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Exogenous insulin does not increase muscle protein synthesis rate when administered systemically: a systematic review

Jorn Trommelen, Bart B L Groen, Henrike M Hamer, Lisette C P G M de Groot¹
and Luc J C van Loon

Department of Human Movement Sciences, Faculty of Health, Medicine and Life Sciences, NUTRIM School for Nutrition, Toxicology and Metabolism, Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands and

¹Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands

Correspondence
should be addressed
to L J C van Loon

Email
l.vanloon@
maastrichtuniversity.nl

Abstract

Background: Though it is well appreciated that insulin plays an important role in the regulation of muscle protein metabolism, there is much discrepancy in the literature on the capacity of exogenous insulin administration to increase muscle protein synthesis rates *in vivo* in humans.

Objective: To assess whether exogenous insulin administration increases muscle protein synthesis rates in young and older adults.

Design: A systematic review of clinical trials was performed and the presence or absence of an increase in muscle protein synthesis rate was reported for each individual study arm. In a stepwise manner, multiple models were constructed that excluded study arms based on the following conditions: model 1, concurrent hyperaminoacidemia; model 2, insulin-induced hypoaminoacidemia; model 3, supraphysiological insulin concentrations; and model 4, older, more insulin resistant, subjects.

Conclusions: From the presented data in the current systematic review, we conclude that: i) exogenous insulin and amino acid administration effectively increase muscle protein synthesis, but this effect is attributed to the hyperaminoacidemia; ii) exogenous insulin administered systemically induces hypoaminoacidemia which obviates any insulin-stimulatory effect on muscle protein synthesis; iii) exogenous insulin resulting in supraphysiological insulin levels exceeding 50 000 pmol/l may effectively augment muscle protein synthesis; iv) exogenous insulin may have a diminished effect on muscle protein synthesis in older adults due to age-related anabolic resistance; and v) exogenous insulin administered systemically does not increase muscle protein synthesis in healthy, young adults.

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Introduction

Aging is associated with a progressive loss of skeletal muscle mass and function, coined sarcopenia (1). Age-related muscle loss is associated with many adverse health outcomes including increased falls, need for institutionalization, early onset of disabilities, decline in quality of life, increased likelihood of developing chronic metabolic diseases, and increased mortality (2). Owing to the global expansion of the elderly population, sarcopenia is becoming an increasing burden on our healthcare system (3).

The etiology of muscle loss with aging remains far from understood. While multifaceted in nature, the observed decline in muscle mass must stem from a structural imbalance between muscle protein synthesis and breakdown rates. The loss of muscle mass may be attributed to a decrease in muscle protein synthesis, an increase in muscle protein breakdown, or a combination of both. Previous work suggests that post-absorptive muscle protein synthesis rates are not compromised with

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aging (4). Therefore, investigators have shifted their focus to possible age-related blunted responses to anabolic stimuli such as food intake (1, 5, 6). Several studies have now shown that the postprandial muscle protein synthetic response to a single dose of amino acids is attenuated in senescent muscle, a phenomenon that has been termed 'anabolic resistance' (1, 6). One of the potential mechanisms underlying anabolic resistance may include an increase in insulin resistance with aging (7, 8).

Insulin is well recognized as a key hormone responsible for increasing endogenous carbohydrate and fat storage. However, its role in protein metabolism is more controversial. *In vitro* studies have shown that insulin stimulates muscle protein synthesis by direct activation of the translation machinery through the PI3K→Akt→mTORC1 pathway (9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19). Insulin may also affect protein metabolism *in vivo* due to its vasoactive properties. The postprandial rise in circulating insulin stimulates endothelial-dependent vasodilation by virtue of its action on endothelial nitric oxide synthase, resulting in greater capillary recruitment, increased microvascular volume, and nutritive blood flow to skeletal muscle tissue (20). It could be speculated that the greater post-prandial perfusion will increase the exposure of muscle tissue to nutrients and growth factors and increase muscle protein synthesis. However, whether insulin has a stimulatory effect on post-prandial muscle protein synthesis in humans has been the subject of much debate (21, 22). Many believe that circulating insulin concentrations are simply permissive, as opposed to modulatory, to allow an increase in muscle protein synthesis in healthy young subjects (21). Specifically, only a small amount of insulin is thought to be necessary to 'prime' the system and it is the subsequent increase in amino acid availability that drives the post-prandial muscle protein synthesis response (23). However, it is speculated that older adults are more resistant to the effect of insulin on muscle protein synthesis, a defect associated with endothelial dysfunction (24, 25, 26). This systematic review examines the existing literature regarding the proposed effect of increasing circulating insulin levels on muscle protein synthesis *in vivo* in humans and tries to define whether such an effect differs between young and older adults.

Methods

Search strategy

A systematic review was performed according to PRISMA guidelines (27). In short, a computerized search of the

literature was performed in January 2014 by using the PubMed database (<http://www.ncbi.nlm.gov/pubmed/>) and by hand searching reference lists of the identified studies and key literature reviews. The following search terms were used: insulin; hyperinsulinism; muscle; leg; forearm; myofibrillar; anabol*; protein synthe* and protein accretion and the Boolean functions AND and OR. The final electronic search was performed on 8th August 2014.

Eligibility criteria

Types of studies ► Clinical trials studying the administration of insulin to healthy humans. Studies were restricted to those written in the English language. No publication date restrictions were imposed.

Types of participants ► Healthy human participants of any age receiving exogenous insulin were considered and stratified in healthy young adults (group mean age between 18 and 65 years) or older adults (group mean age ≥ 65 years). To study the effect of age on insulin-mediated muscle protein synthesis *per se*, subjects with any apparent co-morbidity, including diabetes, were excluded.

Types of intervention ► This review was limited to studies examining exogenous insulin administration.

Types of outcome measures ► The primary outcome measure is qualitative assessment of muscle protein synthesis, i.e. a significant increase or no effect. The included studies assessed muscle protein synthesis as measured by precursor rate of disappearance (leg Rd) in the two-pool arteriovenous balance method, intracellular precursor utilization ($F_{o,m}$) in the three-pool arteriovenous balance method, or fractional synthetic rate (FSR) in the precursor-product model (28). These measures tended to reach similar qualitative assessments of muscle protein synthesis (data not shown), therefore no distinction between models was made for interpretation of the reported effect of insulin on muscle protein synthesis.

Study selection

Eligibility assessment was performed individually by two authors (J Trommelen and B B L Groen). Disagreements between reviewers were resolved by consensus. Titles and abstracts identified by the search strategy were screened for relevance, defined by meeting all of the following criteria: i) human subjects, ii) study design was a clinical trial, iii) intervention included exogenous insulin

administration in at least one of the study arms, iv) assessed mixed muscle protein synthesis (Rd, $F_{o,m}$, or FSR), and v) full-text accessibility.

Data items and collection process

Two authors (J Trommelen and B B L Groen) extracted data from included studies individually. Disagreements between reviewers were resolved by consensus. Information was extracted from each included trial on: i) subject characteristics, including age and number; ii) muscle protein synthesis assessment model (data not shown); iii) type of intervention, including dose, co-intervention, infusion compartment, and comparator groups; and iv) study outcome, including effect of exogenous insulin on muscle protein synthesis, clustered in significant increase, or no effect.

Data synthesis

The trials were reviewed with full tabulation of the results of all included studies. Owing to clinical heterogeneity in experimental designs, it was not possible to conduct a meta-analysis. In addition, differences in experimental methods are known to introduce variability in muscle protein synthesis, complicating quantitative analysis between studies (readers are referred to Smith *et al.* (29) for a review on this topic). Therefore, qualitative assessment of muscle protein synthesis, i.e. a significant increase or no effect, was determined for included studies. From these data, we constructed several models in which study arms were excluded based on (biological) rationale. In model 1 study arms with concurrent hyperaminoacidemia are excluded. Amino acids can independently stimulate muscle protein synthesis as well as endogenous insulin release, making it impossible to distinguish between the effect of insulin and that of the amino acid infusion (30, 31). Model 2 additionally excludes study arms with insulin-mediated hypoaminoacidemia. It has been suggested that the effect of insulin on muscle protein synthesis is mediated by insulin-induced changes in amino acid delivery to the muscle (8, 26, 32, 33). Systemic insulin infusion induces hypoaminoacidemia, which may limit muscle amino acid delivery. Therefore in this model, study arms were excluded that allowed amino acid levels to drop below baseline. Model 3 additionally excludes study arms achieving supraphysiological insulin concentrations. This was done to differentiate between supra-physiological levels that can only be achieved by insulin administration (>1200 pmol/l) and physiological levels

within reach of endogenous production in response to a mixed meal (≤ 1200 pmol/l). Model 4 additionally excludes study arms in older subjects, because it has been suggested that elderly are more resistant to the proposed stimulatory properties of insulin on muscle protein synthesis (24, 26).

Results

Study selection

The search of the PubMed database provided a total of 2021 citations (see Fig. 1). From the total of 2025 citations, 1980 studies were discarded, because after reviewing the abstracts these papers did not meet the inclusion criteria. The full text of the remaining 45 citations was examined in more detail. After careful reading of the full text, an additional ten studies did not meet the inclusion criteria as described. A total of 40 studies met the eligibility criteria and were included in the systematic review.

Study characteristics

All selected studies are summarized in Table 1.

Figure 1
Flow diagram of study identification.

Table 1 Summary of included studies evaluating the effect of exogenous insulin on muscle protein synthesis in healthy humans. Data presented as mean $\pm$ s.e.m. or range. Increasing roman subscript numbers indicate increasing levels of administration.

References	Intervention	Comparator	Study design	Compartment	Subjects	Age (years)	n	Concentration (pmol/l)	MPS
(62)	INS	Basal	Single arm	Local: forearm	Young	18–29	7	745 $\pm$ 66	$\leftrightarrow$
(30)	INS + AA _{BASAL}	AA _{BASAL}	Single arm	Systemic	Young	24–42	8	597 $\pm$ 44	$\uparrow$
(63)	INS	Basal	Single arm	Local: forearm	Young	25 $\pm$ 2	8	215 $\pm$ 9	$\leftrightarrow$
(49)	INS	Basal	Single arm	Systemic	Young	37 $\pm$ 4	7	18 755 $\pm$ 2511	$\leftrightarrow$
(50)	INS	Basal	Single arm	Systemic	Young	22–30	7	538 $\pm$ 43	$\leftrightarrow$
(51)	INS	Basal	Single arm	Systemic	Young	53 $\pm$ 3	8	718–861	$\leftrightarrow$
(64)	INS + AA _{BASAL}	Basal	Single arm	Systemic	Young	53 $\pm$ 2	10	509 $\pm$ 36	$\leftrightarrow$
(65)	INS + AA _{BASAL}	Basal	Single arm	Systemic	Young	52 $\pm$ 2	12	502 $\pm$ 29	$\uparrow$
(34)	INS _I	Saline	Parallel arms	Local: forearm	Young		39	198 $\pm$ 24	$\leftrightarrow$
	INS _{II}							240 $\pm$ 30	$\leftrightarrow$
	INS _{III}							402 $\pm$ 48	$\leftrightarrow$
	INS _{IV}							745 $\pm$ 66	$\leftrightarrow$
(57)	INS + AA _{HYPER}	AA _{HYPER}	Single arm	Systemic	Young	49 $\pm$ 6	8	718–861	$\leftrightarrow$
(52)	INS	Basal	Single arm	Systemic	Young	26 $\pm$ 1	6	287	$\leftrightarrow$
(36)	INS + AA _{BASAL}	Basal	Single arm	Systemic	Young	53 $\pm$ 2	12	502 $\pm$ 29	$\uparrow$
(28)	INS	Basal	Single arm	Local: leg	Young	29 $\pm$ 5	6	462 $\pm$ 54	$\uparrow$
(58)	INS + AA _{HYPER}	AA _{HYPER}	Single arm	Local: forearm	Young	20–32	22	600	$\leftrightarrow$
(53)	INS _I	Saline	Parallel arms	Systemic	Young	21–38	24	145 $\pm$ 21	$\leftrightarrow$
	INS _{II}							194 $\pm$ 7	$\leftrightarrow$
	INS _{III}							404 $\pm$ 26	$\leftrightarrow$
(40)	INS _{SUPRA} + AA _{BASAL}	Basal	Parallel arms	Local: forearm	Young	24 $\pm$ 2	14	50 943–81 078	$\uparrow$
	INS _{SUPRA} + AA _{BASAL}								$\uparrow$
	INS _{SUPRA} + AA _{HYPER}								$\uparrow$
(47)	INS + AA _{HYPER}	Basal	Single arm	Systemic	Young	25–32	6	718 $\pm$ 50	$\uparrow$
(39)	INS	Basal	Single arm	Systemic	Young	22–40	7	581 $\pm$ 93	$\leftrightarrow$
	INS + AA _{HYPER}					25–32	6	718 $\pm$ 50	$\uparrow$
(38)	INS	Basal	Single arm	Local: leg	Young	29 $\pm$ 5	5	444 $\pm$ 56	$\uparrow$
(41)	INS	Saline	Parallel arms	Systemic	Young	27 $\pm$ 3	6	159 $\pm$ 8–182 $\pm$ 10	$\leftrightarrow$
	INS + AA _{BASAL}					25 $\pm$ 3	6		$\uparrow$
	INS + AA _{HYPER}					26 $\pm$ 2	6		$\uparrow$
(42)	INS + AA _{HYPER}	Basal	Parallel arms	Systemic	Young	25 $\pm$ 1	6		$\uparrow$
					Older	72 $\pm$ 2	8		$\uparrow$
(54)	INS + high energy	Basal	Parallel arms	Systemic	Young	35 $\pm$ 3	6	240 $\pm$ 24	$\leftrightarrow$
	INS + low energy				Young	23 $\pm$ 1	6	290 $\pm$ 41	$\uparrow$
(55)	INS _I	Basal	Parallel arms	Local: leg	Young	29 $\pm$ 3	6	186 $\pm$ 46	$\leftrightarrow$
	INS _{II}				Young	25 $\pm$ 2	7	275 $\pm$ 38	$\uparrow$
	INS _{III}				Young	28 $\pm$ 2	6	507 $\pm$ 30	$\leftrightarrow$
(26)	INS	Basal	Parallel arms	Local: leg	Young	25 $\pm$ 2	7	242 $\pm$ 6	$\uparrow$
					Older	68 $\pm$ 1	6	501 $\pm$ 7	$\leftrightarrow$
(35)	INS + high energy	Basal	Single arm	Systemic	Young	35 $\pm$ 3	6	240 $\pm$ 24	$\leftrightarrow$
(61)	INS + AA _{BASAL}	Saline	Parallel arms	Local: leg	Young	29 $\pm$ 3	6	132	$\leftrightarrow$
(33)	INS	Basal	Single arm	Local: leg	Older	70 $\pm$ 2	7	501 $\pm$ 31	$\leftrightarrow$
(23)	AA _{HYPER} + INS _I	Basal	Cross over	Systemic	Young	20 $\pm$ 2	8	36	$\uparrow$
	AA _{HYPER} + INS _{II}							215	$\uparrow$
	AA _{HYPER} + INS _{III}							517	$\uparrow$
	AA _{HYPER} + INS _{IV}							1198	$\uparrow$
(37)	INS + high energy	Basal	Parallel arms	Systemic	Young	35 $\pm$ 3	6	240 $\pm$ 24	$\leftrightarrow$
	INS + low energy				Young	23 $\pm$ 1	6	290 $\pm$ 41	$\uparrow$

Table 1 Continued

References	Intervention	Comparator	Study design	Compartment	Subjects	Age (years)	n	Concentration (pmol/l)	MPS
(24)	INS	Basal	Parallel arms	Local: leg	Older	69 ± 1	8	552 ± 43	↕
					Older	72 ± 2	6	920 ± 155	↑
(56)	INS + AA _{BASAL}	Basal	Parallel arms	Systemic	Young	31 ± 2	7		↕
(43)	INS + AA _{HYPER}	Basal	Single arm	Systemic	Young	25 ± 2	8	552 ± 43	↕
(66)	INS + AA _{BASAL}	Basal	Single arm	Systemic	Older	65 ± 1	8	920 ± 155	↕
(44)	INS + AA _{HYPER}	Basal	Parallel arms	Systemic	Young	26 ± 4	8	104	↑
					Young	33 ± 3	7	275 ± 86	↕
(48)	INS + AA _{HYPER}	Basal	Single arm	Systemic	Young	38 ± 2	8	164 ± 13	↑
(7)	INS	Basal	Single arm	Local: leg	Young	37 ± 2	8	184 ± 17	↑
(8)	INS	Basal	Parallel arms	Local: leg	Older	70 ± 8	5	505 ± 54	↑
					Young	32 ± 2	7	248 ± 33	↑
					Older	74 ± 3	6	394 ± 124	↑
(45)	INS + AA _{HYPER}	Basal	Parallel arms	Systemic	Young	32 ± 2	7		↑
					Young	24 ± 1	8	405 ± 43	↑
					Older	73 ± 3	8	300 ± 15	↑
(46)	INS + AA _{HYPER}	Basal	Single arm	Systemic	Older	71 ± 2	7	159 ± 11	↑
					Older	71 ± 1	8	190 ± 19	↑
(67)	INS	Saline	Single arm	Systemic	Young	24 ± 1	9	344 ± 29	↑

AA_{BASAL}, amino acid administration to maintain basal levels; AA_{HYPER}, amino acid administration to achieve hyperaminoacidemia; INS, insulin; INS_{SUPRA}, insulin administration to achieve supraphysiological levels; MPS, muscle protein synthesis; ↑, significant increase; ↕, no significant effect.

Methods ► A high heterogeneity in methodology was observed. Study designs included single-arm, parallel-arms, and crossover studies. Comparator groups varied considerably, including no intervention (basal conditions), saline, alternative insulin dosages, or amino acid administration protocols.

Participants ► The main eligibility criteria entailed good health. Most studies reported preliminary medical history examination and standard blood tests (mainly for glucose tolerance assessment).

Interventions ► The exogenous insulin dosages that were applied resulted in plasma insulin concentrations that ranged from fasting (36 pmol/l) to supraphysiological (81 078 pmol/l) levels. Both systemic and local infusion protocols were commonly used. Local insulin infusion protocols used either the forearm or leg as compartment. Co-intervention varied considerably; most common was co-infusion of amino acids.

Data synthesis

A total of 40 studies were identified for inclusion in the review, varying considerably in the experimental design. Numerous studies applied research designs that included multiple experimental arms with separate interventions and many of which reported opposing findings, making it difficult to draw conclusions on a study level. In addition, several of the identified studies included co-interventions, such as drug administration or exercise protocols, which will influence the results. Therefore, data synthesis was performed on a study arm level (see Table 1).

From the 40 selected studies, after exclusion of nonmixed meal-related intervention (i.e., coadministration of pharmaceuticals), a total of 66 study arms were identified that included insulin treatment. From these 66 study arms, 34 found an insulin-stimulatory effect on muscle protein synthesis, while 32 did not find such an effect (see Fig. 2). Twelve study arms consisted of data reused from other included trials (8, 24, 34, 35, 36, 37, 38, 39). After deduplication, a total of 54 unique study arms were found, of which 28 reported increased muscle protein synthesis rates, whereas 26 did not.

From these data, we constructed several models that excluded study arms based on (biological) rationale as described earlier. In model 1, study arms were excluded in which the insulin administration was combined with amino acid co-interventions that raised plasma amino acid above basal levels and did not have a comparator group to correct for the increase in plasma amino acids

Figure 2

Flow diagram of stepwise models that report the presence or absence of an increase in muscle protein synthesis in response to exogenous insulin. MPS, muscle protein synthesis; ↑, significant increase; ↔, no significant effect.

(23, 40, 41, 42, 43, 44, 45, 46, 47, 48). This criterion excluded 17 study arms, all of which reported increased muscle protein synthesis.

Model 2 additionally excluded study arms in which amino acid levels were allowed to drop below basal levels (41, 49, 50, 51, 52, 53, 54, 55). This criterion excluded an additional ten study arms compared with model 1, none of which reported an effect on muscle protein synthesis.

Model 3 additionally excluded study arms in which a supraphysiological concentration of insulin was achieved (40). This criterion excluded an additional two study arms compared with model 2, which both reported that insulin increases muscle protein synthesis.

Model 4 additionally excluded study arms in older subjects (8, 24, 26, 33, 56). This criterion excluded an additional four interventions compared with model 3, one of which reported an insulin-stimulatory effect of on muscle protein synthesis, whereas the other three did not. After these final exclusions, model 4 included a total of

21 study arms, eight of which reported an increase in muscle protein synthesis, whereas 13 did not.

Discussion

This systematic review examined the literature regarding the proposed age-related effect of exogenous insulin administration on muscle protein synthesis rates *in vivo* in humans. Though ample studies have been performed to assess the impact of exogenous insulin administration on muscle protein synthesis, data do not support a stimulatory role of exogenous insulin administration on muscle protein synthesis rates *in vivo* in humans.

Amino acids are well recognized for their independent capacity to stimulate muscle protein synthesis (30, 31). Therefore, insulin plus amino acid administration has consistently been shown to increase muscle protein synthesis (23, 40, 41, 42, 43, 44, 45, 46, 47, 48). Without an adequate control group with a similar degree of hyperaminoacidemia, it is impossible to differentiate between the proposed anabolic properties of insulin vs amino acid administration. As expected, all 17 study arms combining both insulin and amino acid administration excluded from the analyses by this criterium reported an increase in muscle protein synthesis (see model 1 in Fig. 2). It should be noted that a state of concurrent hyperinsulinemia and hyperaminoacidemia reflects the physiological conditions following ingestion of a mixed meal. Three studies investigated whether exogenous insulin administration can further augment muscle protein synthesis during hyperaminoacidemic conditions, and all of them failed to detect an incremental effect (23, 57, 58). These results suggest that concurrent hyperinsulinemia and hyperaminoacidemia increase muscle protein synthesis but, at least in healthy young subjects, this effect seems entirely attributed to the hyperaminoacidemia.

I.v. insulin administration is followed by a dose-dependent reduction in circulating plasma amino acid levels, with the branched chain amino acids being most sensitive to the increase in circulating insulin levels (59). This insulin-induced hypoaminoacidemia is a reflection of increased amino acid uptake from the plasma in combination with the proposed inhibitory action of increasing insulin levels on endogenous proteolysis (50, 60). It has been suggested that the proposed positive effect of exogenous insulin administration on muscle protein synthesis is mediated by insulin-induced increase in blood flow and subsequent greater amino acid delivery to the muscle. A decline in circulating amino acid concentrations may limit amino acid delivery to the

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muscle and consequently restrict the capacity of insulin to stimulate muscle protein synthesis. To prevent such a decline in amino acid levels, several studies have applied i.v. amino acid infusions in combination with insulin administration or infused exogenous insulin locally in the femoral or brachial artery. In model 2, ten study arms that showed insulin-induced hypoaminoacidemia were excluded. None of these ten study arms found an insulin-stimulatory effect of insulin on muscle protein synthesis, supporting the rationale that insulin-induced hypoaminoacidemia may obviate the proposed stimulating properties of insulin administration on amino acid delivery to the muscle and subsequent increase in muscle protein synthesis.

Insulin has been shown to augment muscle protein synthesis *in vitro* (9, 10, 11, 12, 13, 14, 15, 16, 17, 19). However, from *in vivo* human studies there is much discrepancy on the proposed positive effects of exogenous insulin on muscle protein synthesis rates. It has been suggested that the apparent discrepancy is attributed to the more than tenfold higher supraphysiological insulin concentrations (14 000 pmol/l or above) that are applied in the *in vitro* models when compared with the more physiological increases in plasma insulin levels (up to 1200 pmol/l) applied within most *in vivo* human studies (23, 40). One study administered exogenous insulin locally in the forearm to achieve local supraphysiological insulin levels exceeding 50 000 pmol/l, while clamping amino acids at basal arterial or venous levels, and reported an increase in muscle protein synthesis rates (40). Their results suggest that supraphysiological insulin levels may effectively stimulate muscle protein synthesis.

It has been postulated that the elderly are more resistant to anabolic stimuli, such as increases in circulating plasma insulin and amino acids concentrations, when compared with younger adults (1, 26). Anabolic resistance to amino acid and/or insulin administration in the older population could be attributed to impairments in insulin-stimulated amino acid delivery to the muscle (24). As a consequence, we speculated that exogenous insulin administration may augment muscle protein synthesis in older (more insulin resistant) adults. After stratifying the data in young and older adults, we identified one study that reported a positive effect of exogenous insulin administration on muscle protein synthesis in older subjects (24), whereas three study arms failed to observe such an effect (8, 24, 26, 33, 56). Interestingly, the insulin-stimulated increase in muscle protein synthesis in the older subjects was observed only under relatively high local insulin levels exceeding 900 pmol/l (24). Lower

dosages of local insulin administration, resulting in local plasma insulin levels of ~500 pmol, did not seem to augment muscle protein synthesis in the older adults. These data suggest that older adults may be more resistant to the anabolic properties of insulin, a resistance that could be overcome at higher insulin concentrations.

We applied strict exclusion criteria in model 4 to exclude factors that may modulate the proposed stimulatory effect of exogenous insulin administration in healthy adults, including i) concurrent hyperaminoaminoacidemia, ii) insulin-induced hypoaminoacidemia, iii) supraphysiological insulin concentrations, and iv) older, more insulin resistant, subjects. We identified eight study arms that reported an insulin-stimulated increase in muscle protein synthesis, whereas 14 study arms failed to observe an insulin-stimulated increase in muscle protein synthesis rates in healthy young subjects. A subgroup of model 4 includes eight study arms in which insulin is locally infused into the leg, of which five study arms reported an increase in muscle protein synthesis rate, whereas three did not. Three of these study arms come from a dose-response study, in which both a low dose (0.05 mU/min $\times$ 100 ml of leg) and high dose (0.30 mU/min $\times$ 100 ml of leg) of insulin administration did not increase muscle protein synthesis rates, whereas the intermediate dose (0.15 mU/min $\times$ 100 ml of leg) did (55). This intermediate dose increased muscle protein synthesis rates in all five study arms it was applied in (7, 24, 28, 54, 55), whereas no increases in muscle protein synthesis rates were observed in the three study arms applying alternate dosing (55, 61). This suggests a U-shaped dose-response effect of exogenous insulin administration on muscle protein synthesis where a dose of ~0.15 mU/min $\times$ 100 ml of leg may stimulate muscle protein synthesis. Interestingly, exogenous insulin administration does not seem to stimulate muscle protein synthesis when infused locally in the forearm. In this subgroup of model 4, all six interventions reported no increase in muscle protein synthesis rates, despite a wide range of investigated dosing protocols (34, 58, 62, 63). In an attempt to further delineate the results, the data were reviewed for other potential modulating factors. The presence or absence of a stimulating effect in these studies could not be attributed to differences in levels of circulating insulin or the choice of amino acid tracers (data not shown).

In all of the presented work, exogenous insulin has been administered in a clamp-based approach. This approach may have its limitations as it could be speculated that the steep postprandial rise in circulating insulin level

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serves a regulatory function to activate various physiological processes that facilitate the post-prandial increase in muscle protein synthesis rate. Such temporal changes in insulin secretion, amino acid delivery and uptake, and intramyocellular signaling must be tightly regulated to support the post-prandial anabolic response. Furthermore, we should note that there may be differences in the relevance of circulating insulin levels on modulating the synthesis of various (sets of) proteins in skeletal muscle tissue (60).

From the presented data in the current systematic review, we conclude that: i) exogenous insulin and amino acid administration effectively increase muscle protein synthesis; however, this effect is attributed to the hyperaminoacidemia; ii) exogenous insulin administered systemically induces hypoaminoacidemia, which obviates any insulin-stimulatory effect on muscle protein synthesis; iii) exogenous insulin resulting in supraphysiological insulin levels exceeding 50 000 pmol/l may effectively stimulate muscle protein synthesis; iv) exogenous insulin may have a diminished effect of muscle protein synthesis in older adults due to age-related anabolic resistance; and v) exogenous insulin administered systemically does not increase muscle protein synthesis in healthy, young adults. Based on the existing literature, we conclude that exogenous insulin administration does not increase muscle protein synthesis rates in healthy, young, or older adults.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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Author contribution statement

J Trommelen wrote the manuscript, developed research strategy, and extracted data; B B L Groen extracted data and made manuscript revisions; and H M Hamer, L C P G M de Groot, and L J C van Loon made manuscript revisions.

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