Muscle protein anabolism in type 2 diabetes

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance. This is classically defined as blunted insulin-induced suppression of hepatic glucose production and uptake into peripheral tissues, including muscle, and of lipolysis in adipose tissue. As insulin also modulates protein metabolism by stimulating synthesis and suppressing breakdown, derangement in muscle protein anabolism in T2DM is expected, albeit not found using some experimental protocols. In this review we explore the potential clinical manifestations of altered muscle protein anabolism in T2DM and discuss recent studies of whole body and muscle protein anabolism in response to insulin, amino acids, and exercise.

MUSCLE MASS AND STRENGTH

Clinically, obese people with T2DM are not perceived to have muscle loss, a reason for protein metabolism abnormalities not having been considered until recently as significant. Despite usually having greater muscle mass than lean controls due to larger body size and obesity, T2DM appears to cause poorer muscle performance, a factor contributing to increased disabilities in older age. Lower leg and arm muscle strength have been reported in a cross-section of older men with T2DM compared with healthy controls [2]. Similarly, older patients with T2DM had a 30% greater decline over 3 years in leg muscle strength and quality than healthy age-matched controls [3]. Impairments in muscle strength in T2DM have been strongly correlated with intramuscular fat storage which is twice that found in controls [4]. However, intramyocellular triglyceride content is not an independent biomarker of insulin resistance, as its increase in
**KEY POINTS**

- T2DM is associated with impaired muscle function and accelerated loss of muscle mass in older adult age.
- Protein anabolism is blunted in response to hyperinsulinemia in obese hyperglycemic men with T2DM.
- Aminoacidemia simulating ample protein intake ‘normalizes’ insulin resistance of protein without worsening the already abnormal glucose uptake.
- Muscle protein anabolic responses to exercise are normal in T2DM.
- It is promising that possible nutritional strategies are emerging, namely leucine supply combined with exercise that might protect from age-related loss of muscle mass and function in T2DM without worsening the already abnormal glucose uptake.

**RESPONSE TO INSULIN**

Insulin stimulates protein anabolism in muscle fibers from healthy individuals by increasing protein synthesis and suppressing breakdown [10]. Thus, insulin resistance in T2DM should impair protein metabolism concurrent with that proven to occur for that of glucose and lipids. Under in-vivo physiological conditions, insulin increases postprandially, but meal studies cannot distinguish its effect on protein anabolism from that of absorbed nutrients. To isolate the effect of insulin, the hyperinsulinemic, euglycemic clamp has been used, with serum concentrations maintained at typical postprandial concentrations (500–600 pmol/l) and glucose is infused to maintain glycemia at 5.5 mmol/l. Amino acid tracers ([1-13C] or [1-14C] leucine, L-ring [1-2H5] phenylalanine) are infused to assess whole body and/or muscle protein kinetics. Ideally, a mixture of amino acids is also infused to maintain basal fasting plasma concentrations (isooaminoacidemia). This prevents plasma amino acids from falling below fasting levels due to suppression of protein breakdown by insulin, and thus creating a nonphysiological limitation of amino acid availability for synthesis [11]. Using hyperinsulinemic euglycemic, isooaminoacidemic clamps, we found insulin resistance of whole body protein anabolism, mostly due to blunted stimulation of synthesis, in overweight and obese men with poorly controlled T2DM compared with weight matched non-diabetic men (Fig. 1) [12]. Women studied in the same manner did not show this added diabetes effect possibly because the obese women have more insulin resistance of protein anabolism versus lean patients [13]. Similar results were found in obese, hyperglycemic men with T2DM with glycemia clamped at 8 mmol/l to simulate the more typical postabsorptive milieu of T2DM, instead of being lowered to 5.5 mmol/l to begin the clamp [1*]. This hyperglycemia was associated with a more accelerated turnover rate (higher protein synthesis and breakdown) than during euglycemia. These whole body protein data suggest that most of the impairments exist in muscles, as they account for most of the insulin-induced protein anabolism [14], and thus wherein most insulin resistance takes place.

Our findings contrast with those of others reporting normal whole body [15] and muscle [16] protein anabolism in T2DM in response to insulin. This discrepancy might be due to the lack of controlling for sex, body composition, previous protein and energy intake differences, or especially the absence of concurrent amino acid infusion to maintain fasting levels during these clamps. Inconsistent findings might also stem from differences in prior glucose control as hyperglycemia was normalized by insulin infusion for 11 days [16] or overnight [15] before assessing insulin-induced protein anabolism in some protocols, but not others [1*,12]. It remains to be determined with well designed clamp studies that control for these confounding variables, whether good prior glycemic control improves muscle insulin resistance of protein metabolism.

**RESPONSES TO AMINO ACIDS**

Amino acids not only stimulate muscle protein anabolism by serving as substrates for protein
synthesis but also as nutritional signals in the mRNA translation initiation pathway leading to protein synthesis. Among amino acids, the essential branched-chain, leucine, is the most potent by activating the mammalian target of rapamycin–complex 1 (mTORC1), which then triggers signal transduction events that promote protein synthesis in skeletal muscle [17]. The exact mechanisms of leucine action are still not clear but appear to involve Rag proteins, a family of guanosine triphosphatases that interact with mTORC1. This promotes its intracellular perinuclear localization, which favors its activation by Rheb [18]. Furthermore, leucine may also contribute to anabolism by suppressing muscle protein breakdown via inhibition of the ubiquitin–proteasome pathway [19] and possibly autophagy, through mTORC1 activation or independently [20].

Amino acids stimulate muscle protein anabolism in an independent and synergistic fashion with insulin [21]. This is why under normal physiological conditions, the greatest protein anabolism occurs in the fed state, during which concentrations of insulin and amino acids are elevated. Thus, one might anticipate that insulin resistance of protein anabolism in T2DM would be predicted to be maximal in this state. However, postprandial muscle protein synthesis was found to be normal in hyperglycemic T2DM men after the consumption of carbohydrate and protein hydrolysate taken in repeated boluses [22]. We have also recently reported ‘normalized’ whole body protein anabolism in T2DM during clamp simulating fed state concentrations of insulin and amino acids [1*]. Obese men with T2DM had a whole-body protein anabolic response comparable to that found in lean men during the hyperinsulinemic, hyperglycemic, hyperaminoacidemic clamp. This was supported by signaling data from muscle biopsies collected during the clamps [23**], in which the magnitude of increase in phosphorylation of mTOR and its downstream substrate, ribosomal protein S6 (rpS6) in response to hyperinsulinemia and hyperaminoacidemia (Fig. 2a and b) was comparable to that of lean men studied under similar conditions [21]. Thus, we concluded that postprandial hyperaminoacidemia can overcome insulin resistance of protein anabolism in T2DM.

One possible explanation for these findings is the ample amino acid or protein administered, which if extrapolated over one day exceeds the recommended daily adult protein intake. Although the total amount of amino acid infused during the clamp study (30 g) [1*] corresponds to a typical adult meal, the total amount of protein consumed during the study by Manders et al. [22] was very high (134 g protein hydrolysate taken in 12 repeated boluses over 6 h). Thus, it remains to be determined at what levels and durations of hyperaminoacidemia muscle protein anabolism become normalized in T2DM, as there could be a shift in dose-response to the right, requiring larger amounts of exogenous protein than
recommended requirements. This could explain the clinical signs of abnormal muscle protein anabolism in T2DM that start to become apparent in old age concurrent with a decrease in protein intake [24] and specifically in serum branched-chain amino acids (BCAA).

**THE DILEMMA OF AMINO ACIDS/PROTEIN IMPROVING VERSUS IMPAIRING METABOLIC CONTROL**

Increasing dietary protein/amino acid intake has been recommended as part of the dietary management to improve insulin sensitivity and blood glucose control in T2DM [25]. High protein, hypenergetic diets can cause weight loss with maintenance of lean body mass. Furthermore, certain individual amino acids including leucine augment insulin secretion, despite the blunted glucose insulino tropic response in T2DM [26]. This is likely to be a factor in low carbohydrate, high protein diets improving glycemic control in untreated T2DM [27]. Increasing protein and amino acid intake, leucine in particular, has been suggested as a nutritional strategy to improve muscle protein anabolism in T2DM, especially in the elderly with decreased muscle mass [28,29]. This is thought to increase muscle protein anabolism both directly by activating protein synthesis and by stimulating insulin secretion. Amino acid composition and digestibility of specific protein sources may also play roles. Muscle protein synthesis was stimulated significantly more in response to 20 g whey versus casein in prediabetic older men [30]. This was partly attributed to whey’s higher leucine content. A high protein (30% of energy mostly from animal food sources), low carbohydrate (30%), weight maintaining diet for 5 weeks in men with untreated T2DM was associated with improved nitrogen balance with no change in body composition [31]. Notable is that integrated 24 h plasma concentrations of leucine and the other BCAA, tyrosine, and phenylalanine were increased up to 12-fold for leucine. In contrast, long-term leucine supplementation (7.5 g per day) did not affect muscle mass in older men with T2DM. However, these patients did not have compromised muscle mass at baseline, and their diet composition was not controlled, though protein intake was ‘adequate’ (~1.0 g per day) [32]. It remains to be established whether persons with T2DM with reduced protein intake and/or muscle mass would benefit from leucine supplementation,

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**FIGURE 2.** Phosphorylation of mTOR (a), rpS6 (b), IRS-1 at Ser636/639 (c) and Ser1101 (d) at baseline and during hyperinsulenic, hyperglycemic clamps, with amino acids clamped at postabsorptive concentrations (isoAA) and postprandial concentrations (hyperAA). Data are expressed as the ratio of phosphorylated to total protein. Representative western blots are shown. *P < 0.05 versus baseline, †P < 0.05 versus isoAA. Reproduced with permission from [23**].
as was recently demonstrated in the healthy elderly consuming protein at the RDA level (0.8 g/kg body weight per day) [33].

In contrast, several lines of evidence have been published that have been interpreted as protein, especially leucine, worsening glucoregulation. A plethora of recent metabolomic studies have all come up with serum BCAA as a signal of diabetes risk, including prediction of later T2DM [34]. Epidemiological studies have revealed a strong positive association between increased intake of animal protein rich in BCAA and the risk of insulin resistance and T2DM [35]. Furthermore, raising serum amino acids to postprandial concentrations blunts glucose uptake in healthy humans [21,36]. At the molecular level, this was attributed to leucine-induced overactivation of ribosomal protein (rp) S6 kinase 1 (S6K1), a substrate of the mTORC1 signaling pathway [36], which phosphorylates the insulin receptor substrate (IRS)-1 at specific serine residues [37]. This, in turn, inhibits IRS-1 and deactivates Akt signaling resulting in attenuated translocation of GLUT4 to the plasma membrane, and hence, reduced glucose uptake.

These mechanisms were extrapolated to insulin resistant states to postulate that increased protein intake in T2DM could further contribute to peripheral insulin resistance of glucose metabolism [38]. However, we have recently demonstrated in hyperglycemic men with T2DM during hyperinsulinemic clamps that whole-body glucose uptake was not further impaired by hyperaminoacidemia. This was supported by unchanged IRS-1 serine phosphorylation during hyperaminoacidemia despite increased mTORC1 phosphorylation (Fig. 2c and d) [23**]. We suggest that in such hyperglycemic T2DM, insulin-stimulated glucose uptake is already highly attenuated, as is hyperphosphorylation of IRS-1 serine residues, such that hyperaminoacidemia does not aggravate it. Another key metabolic abnormality of T2DM that is thought to be worsened by excess leucine intake is pancreatic β cell apoptosis. Leucine-induced mTORC1 phosphorylation in cultured β cells, concurrent with high levels of glucose, insulin, and insulin-like growth factor (IGF)-1 in T2DM might ‘overstimulate’ β cell proliferation and precipitate β cell senescence [39]. However, there exists no evidence to support this hypothesis in humans. In summary, inconsistent findings arise among those with unambiguous clinical benefits favoring higher protein/amino acid intakes with negative associations derived from epidemiologic/metabolomic and some more fundamental studies. Reconciliation of these findings requires highly targeted whole-body and organ/tissue studies at a mechanistic level, in persons with T2DM.

## RESPONSES TO EXERCISE

Resistance exercise generates anabolic signals that stimulate muscle protein synthesis and is also known to improve insulin sensitivity independently of any effect it might have on weight loss. Physical activity has been recommended to prevent age-related loss of muscle mass. In a recent report, Wall et al. [40*] demonstrated that electrical stimulation of leg muscle contraction was sufficient to increase muscle protein synthesis in older T2DM men during a 4 h recovery period. Exercise prior to ingestion of a protein bolus (20 g) led to higher muscle protein synthesis versus no exercise in prediabetic aged men [41], at a magnitude that was comparable to that observed in young men. Longer term exercise regimens over 3–4 months have also been proven to increase muscle mass [42] and strength [43*,44*] in T2DM and improvements were comparable with those of healthy controls [44*].

## CONCLUSION

Age-related loss of muscle mass and function is accelerated with T2DM that might be due to insulin resistance of protein anabolism with concurrent insufficient protein intake and physical activity. Indeed, in T2DM, muscle protein anabolism is normal in response to ample supply of BCAA, especially leucine, or to exercise. More research is required on the human muscle cellular and molecular mechanisms that reveal strategies for intervention to concurrently correct glucose and protein metabolic abnormalities. The dilemma is to identify the right balance, that is, optimal protein intake combined with exercise prescription to protect from muscle loss, while not aggravating good glycemic control.

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## Conflicts of interest

There are no conflicts of interest.

## REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
* of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 116–117):

Protein, amino acid metabolism and therapy


Data from Third National Health and Nutrition Examination Survey showing inverse relationship between muscle mass and risk of type 2 diabetes.


Increased expression of myostatin known to downregulate muscle mass in diabetic muscle.


Molecular data from muscle biopsies showing normal phosphorylation of mTORC1 pathway in response to hyperaminoacidemia in T2DM supporting previous data of normal whole-body leucine kinetics in T2DM.


Leucine rich whey protein versus casein results in higher stimulation of muscle protein synthesis in prediabetic men.


High protein, low carbohydrate diet for 5 weeks induces a positive nitrogen balance in type 2 diabetes compared with control diet.


First long-term (6 months) leucine supplementation studies in T2DM that showed no change in muscle mass.


First study on effective muscle protein synthesis stimulation in elderly T2DM by electrical stimulation.


Improvement in muscle strength after 3 months exercise regimen in T2DM.


Similar Improvements in muscle strength in elderly T2DM and healthy controls after 4-month exercise regimen.

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