for one hour at 37° C., then each was homogenized in 1 ml. of the buffer used for the incorporation studies;[25] 0.2 ml. of the resulting sap was incubated with c. 200 μg. of hepatic ribosomes and the other usual constituents. Each figure is the average of three observations. A similar proportional change was observed in several experiments.

motion by insulin of incorporation in the isolated muscle may be (as suggested in recent experiments with the in vitro action of growth hormone[38]) primarily an increase in rate of ribosome movement along the messenger as much as control of initiation. It is perhaps noteworthy that the sap activity of diaphragm muscle appears also to rise during the transient hypertrophy that accompanies unilateral denervation.[39]

CONCLUSIONS

It is clear that we still know little about the way in which insulin affects protein synthesis. Although a defect in initiation occurs in skeletal muscle of acute alloxan-diabetic rats, it is open to question whether this is due to lack of availability of insulin as such, since the defect is minimal in liver and cardiac muscle.[26,27] In fact, the defect is also much less severe in muscle of rats made diabetic with streptozotocin.[40] Perhaps in the acute alloxanized animals the intense mobilization of fatty acids adversely affects skeletal muscle metabolism.* Because of the difficulty of interpretation of the results with intact animals, the effect of insulin to stimulate incorporation of amino acids into protein of isolated tissues independent of a requirement for added glucose or amino acids still remains one of the most significant observations in the field.

Some years ago, Krahl[43] speculated as to the primary action of insulin in terms of a conformational change in responsive tissues. There is no question that in several ways insulin affects the "condition" of isolated tissues and cells in culture,[44,45] but identification of the specific point of action has proved difficult. Research of the past fifty years has constantly emphasized the great importance of the insulin molecule. It is to be hoped it will not take another fifty years to understand in biochemical terms how it works.

*Diaphragm muscle, which can readily metabolize fatty acids, actually shows an enhanced capacity for protein synthesis[41] and uptake of amino acids[42] after acute alloxan treatment.

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