Dr Ketan Dhatariya MBBS MRCP(UK) MSc

Research Fellow, Endocrine Research Unit, Mayo Clinic and Foundation, 200 First St SW,

Rochester, Minnesota. USA 55905

Dr K Sreekumaran Nair. MD PhD, FRCP (London)

David Murdock-Dole Professor of Nutrition and Consultant in Endocrinology, Endocrinology

Division, Mayo Clinic and Foundation, 200 First St SW, Rochester, Minnesota. USA 55905

Chapter 25 – Diabetes Mellitus – Amino Acid and Protein Metabolism

A chapter for 'Amino acid metabolism and therapy in health and nutritional diseases'. Ed

Cynober LA. CRC press. Boca Raton. Fla.

I. INTRODUCTION

Diabetes mellitus is becoming an increasingly common metabolic disorder [1]. The condition is characterized by impaired glucose metabolism manifested by hyperglycemia. Diabetes also affects fat and protein metabolism. Studies in humans have demonstrated that effects on protein and amino acid metabolism differ in subjects with type 1 and type 2 diabetes. In addition, the effects on protein metabolism in these conditions differ in humans from the effects seen in vitro and in animal models of type 1 and type 2 diabetes.

In people with type 1 diabetes cachexia and muscle wasting is completely prevented by insulin replacement. No such dramatic changes in body composition are noted in people with type 2 diabetes. Circulating amino acid levels, amino acid kinetics, protein synthesis and breakdown are different between people with type 1 diabetes and those with type 2 diabetes. The current review will focus on the alterations in amino acid and protein metabolism that occur in people with type 1 and type 2 diabetes.

II. MECHANISMS OF INSULIN ACTION ON PROTEIN METABOLISM

Insulin plays a pivotal role in the regulation of amino acid and protein metabolism in health [2]. In individuals without diabetes, plasma insulin concentration varies from 2 mU/l to12 mU/l in the fasting state, to 30 mU/l to 100 mU/l in the postprandial state. In common with many other hormones, insulin acts on the cells after binding to a specific receptor. The receptor is a tetrameric glycoprotein, consisting of 2 α subunits that anchor the receptor to the cell membrane, and 2 intracellular β subunits, to which tyrosine kinase domains are attached. The α

and β subunits are linked by sulphydryl bonds. Insulin binding causes a conformational change to the receptor, leading to autophosphorylation of the tyrosine residues. Other amino acid residues on the receptor, such as threonine and serine, may also become phosphorylated.

Liver, fat, and skeletal muscle are the tissues in which insulin has its main effects. The actions of insulin in the liver include changes in hepatic gluconeogenesis by its effect on key enzymes such as phosphoenolpyruvate carboxykinase (PEPCK) [3], and glucokinase [4]. Within the nucleus, insulin acts on a number of genes via the insulin response element. Insulin also acts on several pathways, possibly through it's stimulatory effect on the Insulin Receptor Substrate 1 and 2 gene. These genes produce intracellular signaling proteins that trigger the cascade that is responsible for many of the actions of insulin [5]. In type 2 diabetes, the chronic hyperinsulinemia leads to a down regulation of Insulin Receptor Substrate 2 gene transcription, thus leading to abnormalities in many intracellular processes, including a failure to suppress hepatic gluconeogenesis [6].

A.   AMINO ACID TRANSPORT INTO SKELETAL MUSCLE

In the postabsorptive state, intracellular transport of amino acids into skeletal muscle is well below the maximum capacity of the transport systems. However, optimal amino acid transport across the blood brain barrier takes place at concentrations similar to those found during fasting. This implies that when plasma amino acid concentrations rise after a meal, transport of amino acids into skeletal muscle can increase relative to transport across the blood brain barrier. This allows for greater intracellular amino acid availability for protein synthesis in skeletal muscle [7,8]. There are several different amino acid transport mechanisms, of which four, A, ASC, N_m and X_c, are insulin responsive [7-9]. These four mechanisms are primarily

responsible for the transport of nonessential amino acids. However, the mechanism responsible for the transport of some of the large neutral branched chain amino acids, L, is not sensitive to insulin. Insulin also effects amino acid transport in several tissues, including cardiac and skeletal muscle, liver and adipose tissue [10-12].

Insulin has most of its effects on intracellular amino acid metabolism in a dose dependant manner. This occurs by altering cell surface receptor binding. This change in binding inhibits the inactivation of amino acid uptake by the sodium and pH dependant, small neutral amino acid transport mechanism known as system A. Although these transport systems are important in regulating the intracellular concentrations of amino acids, no evidence is currently available to show that they limit protein synthesis in any way.

B.   PROTEIN SYNTHESIS

Insulin plays a major regulatory role in vivo protein synthesis in human and animals; however the precise role that insulin plays at various stages of regulation protein synthesis, and the interactions of insulin with other factors remains to be clearly defined. Stimulation of protein synthesis in various in vitro cell lines and tissue models have been clearly documented [3,13-17]. These researchers reported divergent findings, largely related to methodological limitations. For example, is it hard to demonstrate insulin effects separate from the changes in concentrations of substrates such as amino acids, glucose and free fatty acids, or other hormones such as glucagon, cortisol, growth hormone, etc. Recent advances in molecular techniques allow a better understanding of insulin's specific effects at different levels of protein synthesis.

Insulin has been shown to be a regulator of gene expression by altering mRNA production, maintenance of mRNA stability, ribosomal biogenesis, imitation and elongation of

mRNA translation and regulation of pre-existing enzymes [3,16]. Table 1 summarizes some of the steps where insulin has been shown to act.

The effect of insulin on gene transcription is thought to be based on intracellular *trans*-acting factors bound to *cis*-acting DNA sites in the nucleus. The specific gene sites affected by insulin are referred to as insulin response sequences or elements (IRSs/IREs) located in the gene promoter region. Specific IREs such as glyceryldehydro-3-phosphate dehydrogenase, pyruvate kinase, glucagon, PEPCK, and insulin-like growth factor protein-1 have been identified [3]. Insulin has been shown to selectively increase mRNA of eukaryotic elongation factor-2 (eEF-2) [18], phosphorylated heat-acid stable protein (PHAS-1) [19], and myosin heavy chain alpha [20]. Recent studies using the gene array approach have demonstrated that insulin downregulates and upregulates expression of several genes in human muscle [21].

Insulin has been shown to reduce the degradation of transcripts (mRNA) of glyceryl-3-glycogen phosphate dehydrogenase, glycogen phosphorylase and GLUT-1 [3,22]. These are examples of the role of insulin in stabilizing mRNAs. In contrast, there are examples of insulin destabilizing mRNA's of PEPCK and GLUT-4 [23,24]. These data show that insulin can stabilize some mRNA while destabilize others within the same cell. These animal and in vitro results are intriguing as they show insulin's divergent effects on the transcription of many genes in skeletal muscle. Work has recently been done looking at these differences in gene expression in humans [21].

The mechanism of insulin's effect on translation of messages has been extensively reviewed [16,25,26]. Insulin has been demonstrated to regulate translation initiation, in part, by enhancing mRNA binding to the 40S ribosomal subunit. In this process, a key step is the binding of the eIF-4E (cap binding protein) and eIF-4E binding protein (PHAS-I or 4E-BP$_1$) and

eIF-4G to the 7-methylguanosine 5'-triphosphate cap structure at the 5'end of eukaryote mRNAs [15,27-29]. Insulin enhances PHAS-1 action by dissociating it from eIF-4E, so that eIF-4E forms a complex with eIF-4G that associates with the 40S ribosomal subunit [27]. Insulin deficiency has been shown to decrease eIF-4E binding to eIF-4G and increase eIF4E-4E-BP$_1$ complex formation in skeletal muscle [30].

Insulin selectively upregulates translation initiation of mRNAs containing polypyrimidine at the 5'transcriptional start-site. These mRNAs encode important components of translational elongation factors that would increase overall capacity for protein synthesis.

Insulin appears to promote elongation by inactivation of eukaryotic elongation factor-2 kinase through pathways sensitive to the antifungal agent rapamycin [31].

Insulin thus promotes important steps in translation initiation and elongation. In addition, insulin promotes the capacity of cells to synthesize proteins by increasing synthesis of ribosomal proteins [32]. The synthesis of mRNA species has also shown to be stimulated by insulin as well as the maintenance of ribosomal numbers [32-35]. Insulin also may diminish the rate of ribosomal degradation [16].

C.   PROTEIN BREAKDOWN

The regulation of cellular protein breakdown is a highly complex process and the regulation is variable in different tissues. The available information on insulin control of protein breakdown is minimal. Insulin is known to influence cellular protein breakdown via several mechanisms including lysosomal pathways, ATP-dependent ubiquitin-proteosome pathway, calcium dependent proteosomes and ATP-independent pathways. Approximately 10-20% of protein breakdown occurs via lysosomal pathways and this pathway is primarily responsible for

degrading extracellular membrane and organellar proteins. The majority of intracellular proteins are degraded by the ubiquitin-proteosome pathway. The ubiquitin-proteosome pathway is involved in accelerated protein breakdown in animal models of diabetes [36]. Inhibition of ATP synthesis reduces the increased muscle protein breakdown during insulin deficiency. Selective inhibition of lysosomal functions or calcium dependent proteases do not result in reduction of protein breakdown during insulin deficiency. However, involvement of both ubiquitin-proteosome and calcium dependent pathways are also reported in streptozotocin-treated rats [37]. Studies also have shown that adrenalectomy prevented the increased muscle protein breakdown in streptozotocin-induced rats, with muscle protein breakdown being restored with subsequent glucocorticoid administration [38]. It remains to be established whether insulin has any direct control on the ubiquitin-proteosome pathway, and the underlying mechanism.

III. DIABETES MELLITUS

A.   IN VIVO STUDIES – ANIMAL MODELS

In the rodent diabetic model it has been shown that protein synthesis is depressed in many tissues [39,40]. The effects of insulin deprivation on muscle protein breakdown in these animal models were determined indirectly, as there is evidence for an increase in whole body leucine oxidation in streptozotocin treated rats [41]. The loss of muscle, disproportionate to the decrease in synthesis, is assumed to be due to increased breakdown. Even within muscle, the rates of protein degradation vary, with the relative preservation of muscle with high oxidative capacity, such as the soleus [42].

In rats, starvation has similar effects on muscle protein metabolism as insulin deprivation. There is loss of muscle protein and a depression in muscle protein synthesis. In studies looking at

the effects of refeeding after a period of prolonged starvation, muscle protein synthesis is rapidly restored, with the rate of response proportional to the length of starvation [43,44]. As insulin can mimic this effect by stimulating protein synthesis in fasted animals, it is thought that insulin may also be responsible for the increase in protein synthesis seen in the fed state [45]. Two studies support this hypothesis. The use of anti-insulin antibodies have been shown to prevent the protein synthesis seen with insulin infusions [46]. In addition it was demonstrated that the infusion of supraphysiological doses of insulin in fed rats does not increase protein synthesis further, suggesting a plateau to the dose response relationship between insulin levels and protein synthesis rates [47,48]. However, the addition of amino acids alters these variables, allowing maximal protein synthesis rates to occur at lower insulin concentrations. This suggests that amino acids increase the sensitivity of muscle protein synthesis to insulin [48]. The infusion of amino acids without insulin did not increase protein synthesis rates, showing that both insulin and amino acids are necessary for synthesis to occur [46,48]. This is the situation in nondiabetics during feeding, both amino acid concentrations and insulin levels increase, providing optimal conditions for protein synthesis. Further work by Garlick et al showed that it was only the branched chain amino acids, with leucine in particular, that stimulated protein synthesis in the presence of insulin [49].

Aging also has an effect on protein synthesis rates. There is a divergent effect of diabetes on protein synthesis in growing rats vs. old rats. Later studies have shown that in adult rats the response to insulin is altered, suggesting that age is a determinant in insulin action on muscle protein synthesis [50]. These results are more consistent with those from humans. Studies performed in piglets demonstrated that there is an age-related alteration of stimulation of muscle

protein synthesis in response to insulin [51]. These studies clearly indicated that age, or stage of development, are important in determining the effect of insulin on muscle protein synthesis.

Further work has suggested that the effects of insulin are tissue specific, as illustrated in Figure 1A and 1B. Work in pigs has shown that insulin has a regulatory role in mitochondrial protein synthesis [52]. This effect was most pronounced in skeletal muscle, and was not found in other highly active tissues, such as liver or heart [52]. This is shown in Figure 1A. These studies also have shown that insulin has no effect on liver tissue protein synthesis, whereas Figure 1B shows that insulin has an inhibitory effect on fractional synthesis rates of fibrinogen and albumin [53]. The same study indicated that insulin administered at a high physiological level, failed to alter the rate of liver tissue protein synthesis [53]. However, there are differences in protein synthesis rates between tissue beds, and also within specific tissues [54].

There is some support for the hypotheses that tissue protein synthesis may be driven by the high intracellular amino acid concentrations as a stimulus. There has been recent work showing that the provision of leucine in postabsorbative rats had a stimulant effect on skeletal muscle protein synthesis [55,56].

B.   TYPE 1 DIABETES

1. Amino Acid Metabolism During Insulin Deficiency

Unlike in animal models, the stimulatory effect of insulin on protein synthesis has been more difficult to demonstrate in humans. The effects of insulin withdrawal and restoration on whole body protein balance were originally studied in humans in the early 1930s by Atchley et al. [57]. This is illustrated in Figure 2. It has been known for some time that stable insulin doses allow the maintenance of neutral nitrogen balance, however, on withdrawal of insulin, nitrogen

balance quickly becomes negative, indicating rapid net protein breakdown [57]. Restarting insulin therapy restored a neutral nitrogen balance within 48 hours. This is also illustrated in Figure 2. The changes associated with protein catabolism occur within hours of changing the insulin status. In humans, as with animals, the postabsorptive, insulin deprived state is associated with a rise in plasma amino acid levels in type 1 diabetic patients [58]. These levels fall with the reintroduction of insulin [59-61]. It is the catabolism of these increased levels of circulating amino acids that account for higher nitrogen excretion.

In contrast to animal models, where insulin stimulates protein synthesis as well as preventing protein breakdown, human studies using isotopically labelled leucine, phenylalanine and tyrosine have shown that insulin deprivation in subjects with type 1 diabetes leads predominantly to an increase in whole body protein breakdown [60,62-65]. Although there is an associated increase in protein synthesis during insulin deprivation, as measured by a high non-oxidative leucine disposal rate, the rate of breakdown far exceeds that of synthesis, resulting in net protein loss [60-68].

With the addition of amino acids to insulin, studies of healthy volunteers showed that there are further reductions in protein breakdown and increases in whole body protein synthesis rates than with insulin alone [69,70]. These results have been reproduced in subjects with type 1 diabetes [63,71]. This suggests that the decline in protein synthesis seen with insulin infusions in early studies was due to the lack of substrate availability.

These acute and subacute changes seen in protein turnover with underinsulinization can lead to changes in body weight, such that children with poorly controlled type 1 diabetes may present with growth retardation, despite normal growth hormone levels [72].

2. Amino Acid Metabolism During Insulin Therapy

Previous work by Luzi et al., showed that insulin had a differential effect on type 1 diabetes with respect to glucose and amino acid metabolism [63]. The responses of leucine flux, leucine oxidation and non-oxidative disposal in response to insulin infusion were similar in both diabetic and non-diabetic subjects. However, glucose uptake remained initially suppressed by over 50%, suggesting differences in cellular response of glucose to insulin [63]. The baseline increase in leucine kinetics in poorly controlled type 1 diabetic patients were normalized by one to two months of intensive insulin treatment.

Small changes in plasma insulin levels, e.g., from 20 pmol/l to 70 pmol/l, can reduce the rate of skeletal muscle protein breakdown by up to 40% [65,73].

Although intensified insulin regimes may normalize glycemic control, the abnormalities in leucine metabolism may take hours or days to resolve [64,73]. This delay may be due to the effects of insulin on inhibiting enzymes such as branched chain ketoacid dehydrogenase, which is the rate-limiting enzyme for the catabolism for branched chain amino acids, that determine the rate of leucine oxidation in the insulin deprived state. Alternatively, the delay may be due to the time taken to restore levels of counterregulatory hormones once euglycemia is achieved. However, these effects may be additive, because glucagon activates branched chain ketoacid dehydrogenase, and levels of this hormone rise in absolute insulin deficiency [74].

Studies in type 1 diabetic patients and in non-diabetic people have shown that insulin's main effect on leucine oxidation is at the level of leucine transamination [65]. During insulin deficiency leucine transamination increases several fold and account for the accelerated decarboxylation (oxidation) of leucine.

In the fed state, protein metabolism reflects the degree of insulinization and amino acid availability. In subjects without diabetes, approximately 50% of ingested amino acids are taken up and metabolized by the splanchnic bed after a meal. The branched chain amino acids, leucine, isoleucine, and valine, are not preferentially taken up by the liver, leading to a greater rise in plasma levels with respect to other amino acids. These branched chain amino acids represent the major form of nitrogen transport between the gut and the skeletal muscle after a meal. In underinsulinized subjects, this response is exaggerated, with the liver failing to take up the majority of amino acids, leading to an approximately 50% rise in systemic plasma levels compared with either adequately insulinized subjects or healthy controls [75]. How insulin status alters plasma amino acid levels across the splanchnic bed and across the leg is shown in Table 2.

There are other differences in the types of amino acid found in the plasma of subjects with poorly controlled type 1 diabetes. Within the circulation, the gluconeogenic amino acid to branched chain amino acid ratio changes with poorly controlled type 1 diabetes, possibly due to increased hepatic or renal uptake to maintain the high rate of gluconeogenesis [75,76].

In humans, the results suggesting that intracellular amino acid levels contribute to muscle protein synthesis rates have been conflicting. They have shown that although amino acid administration has an effect on decreasing protein breakdown and increasing leucine oxidation [77-79], the effects on whole body nonoxidative leucine disposal, i.e. protein synthesis, have not been consistent [63,71,80-82]. This issue remains to be fully clarified.

There have been several studies in healthy individuals to suggest that there is an decline in whole body protein breakdown with insulin [67,83-85]. These findings have also been

reported in subjects with type 1 diabetes [65,86,87]. Taken together, these studies suggest that insulin inhibits skeletal muscle protein breakdown.

3. Regional Differences In Amino Acid Metabolism

Whole body studies do not allow assessments to be made of the relative contribution made to protein synthesis and breakdown by individual tissue beds. Regional differences in protein flux have been demonstrated, based on isotopic and amino acid balance studies performed in people with type 1 diabetes. There is a differential response of protein dynamics in splanchnic and muscle beds [65,88]. During the postabsorptive state, in both non-diabetic people and patients with type 1 diabetes (insulin-deprived), muscle protein breakdown exceeds that of muscle protein synthesis. The difference between synthesis and breakdown is greater in type 1 diabetic patients during insulin deprivation than in non-diabetic people. Work looking at protein synthesis in mucosal tissue of the small intestine, showed that in the insulin deprived state there was a 30% decline in protein synthesis compared with the insulin replete state [89]. This is in contrast to an 82% increase in protein synthesis across the whole of the splanchnic bed with insulin deprivation in subjects with type 1 diabetes [65]. Other work has shown that in vitro and in vivo the absence of insulin led to a declined in albumin synthesis [16,90] with the restoration of albumin mRNA expression being reliant in a dose dependent fashion, on the amount of insulin present. At the same time, restoring insulin levels in insulin deprived subjects with type 1 diabetes can normalize fibrinogen levels [91].

Muscle protein breakdown decreased in subjects with type 1 diabetes and in non-diabetic subjects with insulin treatment [65,83,88]. In both groups there was minimal effect on muscle protein synthesis. In contrast, splanchnic protein synthesis and breakdown decreased with

insulin in people with type 1 diabetes [65]. This decrease in splanchnic protein synthesis can explain the entire change that occurred in the whole body protein synthesis in people with type 1 diabetes. Although insulin did not stimulate muscle protein synthesis in these experiments, the relative contribution of muscle protein synthesis rate to whole body protein synthesis increased significantly with insulin [92]. These changes are illustrated in Figures 3A and 3B.Another example of this differential effect includes myosin heavy chain [93] as well as mitochondrial and sarcoplasmic proteins found in skeletal muscle [52]. In type 1 diabetes, insulin deprivation in the short term had no effect on muscle myosin heavy chain synthesis rates. Although the effects of insulin on the synthesis rates of individual proteins have not been studied, the effects of insulin in different tissues have been done [92].

Although the synthesis rates of individual proteins are measurable, there are no data on the breakdown rates nor of the effect insulin has on them, because there are no methods currently available for such measurements. Although studies have been done to help confirm the differential effects that insulin and amino acids have on the synthesis rates of individual proteins, how this differential effect is regulated has yet to be elucidated. It is possible that part of the differences seen with regional protein turnover may be due to the differential effects of insulin on protein subfractions within a tissue bed. Examples of this include the decrease in albumin synthesis with a simultaneous rise in fibrinogen synthesis, as well as the effects on apolipoprotein B 100 and antithrombin III [94].

C.   TYPE 2 DIABETES

Type 2 diabetes, formerly known as either maturity onset diabetes or noninsulin dependant diabetes, constitutes approximately 85% of all cases of diabetes. It is most commonly associated with obesity and may occur associated with a constellation of other conditions, including hypertension and dyslipidemia, when it may be referred to as part of the metabolic syndrome or 'syndrome X' [95]. Type 2 diabetes is characterized by the altered use of fuel, with glucose being overproduced by the liver and underused by the peripheral tissues. Although this condition is predominantly associated with abnormalities in carbohydrate metabolism, individuals with type 2 diabetes may still have some changes in protein metabolism that are associated with chronic complications seen with long term, poor glycemic control. Multiple hormones, and possibly substrates such as free fatty acids, may affect protein metabolism in type 2 diabetes. Since type 2 diabetes increases with age, aging per se may be a factor involved in protein metabolism in type 2 diabetes.

Although there is a rapid loss of lean body mass in type 1 diabetes when insulin is withdrawn, there is no such lean tissue to loss in type 2 diabetes. This may be due to the protein conserving effects of the insulin that is present. The quantity of insulin secreted may be the same as that in subjects who do not have diabetes, but they have an inappropriately low level in the face of high blood glucose. Only very low concentrations of insulin may be required to prevent proteolysis compared to those levels needed to stimulate glucose uptake into cells in people with type 2 diabetes. 10 mU/l of insulin is also the plasma concentration needed to prevent ketosis from hepatic nonesterified fatty acid metabolism by its effect on the mitochondrial carnitine shuttle, and this level may be sufficient to normalize amino acid metabolism.

Protein metabolism in type 2 diabetes is relatively preserved. Henry et al., showed that despite prolonged periods of hyperglycemia and dietary restriction, subjects with type 2 diabetes preserve lean body mass [96]. Furthermore, in age matched controls, fasting whole body protein metabolism is no different in subjects with or without type 2 diabetes [97-99]. Leucine flux, leucine balance and oxidation rates are normal in obese and nonobese subjects with type 2 diabetes and are unaffected by an improvement in glycemic control [97-101]. In addition, improving glycemic control by the use of sulphonylureas, biguanides or insulin has not been shown to improve either splanchnic or whole body protein turnover [99,102]. More evidence for this lack of difference came from work comparing amino acid disposal in diabetic and nondiabetic subjects undergoing simultaneous amino acid and insulin infusions. In both sets of subjects, whole body protein turnover was reduced suggesting that the antiproteolytic effect of insulin in people with type 2 diabetes remains intact [98]. There is some evidence showing that protein turnover is in fact increased in type 2 diabetes [103,104]. Several previous studies have demonstrated a nonsignificant trend towards increased whole body protein turnover. However, one study showed a significant 21% rise in breakdown with a corresponding 16% increase in protein synthesis when comparing protein kinetics during a period of poor glycemic control and then after 42 days of a very low energy diet [105]. The methodological differences among the studies may explain the differences. Cross sectional comparisons between type 2 diabetic subjects and controls are not easy because of the difference in body composition and fat distribution. Comparison of lean and obese people has demonstrated that obese people have higher whole body protein turnover than lean people [62]. However, at the baseline state there are no reported differences between obese (non-diabetic) controls and type 2 diabetic people.

Levels of branched chain amino acids in subjects with type 2 diabetes vary according to body habitus. Branched chain amino acid levels in lean diabetic subjects are the same as lean controls [97]. In addition, although levels are different to those seen in lean subjects, branched chain amino acid levels were reported to be similar [97], or higher [106] in obese subjects with and without type 2 diabetes. When subjects were maintained on a similar diet prior to the measurements, no differences between type 2 diabetic patients (on and off treatment), obese and lean controls could be detected [101]. This study, however, demonstrated that leucine transamination rates are slightly higher in type 2 diabetic people on poor glycemic control [101]. Furthermore, the above study showed that synthesis rates of various fractions of muscle proteins were unaffected by type 2 diabetes or its treatment state [101]. It may also be that, although the antiproteolytic effects of insulin at the whole body level may be similar in subjects with type 2 diabetes and controls [98,101], the action of insulin in specific tissues to produce an antiproteolytic effect seems to be significantly different. In lean control subjects, reductions in whole body proteolysis by high dose insulin primarily results from suppression of proteolysis in skeletal muscle. This is in contrast to type 2 diabetes, where despite the improvement in glycemic control to almost normal levels, the hyperinsulinemia seems only to suppress nonmuscle proteolysis [83]. This could be because protein and glucose metabolism in skeletal muscle is resistant to the effects of insulin in type 2 diabetes. An alternative explanation is that there is a dose response curve in different tissues for each of these effects, and that suppression of proteolysis may be maximal with the hyperinsulinaemic state seen in type 2 subjects and in hyperinsulinaemic clamp studies. This would explain the apparent low basal rates of skeletal muscle proteolysis and the inability of higher doses of insulin to produce any further suppression [107].

Skeletal muscle protein turnover in healthy individuals is reduced with insulin infusions [67,83], but in individuals with type 2 diabetes the effects of insulin on muscle protein turnover are less clear [97,99,101,103]. Gougeon et al., found that with high protein isoenergetic feeding of subjects with moderately well controlled type 2 diabetes, insulin therapy sufficient to normalize plasma glucose levels was enough to induce nitrogen retention [108].

D.   THE EFFECTS OF OTHER HORMONES ON PROTEIN TURNOVER IN DIABETES

Insulin deficiency is associated with increase in glucagon in all cases. Glucagon in the post-absorptive state increases energy expenditure and leucine oxidation, especially when insulin levels are low [109]. When amino acids are administered, glucagon plays a pivotal role in their disposal [110]. Glucagon also plays a key role in amino acid catabolism in people with type 1 diabetes [111]. Growth hormone levels may increase in some cases. When given systemically, growth hormone increases whole body protein synthesis but it's effects on muscle protein synthesis remain to be clarified [112,113]. IGF 1 has also been shown to increase muscle protein synthesis [114]. IGFBP 1 levels increase resulting in a decrease of free IGF-1 levels. In extreme situations, epinephrine, norepinephrine, and cortisol levels also increase. Catecholamines are not thought to be protein catabolic [115], and although cortisol levels do not rise in acute insulin deficiency, increased circulating levels have been associated with increased protein breakdown [116]. Glucocorticoids enhance proteolysis via the induction of the ATP-ubiquitin pathway in muscle, leading to a decrease in intracellular muscle proteins with little or no effect being seen in the liver.

VI. NEWER TECHNOLOGIES

Over time, the ability of researchers to better understand protein metabolism has advanced. Early work relied on crude estimations of whole body nitrogen balance [57], followed by the introduction of labelled tracers that allowed measurements at the whole body level and across tissue beds [117]. Further refinement allowed the measurement of individual protein subfractions within a tissue, such as myosin heavy chain, sarcoplasmic, and mitochondrial proteins within skeletal muscle. Other advances allowed the measurements of synthesis rates of circulating proteins [91,118-120]. It is currently possible to better understand the specific molecular level regulation of protein synthesis and breakdown. To understand the regulation of amino acid and protein metabolism it is important to study different tissues and the regulation of transcriptional and translational levels by hormones, substrates and other factors such as aging, exercise, etc. In addition, the study of amino acid and protein metabolism is not complete until the factors involved in the post-translational changes in proteins are better understood. Furthermore, integration of studies of body functions with changes in protein metabolism is also important. With the recent advances in genomics and proteomics, the potential for studies in this area has never been greater. The new microarrays and gene chips allow the assessment of several thousand gene products simultaneously. This can be done without having to specify which gene in particular need so to be studied. These global approaches will help to further focus studies on pattern of changes in gene transcript profiles [121], and on specific genes involved in the regulation of protein synthesis and breakdown in specific tissues. These changes can be followed over time in the same subjects, leading to a better understanding of what changes occur at the level of mRNA expression when insulin or other hormone or substrate

levels alter. However, mRNA expression often may not correspond to protein expression or function. It also remains to be determined what constitutes 'normal' and what is 'abnormal'. The normal, small differences in the genetic makeup of populations and individuals need to be distinguished from the small differences seen in disease states such as diabetes. Although there are challenges in data interpretation, it is the progress made in the data management and informatics areas of this technology that has enabled rapid improvement of our understanding. This issue is discussed in detail elsewhere [122].

VII. CONCLUSIONS AND SUMMARY

Absolute insulin deficiency is associated with profound metabolic changes including increased circulating amino acid levels (especially branched chain amino acids), net protein loss and increases in whole body protein catabolism. These effects can be reversed by the administration of insulin. The cause of increased amino acid levels and nitrogen loss associated with insulin deficiency have been investigated using isotopic techniques. Most of the findings suggest that, in humans, the anabolic actions of insulin are mainly in the prevention of protein breakdown, although not having an overall stimulatory effect on whole body protein synthesis unless in the presence of supplemental amino acids. It is the plasma amino acid concentrations that determine the ultimate effects that insulin has on protein synthesis rates. However, insulin is a key hormone involved in the regulation of gene transcription and translation and therefore plays a pivotal role in the regulation of protein synthesis and breakdown. Insulin specifically stimulates synthesis of proteins such as mitochondrial protein. In contrast to the dramatic changes of protein turnover in type 1 diabetic patients, the changes in protein turnover are rather

minimal in type 2 diabetic people. In type 2 diabetes these effects on protein metabolism are less impressive due to the probable high circulating levels of insulin already present.
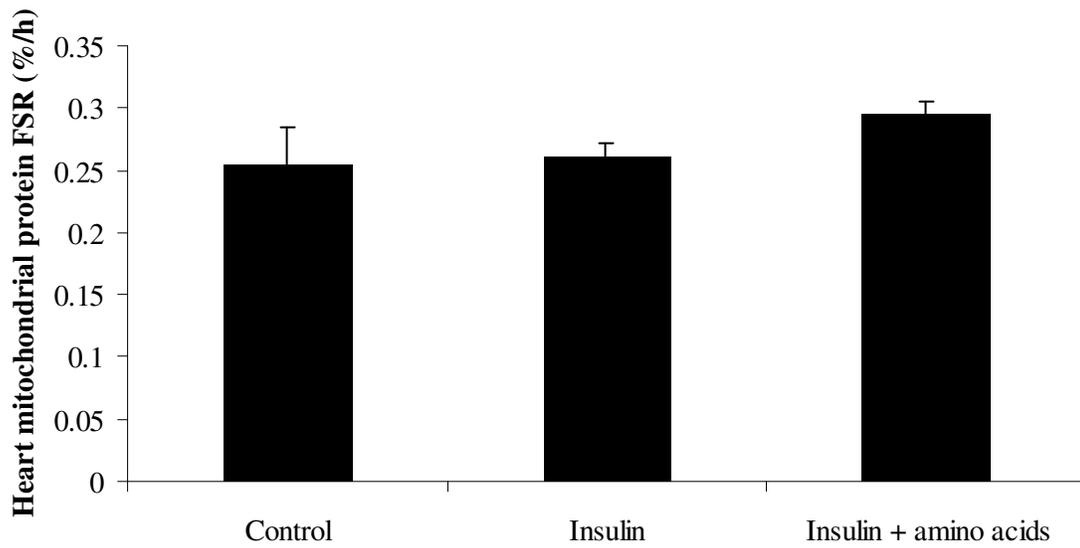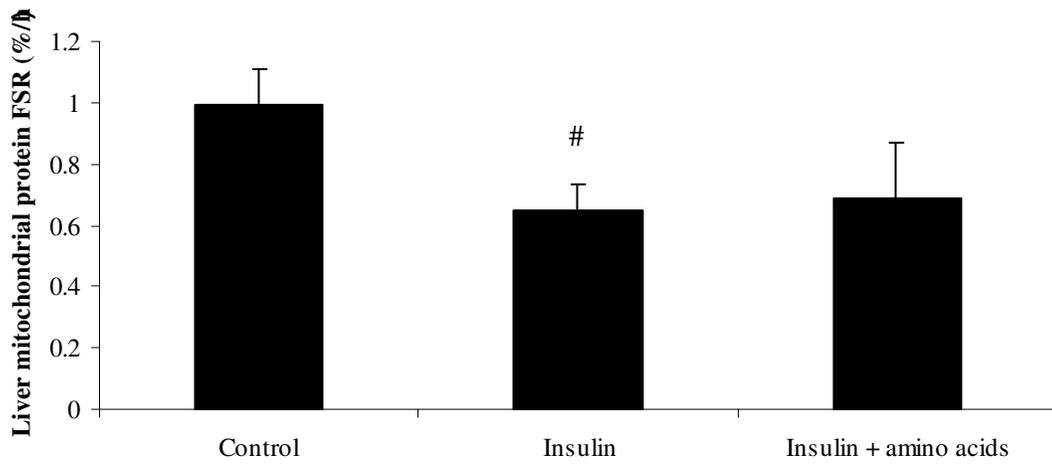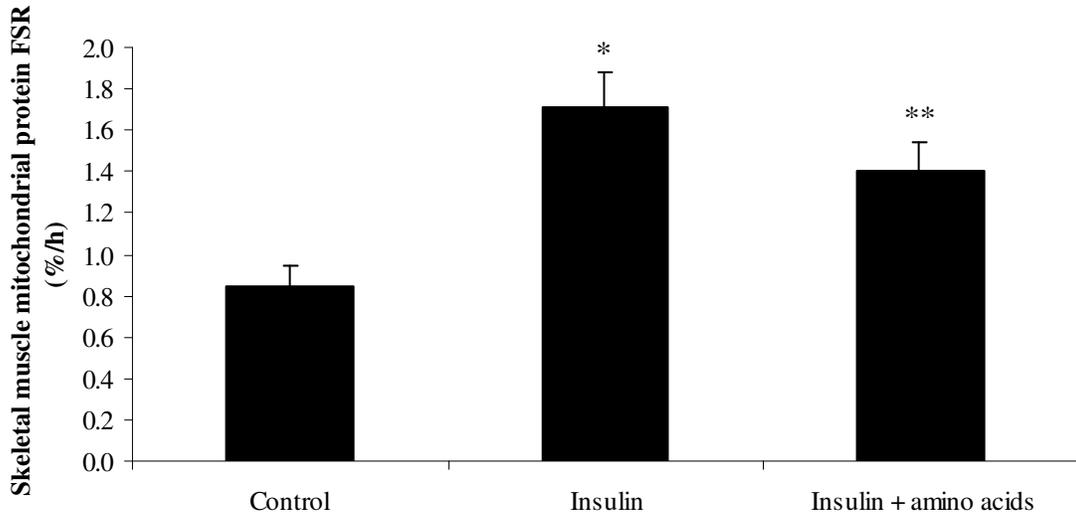
Figure 1A. Skeletal muscle, liver and heart mitochondrial protein fractional synthesis rates using [^{13}C] leucine. The upper panel represents skeletal muscle. * indicates a significant difference between the control group and the insulin group (P<0.04). ** indicates a significant difference between the control group and the insulin with amino acid group (P= 0.05). The middle panel represents liver mitochondrial protein fractional synthesis rates. # indicates a significant difference between the control group and the insulin group (P<0.02). There was a nonsignificant difference between the control group and the insulin with amino acid group (p<0.08). The lower panel represents heart mitochondrial protein fraction synthesis rates. There was no significant difference between the control group and the insulin group, nor was there any significant difference between the control group and the insulin with amino acid group (p>0.1 for both). Data from [52].
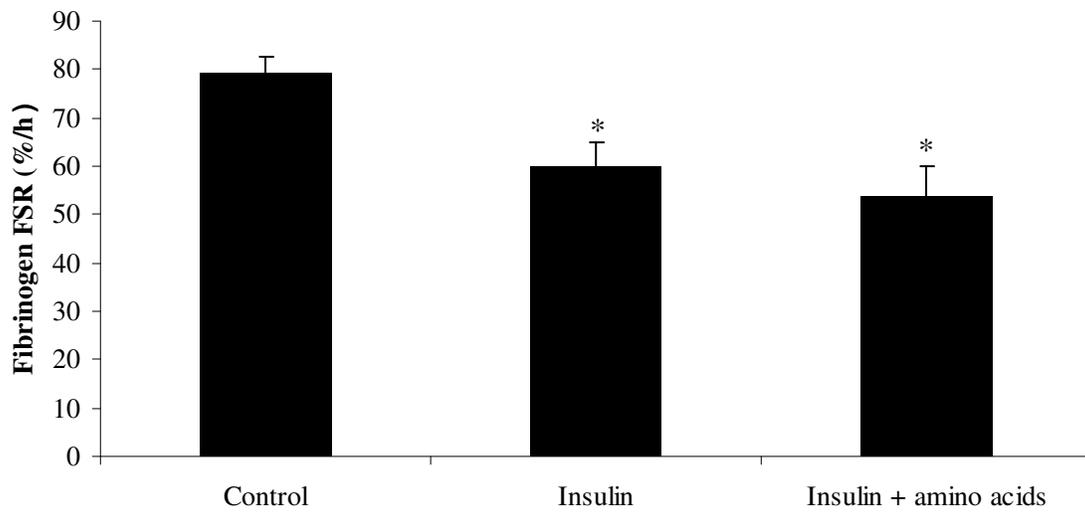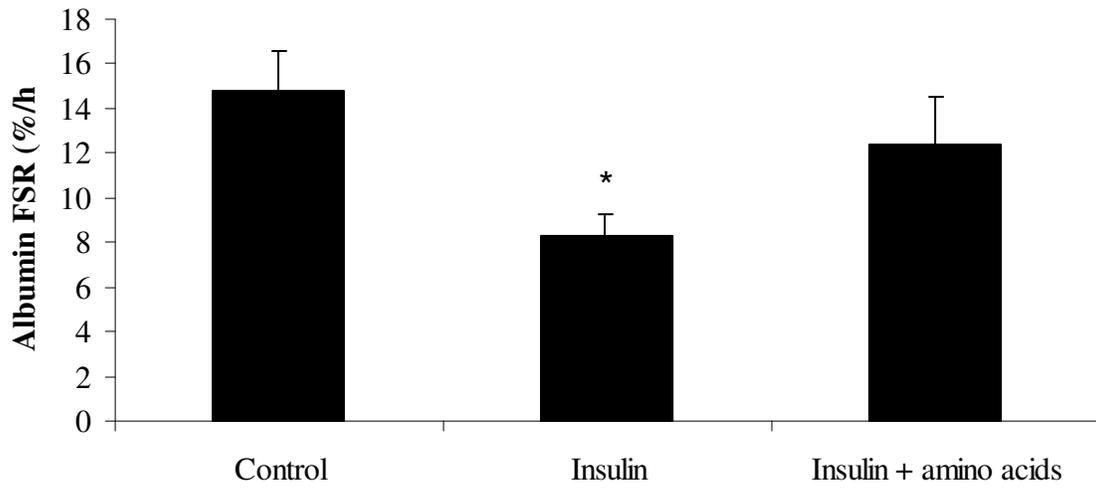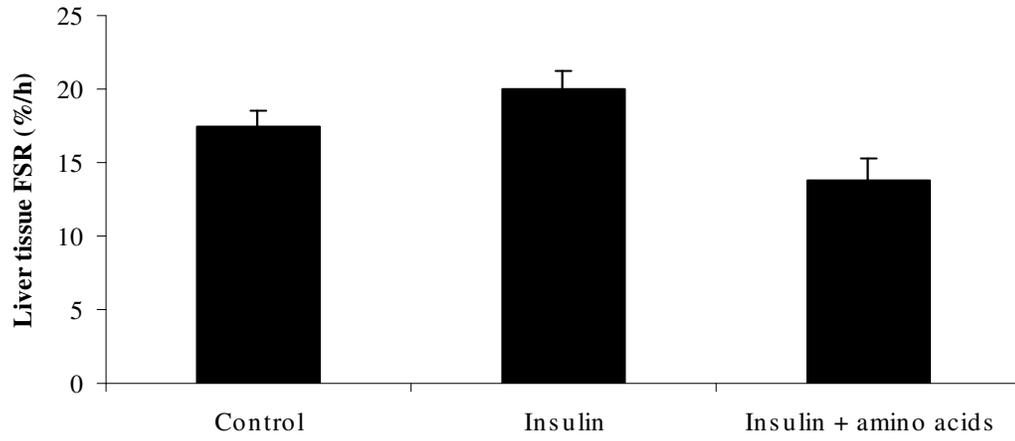
Figure 1B. Liver tissue, albumin and fibrinogen fractional synthesis rate in a swine model using leucyl tRNA as the precursor. The upper panel shows that total liver protein synthesis is not significantly different under control conditions or when insulin alone or with amino acids is infused. The middle panel shows the albumin fractional synthesis rate. This shows that, unlike liver, albumin synthesis is significantly decreased by insulin infusion, but restored if amino acids are added ($p<0.05$). The lower panel shows fibrinogen fractional synthesis rate. This differs from the effects on albumin. It can be seen that an insulin infusion significantly reduces fibrinogen fractional synthesis rate), but that amino acid infusion does not restore this ($p<0.05$ for both). Data from [53].

Figure 2. Effects of insulin deprivation on whole body nitrogen balance. Data from [57].

Figure 3A. Effects of insulin on skeletal muscle protein dynamics. Insulin does not significantly increase the rate of protein synthesis, but decreases the rate of protein breakdown. Data from [65].
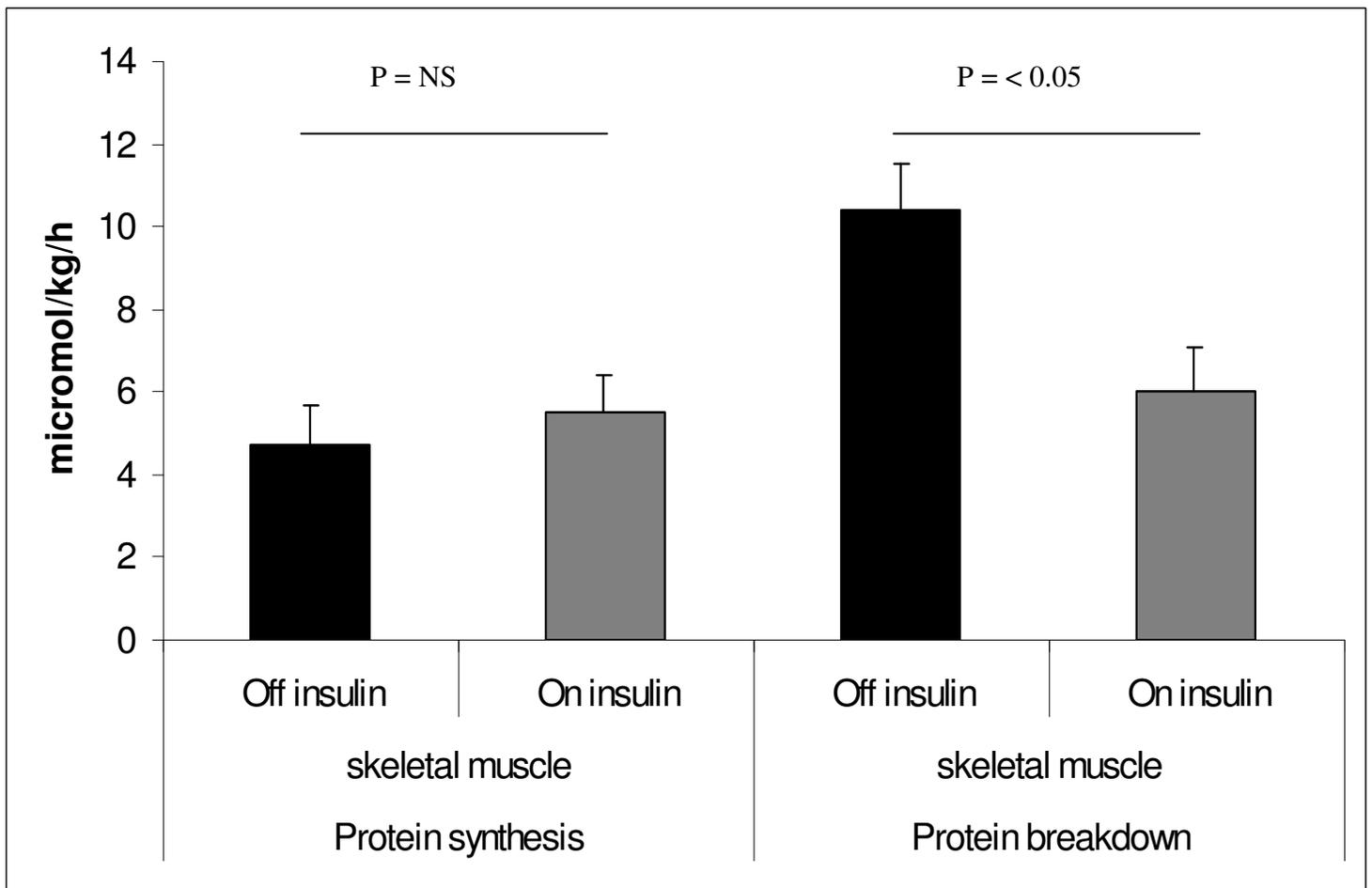
Figure 3B. Protein turnover in the splanchnic bed. This figure demonstrates that in the splanchnic bed, insulin deprivation leads to higher protein synthesis rates and higher protein breakdown rates. See text for explanation. Data from [65].
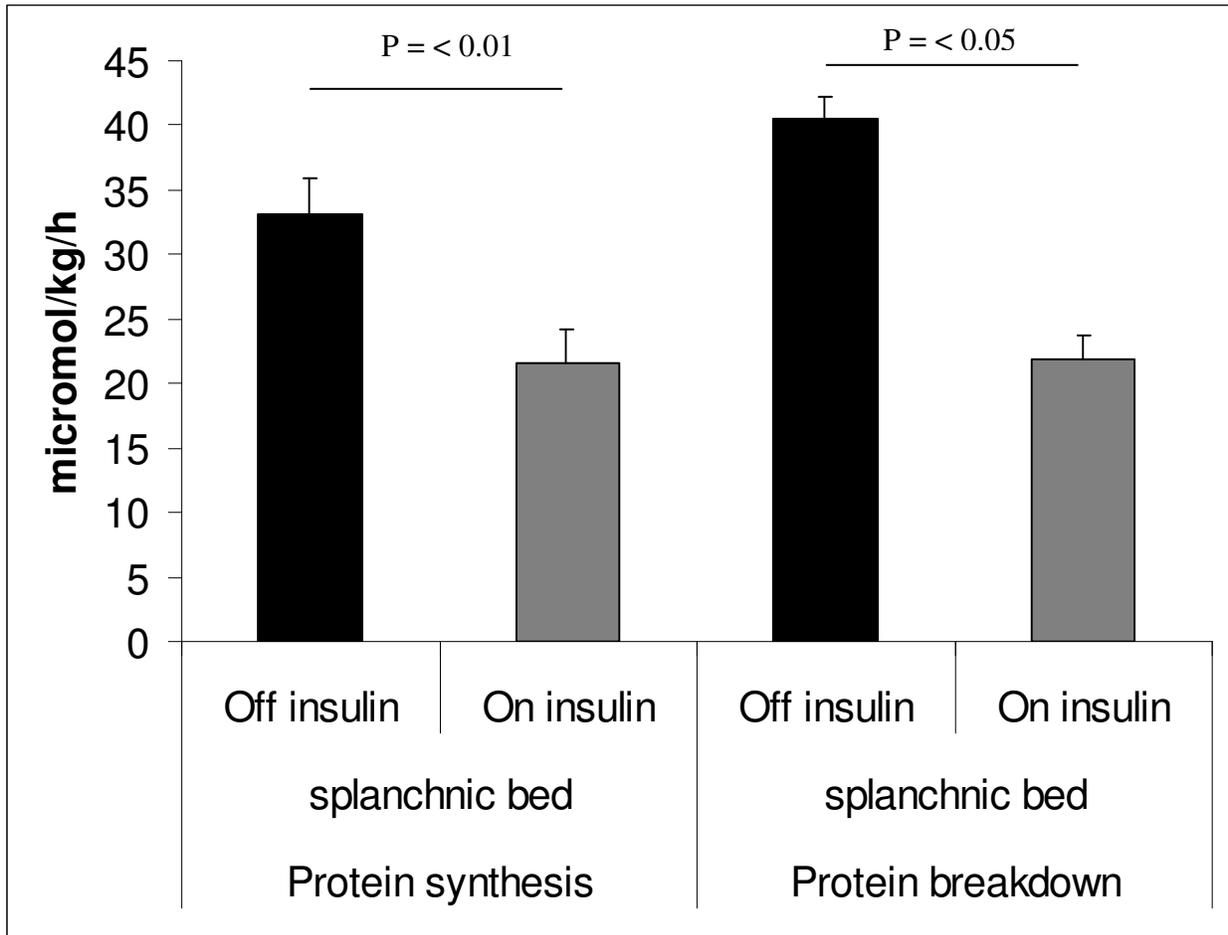
Table 1 Mechanisms by which insulin has effects on protein synthesis. Adapted from [16].

Total cellular protein synthesis can be enhanced by:
Stimulation of rRNA transcription
Increased translation of ribosomal protein mRNA with selective translation of previously transcribed mRNA
Promotion of nuclear mRNA release
Increasing association of polysomes with cytoskeleton
Regulation of initiation and elongation of mRNA translation
Specific proteins can be effected by:
Specific gene transcription can be either up or down regulated by altering the activity of a variety of transcription factors that are dependant on the hormone response element on the 5' region of the gene
Increased association and enhanced efficiency of translation of specific mRNA with polysomes
Changes in the stability of specific mRNA molecules

Table 2 Percent change in amino acid balance comparing insulin deprivation with insulin treatment across the leg and splanchnic beds. A negative number signifies a reduction in arterio – venous differences with insulin treatment, consistent with a decrease in amino acid output, i.e., a net decrease in protein breakdown.   a = p < 0.05, b = p < 0.01, c = p < 0.001, d = not significant. Data from [65].

	Across leg	Across splanchnic bed
Aspartate	187.5 [a]	-49.5 [d]
Glutamate	37.2 [d]	-39.4 [d]
Serine	-192.9 [c]	109.6 [c]
Glutamine	-70.7 [d]	111.0 [d]
Histidine	-91.2 [a]	129.8 [b]
Glycine	-64.7 [a]	359.9 [a]
Theronine	-84.0 [c]	145.4 [c]
Alanine	-58.8 [c]	75.8 [a]
Tyrosine	-74.9 [a]	30.2 [b]
Valine	-79.0 [d]	108.3 [d]
Methionine	-63.1 [a]	93.5 [a]
Phenylalanine	-83.4 [b]	194.5 [b]
Isoleucine	-93.1 [a]	-174.2 [d]
Leucine	-79.0 [a]	937.0 [b]
Lysine	-89.9 [c]	369.9 [b]
α ketoisocaproic acid	-83.0 [b]	-2000.0 [b]

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