Exploring the multifactorial causes and therapeutic strategies for anabolic resistance in sarcopenia: A systems modeling study

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Abstract

Background: Sarcopenia is the progressive loss of skeletal muscle mass, strength, and function with age, driven by dysregulation in the rates of muscle protein synthesis (MPS) and breakdown (MPB). Although MPB contributes to net protein balance (NB), a primary contributor of sarcopenia is anabolic resistance, defined as the blunted MPS response to anabolic stimuli such as feeding. While candidate mechanisms of anabolic resistance have been identified, none singularly explains the reduction in MPS. Instead, multiple mechanisms likely act simultaneously and interactively to suppress MPS. Studying these interactions experimentally is challenging. Alternatively, mathematical modeling is well suited to analyzing complex biological phenomena such as anabolic resistance.

Methods: We analyzed a previously developed kinetic model of leucine-mediated signaling and protein metabolism in human skeletal muscle to systematically investigate potential mechanisms contributing to anabolic resistance. A global sensitivity analysis identified key controllers of MPS, MPB, and NB. We then simulated an essential amino acid feeding intervention in older adults, classified the responses as either anabolic sensitive or resistant, and compared the resulting parameter distributions of the two groups. A targeted analysis evaluated the effects of individual and combined dysregulated putative mechanisms of anabolic resistance on muscle metabolism. Finally, we simulated therapeutic interventions aimed at restoring muscle metabolism.

Results: Sensitivity analysis revealed that MPS and MPB are primarily controlled by their proximal signaling controllers, while NB is largely driven by MPS dynamics. Exploratory simulations showed that several parameters and signaling protein concentrations, particularly those controlling MPS, differed significantly between anabolic sensitive and resistant groups. Targeted simulations indicated that multiple dysregulated mechanisms were required to account for the experimentally observed reductions in MPS in older adults. Therapeutic simulations showed that while single-target interventions could largely restore MPS when isolated mechanisms were perturbed (e.g., enhancing p70S6K levels, increasing mTORC1 sensitivity), a multifactorial approach was required to recover muscle metabolism when all anabolic resistance mechanisms were present.

Conclusion: This study demonstrates the multifactorial nature of anabolic resistance. We provide a framework for the experimental manipulation of independent and related factors that could underpin age-related anabolic resistance. The results motivate new hypotheses regarding the mechanisms most likely to be impaired and support the development of multi-target therapeutic strategies to help restore muscle protein metabolism in aging.

Keywords

Skeletal muscle, protein synthesis, mTOR, signal transduction, mathematical model, computational biology

Introduction

Sarcopenia is the progressive decline in skeletal muscle mass, strength, and function that accompanies aging [1]. Those with sarcopenia experience higher hospitalization rates [2] and contribute to increased public healthcare costs [3–5]. Given the impact of sarcopenia on health and quality of life, further research is needed to enhance our understanding of age-related muscle loss and to develop effective strategies for its prevention and management.

Skeletal muscle mass is largely determined by its protein content, which undergoes continuous turnover through concurrent synthesis and degradation processes [6]. The balance between the rates of muscle protein synthesis (MPS) and muscle protein breakdown (MPB) governs overall muscle protein levels, with the difference between MPS and MPB termed *net protein balance* (NB). MPS increases in response to anabolic stimuli such as nutrient intake [7], with amino acid availability – particularly leucine [8–10] – playing a key stimulatory role. In contrast, the attenuation of MPB during the postprandial period is primarily mediated via an increase in plasma insulin levels [11]. In the postabsorptive state, MPS and MPB rates are generally similar between young and older adults [12–15]. However, aging muscle exhibits *anabolic resistance*, characterized by a blunted stimulation of MPS and impaired suppression of MPB following anabolic stimuli [16–18]. Over time, this anabolic resistance drives the gradual loss of skeletal muscle.

Anabolic resistance of aging has been suggested to be underpinned by a range of factors including reduced amino acid availability to muscle due to increased splanchnic extraction [19–21], impaired transport from blood into muscle [20,22], impaired insulin-mediated protein signaling [23,24], reduced mTOR and p70S6K protein levels [18,25], and diminished mTORC1 signaling sensitivity to protein intake [18,26]. Sustained MPB rates are due to reduced insulin-mediated blunting of proteolysis or other age-related factors, such as chronic inflammation [27–29]. Effectively understanding and treating anabolic resistance therefore requires quantifying the relative contributions of these mechanisms and identifying targets for intervention.

The limited long-term success of treatments for anabolic resistance may stem from the simultaneous dysregulation of multiple mechanisms affecting muscle protein metabolism. Nutritional and pharmacological interventions tend to target individual pathways rather than addressing the broader complexity of these metabolic disruptions. Therefore, strategies that

consider multiple interacting factors influencing muscle protein metabolism may be needed to develop more effective treatment strategies.

Mathematical models are valuable tools for studying complex biological systems. They could be applied to identify the most likely combination of dysregulated mechanisms underlying anabolic resistance in older adults, without relying on logistically challenging and costly human clinical research needed to fully characterize the myriad potential biological mediators. We recently developed a kinetic model of leucine-mediated skeletal muscle signaling and protein metabolism, incorporating key leucine- and insulin-mediated pathways that control muscle metabolism [10]. Although originally developed using data from healthy younger adults, these molecular pathways are also present in older individuals but likely operate with altered reaction rates. Therefore, this kinetic model provides a framework to investigate the multifactorial nature of anabolic resistance and identify key physiological mechanisms driving impaired feeding-induced muscle metabolism.

The purpose of this study was to investigate the multifactorial causes of anabolic resistance by quantifying how dysregulated mechanisms in muscle protein metabolism contribute to impaired muscle mass in older adults. Specifically, we applied sensitivity analyses to the McColl and Clarke kinetic model [10] to identify key parameters controlling muscle metabolism. We pursued two strategies: 1) a "naïve" global sensitivity analysis to identify parameters potentially responsible for reduced MPS, and 2) a targeted sensitivity analysis that simulated previously proposed anabolic resistance mechanisms. These targeted simulations were then used to evaluate therapeutic strategies aimed at restoring MPS and NB. Collectively, our findings provide insight into the principal physiological drivers of anabolic resistance and identify promising targets for therapeutic intervention to mitigate muscle loss in older adults.

Methods

Kinetic model of leucine-mediated signaling and muscle protein metabolism

The McColl and Clarke kinetic model of leucine-mediated signaling and muscle protein metabolism in human skeletal muscle [10] (henceforth, the kinetic model) served as the foundation for this study (Figure 1). This model simulates skeletal muscle protein metabolism in response to leucine ingestion in healthy adults and contains four interconnected modules: 1) the mTOR signaling module, 2) the leucine kinetic module, 3) the digestive system module, and 4) the insulin

121 secretion module [10]. Together, these modules simulate the ingestion of leucine, leucine-
122 mediated stimulation of insulin-and leucine-dependent signaling pathways, and muscle protein
123 metabolism. While originally developed using data from healthy adults, the model can be applied
124 to study anabolic resistance in sarcopenia because the biological mechanisms it represents are
125 conserved in young and older individuals. Differences between young and older adults manifest
126 as differences in parameter values. Further details of the kinetic model are provided in the
127 Supplementary Information.

128 **Defining anabolic resistance**

129 We established an anabolic resistance "threshold" based on two studies by Mitchell et al.
130 [30,31]. Although these studies measured myofibrillar protein synthesis specifically, this protein
131 fraction, which constitutes ~60% of muscle proteins, demonstrates a similar nutrient sensitivity
132 and age-related resistance as sarcoplasmic proteins [18,32]. Both studies used the same
133 intervention: young [30] and older adults [31] received a 15-gram mixed essential amino acid
134 (EAA) bolus (3.59 grams of leucine), with muscle biopsies collected over the subsequent four
135 hours to calculate MPS, measured as fractional synthetic rate (FSR) [30,31]. Using the "total
136 leucine synthesized" calculations from McColl and Clarke [10], the experimentally measured MPS
137 response in young adults was 0.461 ± 0.111 grams of leucine synthesized in muscle over four hours,
138 while in older adults it was 0.267 ± 0.092 grams of leucine. Despite the older adults being classified
139 as healthy [31], the reduced MPS response to EAA feeding indicated the presence of anabolic
140 resistance. To define the anabolic resistance threshold, we used the lower 95% confidence interval
141 of the younger adult MPS response (0.461 ± 0.111 grams of leucine), yielding a threshold of 0.251
142 g leucine synthesized in muscle. Simulated MPS responses below this threshold were classified as
143 *anabolically resistant*, whereas those above it were considered *anabolically sensitive*. This value
144 closely approximates the mean MPS response for the older adult group.

145 **Multi-parametric sensitivity analysis**

146 We applied a multi-parametric sensitivity analysis (MPSA) to generate physiologically
147 plausible parameter sets, with the purpose of identifying model parameters that most influence
148 system-level behaviors representative of anabolic resistance. MPSA is a global sensitivity analysis
149 approach that systematically samples parameter sets and evaluates their relative contribution to

150 model dynamics [33]. Complete details for the MPSA, including the criteria for parameter filtering
151 and analyses applied to the filtered parameter sets, are provided in the Supplementary Information.

152 *MPSA: Kolmogorov-Smirnov test*

153 We applied the two-sample Kolmogorov-Smirnov (KS) test to assess whether parameter
154 sets from the anabolic-sensitive and anabolic-resistant groups originated from the same
155 distribution or represented unique distributions. We performed the test using the R function *ks.test*,
156 which calculated the KS statistic and the associated p-value. To account for multiple comparisons,
157 we applied false discovery rate (FDR) correction using the Benjamini-Hochberg method (R
158 function: *p.adjust*) to compute the *q*-value. A *q*-value less than 0.05 indicated a statistically
159 significant difference between parameter distributions.

160 Targeted approach to evaluate the contributions of anabolic resistance mechanisms 161 to muscle protein metabolism

162 We used a targeted approach to evaluate how the putative mechanisms underlying anabolic
163 resistance in sarcopenia independently influence muscle protein metabolism and how these
164 mechanisms might operate synergistically to further impair muscle protein metabolism. As noted
165 in the Introduction and outlined in Table 1, several mechanisms contribute to anabolic resistance
166 (Figure 1). Of note, elevated postabsorptive levels of phospho-p70S6K have been observed in
167 older adults, which we hypothesized represents a compensatory response to dysregulated
168 mechanisms [10,34] – a phenomenon we term "compensatory signaling". For each mechanism,
169 we curated experimental data quantifying the differences between young and older adults,
170 identified the model parameter(s) corresponding to each mechanism, and estimated both a
171 *consensus fold change* and a *worst-case fold change* in the values of these parameters between
172 young and old based on a subset of relevant studies (Table 1). While several of the studies
173 informing each mechanism did not directly assess the modeled process, they reported
174 measurements of critical pathway components that we used to guide the changes representing
175 anabolic resistance.

176 **Table 1.** Modeling the putative anabolic resistance mechanisms. Summary of putative mechanisms
177 contributing to anabolic resistance in sarcopenia, along with quantitative evidence for the
178 consensus (i.e., average) and worst-case effects identified across a subset of studies. The worst-
179 case effect was selected from the study reporting the greatest deviation in fold-change relative to
180 the young group. The number of studies informing each consensus estimate is indicated by n. The
181 kinetic model parameters that were altered to represent the corresponding mechanism are listed in
182 the rightmost column.

Putative anabolic resistance mechanism	Consensus change	Worst-case change	Integration into model
First-pass splanchnic extraction	1.76±0.25 F.C. extraction in elderly (n=3) [19–21]	2.20±0.52 F.C. extraction in elderly [19]	Increased k68 (first-pass splanchnic extraction)
Reduced delivery of amino acids to muscle	0.73±0.30 F.C. $F_{m,a}$ (n=2) [20,22]	0.69±0.39 F.C. $F_{m,a}$ [22]	Reduced k6 (leucine transport into muscle)
Impaired insulin signalling	0.80±0.24 F.C. Akt activity in elderly (n=3) [17,27,35]	0.66±0.41 F.C. Akt activity in elderly [27]	Reduced k16 (insulin binding mediated insulin signalling)
mTOR protein level	0.79±0.15 F.C. mTOR levels in elderly (n=2) [18,25]	0.50±0.18 F.C. mTOR levels in elderly [18,25]	Reduced both mTORC1 and mTORC2 initial concentrations
Reduced mTORC1 sensitivity#	0.57±0.16 F.C. phospho-p70S6K in elderly (n=3) [17,18,35]	0.43±0.36 F.C. phospho-p70S6K in elderly [18]	Reduced k40 (mTORC1-mediated p70S6K phosphorylation)
p70S6K protein level#	0.78±0.09 F.C. p70S6K levels in elderly (n=2) [18,25]	0.50±0.18 F.C. p70S6K levels in elderly [18,25]	Reduced the p70S6K initial concentration
Elevated postabsorptive phopsho-p70S6K (compensatory signaling)#	1.87±0.55 F.C. phospho-p70S6K per total p70S6K in elderly (n=2) [17,18]	1.70±0.60 F.C. phospho-p70S6K per total p70S6K in elderly [17]	Added a constitutive p70S6K phosphorylation reaction to the ODEs; p70S6K concentrations were unchanged

183 #The combined simulation of reduced mTORC1 sensitivity, reduced p70S6K protein levels, and elevated postabsorptive
184 phospho-p70S6K represents the ***compensatory protein signalling mechanism*** in older adults.
185 F.C., fold change; $F_{m,a}$, inward amino acid transport from artery to muscle.

186 The consensus fold change for each mechanism was calculated in two steps. First, the fold
187 change within each study was calculated as the ratio between the mean response of the young and
188 older adults, with the corresponding standard error (SE) derived using propagation of error.
189 Second, a consensus mean fold change for each mechanism was calculated across studies using an
190 inverse variance-weighted average, which assigns greater weight to estimates with higher
191 precision (Equations 7-9) [36]:

$$w_i = \frac{1}{SE_i^2} \tag{7}$$

$$FC_{consensus} = \frac{\sum_{i=1}^{k} w_i \cdot FC_i}{\sum_{i=1}^{k} w_i} \tag{8}$$

$$SE_{consensus} = \sqrt{\frac{1}{\sum_{i=1}^{k} w_i}} \tag{9}$$

192 where i is each of the k independent studies, FC_i and SE_i are the fold change (FC) and
193 corresponding SE for study i, respectively, w_i is the inverse variance weight of study i, and
194 $FC_{consensus}$ and $SE_{consensus}$ are the consensus fold change and SE across k studies for the
195 respective putative mechanism of anabolic resistance. The worst-case fold change was assigned as
196 the value from the study within the group of studies that reported the greatest deviation from the
197 young adult population.

198 We then used the kinetic model to simulate the effects of each putative mechanism
199 individually, as well as the combined effect of all mechanisms together, in both the presence and
200 absence of elevated postabsorptive phospho-p70S6K (i.e., compensatory signaling), as follows.
201 First, we created a virtual population of 100 anabolic sensitive (i.e., "healthy") models by
202 randomly sampling 100 parameter sets from the anabolic sensitive group of the MPSA (Naïve
203 approach). Performing the simulations for a virtual population was done to foster the
204 generalizability of the results. Second, the anabolic resistance mechanisms were simulated within
205 each of the 100 models by multiplying the relevant model kinetic parameter or initial protein
206 concentration by the fold change corresponding consensus or worst-case estimate. For the
207 simulated compensatory phospho-p70S6K signaling mechanism, an additional reaction was added
208 to the model to simulate the elevated postabsorptive phosphorylation rate, with its kinetic
209 parameter informed by the estimates reported in Table 1. We then simulated the anabolic resistant
210 models in response to a 3.59 g leucine bolus and extracted the MPS, MPB, and NB responses over
211 the 4-hour postprandial period. This dose was chosen to allow comparison with the Mitchell et al.
212 studies, which provided a 15 g EAA bolus containing 3.59 g leucine and informed the anabolic
213 resistance thresholds.

Simulation of therapeutic strategies to mitigate anabolic resistance

Therapeutic strategies to mitigate anabolic resistance were simulated using models developed through the Targeted Approach, which had quantified the effects of individual or combined anabolic resistance mechanisms on MPS, MPB, and NB. Each therapeutic strategy involved restoring one or more dysregulated parameters to their "healthy" value by multiplying the parameters by the inverse of its consensus or worst-case fold-change estimate. Each therapeutic intervention was applied across all anabolic resistance simulation from the Targeted Analysis – including those in which the targeted parameter was not originally perturbed. This approach attempts to mimic the scenario in which one or more druggable targets are treated when the causal mechanism itself is not druggable (cf. [37,38]). The efficacy of each therapeutic intervention was assessed against its ability to recover MPS and NB relative to the healthy response.

Numerical methods and software

The 95% confidence interval was calculated using one-tail and the t-value corresponding to the appropriate degrees of freedom. MATLAB version R2022a was used to perform the MPSA, model simulations, and multiple regression analyses. R (version 4.3.2) was used for statistical testing and the visualization of results using the ggplot2 package [39]. WebPlotDigitizer was used to extract published data (i.e., means, SE) that were only reported in figures [40]. The code generated in this study is freely available in the GitHub repository: https://github.com/tjmccoll/MultifactorialCauseOfAnabolicResistance/.

Results

Multi-parametric sensitivity analysis of the kinetic model

The MPSA resulted in 2,663 physiologically plausible parameter sets for healthy adults. Regression models using $\log_{10}$-transformed outcome measures and $\log_{10}$-transformed parameter values were the most predictive for MPS and MPB, whereas using raw outcomes with $\log_{10}$-transformed parameter values were most predictive for NB. The best-performing regression models for MPS, MPB, and NB include the top 35, 45, and 35 parameters from the main-effect-only models as interaction terms, respectively (Table S2). The p-1 sensitivity analysis of MPS and MPB revealed that parameters most proximal to their respective reactions had the greatest influence on their outcomes (Table S3). For NB, the most influential parameters were a

243 combination of those affecting MPS and MPB, but parameters more proximal to MPS had a greater
244 influence on the NB response (Table S3).

245 **Naïve approach reveals mechanisms contributing to anabolic resistance**

246 We used an unbiased, exploratory approach to assess mechanisms that may contribute to
247 anabolic resistance in sarcopenia and influence muscle protein metabolism. To do this, we repeated
248 the MPSA but with the following modifications. First, we excluded the MPS criterion because the
249 original MPSA was constrained to generate physiologically reasonable MPS responses in healthy,
250 young adults. This criterion likely restricted the range of MPS responses observed in older adults
251 with anabolic resistance (see "Defining Anabolic Resistance" in Methods), thereby removing
252 relevant parameter sets. To better simulate theoretical MPS responses in older adults, we removed
253 the constraint to allow the full breadth of MPS variability to be captured. Second, the original
254 MPSA featured fixed values for the parameters controlling the model's input function. However,
255 one of these parameters controls the rate of splanchnic extraction (k_{68}), which literature suggests
256 is elevated in older adults [19–21]. To comprehensively assess mechanisms contributing to
257 anabolic resistance, we allowed k_{68} to vary as part of the MPSA. We first performed an MPSA in
258 which only k_{68} was varied within a two-fold range to determine its physiologically plausible range
259 (MPS criterion removed). This range was then used to constrain k_{68} in the subsequent MPSA to
260 ensure sufficient passing parameter sets. Simulating 10,000 values for k_{68} we found its plausible
261 range to be 0.0025 – 0.0087 min^{-1}, corresponding to a 2-fold decrease to a 1.75-fold increase from
262 its calibrated value.

263 This MPSA generated 4,054 physiologically plausible parameter sets, including parameter
264 sets producing MPS responses within the healthy range (Table S1), above it, and below it. We
265 simulated the kinetic model using these parameter sets following a 3.59-gram leucine bolus and
266 calculated MPS over the 4-hour postprandial period. The parameter sets were then classified as
267 "anabolic sensitive" (n = 3,328) or "anabolic resistant" (n = 726) based on the anabolic resistance
268 threshold (see "Defining Anabolic Resistance" in Methods). The Kolmogorov-Smirnov tests
269 revealed significant differences in the distributions of ten parameters (Figure 2, Figures S2-4,
270 Table S4). These ten parameters were largely consistent with those identified in the p-1 sensitivity
271 analysis of the MPS regression model (see Supplementary Results and Table S2). Two exceptions
272 were splanchnic extraction, which was not varied in the regression analysis, and hepatic glucose

273 production, which only marginally met the $q<0.05$ threshold for inclusion. Of the ten parameters,
274 eight were proximal to the MPS reaction, while the remaining two reflected systemic processes:
275 first-pass splanchnic extraction and hepatic glucose production (from the insulin secretion
276 module).

277 Targeted simulations reveal a multifactorial basis of anabolic resistance

278 We then simulated the model by integrating the putative mechanisms underlying anabolic
279 resistance – individually and in combination, with or without compensatory signaling – using both
280 consensus and worst-case estimates to evaluate their contributions to blunted MPS responses
281 (Table 1). Simulations using the consensus estimates revealed that increased first-pass splanchnic
282 extraction and reduced mTORC1 sensitivity (as indicated by p70S6K phosphorylation status) each
283 moderately decreased MPS (Figure 3A). In contrast, reducing mTOR and p70S6K concentrations
284 modestly decreased MPS, while reduced blood flow and impaired insulin signaling had minimal
285 effects. When all mechanisms were simulated together, the model predicted a substantial decline
286 in MPS, closely approximating the MPS response in older adults as calculated from Mitchell et al.
287 (2017) [31] (0.267 ± 0.092 grams of leucine synthesized). However, when compensatory signaling
288 was included – simulating the elevated postabsorptive phospho-p70S6K levels observed in older
289 adults – the loss of MPS was largely recovered, though still below the healthy level. Changes in
290 NB closely mirrored those in MPS, as MPB responses remained relatively stable compared to the
291 healthy simulations (Figures 3B and S5).

292 Simulations using the worst-case estimates of the anabolic resistance mechanisms revealed
293 that increased first-pass splanchnic extraction, reduced mTORC1 sensitivity, and reduced mTOR
294 and p70S6K levels each meaningfully reduced MPS, with those of the latter three mechanisms
295 approaching the MPS response calculated from Mitchell et al. (2017) [31] (Figure 3C). Reduced
296 blood flow and impaired insulin-mediated signaling had little effect on MPS. When all
297 mechanisms were simulated together under worst-case conditions, MPS was substantially reduced
298 – falling below the MPS response calculated from Mitchell et al. (2017) [31]. Although
299 compensatory signaling restored much of this loss, the MPS response remained lower than that
300 observed in the older adult data. Similar to the consensus simulations, MPB responses were largely
301 unaffected, such that changes in NB closely reflected the changes in MPS (Figures 3D and S5).

302 Next, we simulated the effect of elevated MPB rates on muscle protein metabolism.
303 Although accurately estimating MPB in older adults is technically challenging and statistically
304 significant age-related increases in MPB have not been consistently observed in healthy aging
305 [12], factors such as chronic low-grade inflammation, which contributes to increased MPB [29],
306 and reduced insulin-mediated suppression of MPB [27,28] have been observed in older adults. To
307 evaluate the potential effects of increased MPB, we simulated the model with a 50% increase in
308 the kinetic parameters controlling Akt- and mTORC1-mediated MPB, both independently and in
309 combination with all anabolic resistance mechanisms, with or without compensatory signaling.
310 Increased MPB led to a slight rise in MPS due to greater availability of free intramuscular leucine,
311 but this effect was offset by a larger increase in MPB, resulting in a net reduction in protein balance
312 (Figure S6). When these elevated MPB effects were added to the combined anabolic resistance
313 simulations – with or without compensatory signaling – MPS increased slightly, but the larger rise
314 in MPB further reduced NB.

315 **Therapeutic strategies to overcome the mechanisms of anabolic resistance**

316 Next, we simulated therapeutic interventions by restoring each dysregulated mechanism to
317 its healthy value under three scenarios: 1) when the mechanism itself was individually
318 dysregulated, 2) when the same magnitude of recovery was applied to other individually
319 dysregulated mechanisms, even though these parameters were not themselves impaired, to
320 evaluate potential compensatory effects, and 3) when all mechanisms were simultaneously
321 dysregulated. The effectiveness of each intervention was evaluated against its ability to recovery
322 of MPS and NB to the healthy responses.

323 Under the *consensus estimates*, recovering mTORC1 sensitivity fully restored MPS and
324 NB when applied to individually simulated mechanisms, while recovery of p70S6K levels also
325 substantially restored both outcomes (Figure 4A-C). However, when all anabolic resistance
326 mechanisms were simulated together without compensatory signaling, no single therapeutic target
327 was sufficient to restore MPS or NB. But when we simulated the combined recovery of splanchnic
328 extraction, mTORC1 sensitivity, and both mTOR and p70S6K levels, near-complete recovery of
329 MPS and NB was achieved.

330 When therapeutic strategies were simulated using the *worst-case estimates* (i.e., the
331 measured values deviating most from the healthy state), recovery of p70S6K levels fully restored

332 MPS and NB when anabolic resistance mechanisms were simulated individually, while recovery
333 of either mTOR levels or mTORC1 sensitivity also largely restored both responses (Figure 4D-F).
334 As with the consensus simulations, no single therapeutic target could overcome the combined
335 effects of all anabolic resistance mechanisms. However, combined recovery of splanchnic
336 extraction, mTORC1 sensitivity, and mTOR and p70S6K levels produced a near-complete
337 restoration of MPS and NB.

338 We then simulated the therapeutic strategies in the presence of compensatory signaling.
339 This analysis was based on the hypothesis that compensatory signaling – reflected by elevated
340 postabsorptive phospho-p70S6K levels in older adults [10,34] – may offset the effects of reduced
341 mTOR and p70S6K concentrations and decreased mTORC1 sensitivity. Accordingly, we
342 simulated these three mechanisms together ("[mTOR] + [p70S6K] + mTORC1 sensitivity"), as
343 well as all anabolic resistance mechanisms together, using the worst-case estimates, in the presence
344 of compensatory signaling and the applied therapeutic strategies (Figure 5). Compensatory
345 signaling alone partially restored MPS and NB toward healthy levels in both simulated conditions,
346 but neither fully reached the healthy level (healthy values: MPS = 0.47 g leucine synthesized, NB
347 = 0.27 g leucine integrated into muscle; Figure 5). When the three mechanisms were simulated
348 together, several therapeutic strategies in the presence of compensatory signaling successfully
349 restored MPS and NB: recovery of p70S6K levels fully restored the MPS response but not NB,
350 while combining p70S6K recovery with either restored mTOR levels or mTORC1 sensitivity was
351 sufficient to recover both responses. When all six mechanisms were simulated with compensatory
352 signaling, additional interventions were required: recovery of mTOR and p70S6K levels along
353 with mTORC1 sensitivity fully restored MPS but fell slightly short of restoring NB. However, the
354 further addition of recovered splanchnic extraction fully restored both MPS and NB.

355 Discussion

356 In this study we used a computational modeling approach to systematically evaluate the
357 mechanisms that most likely contribute to anabolic resistance. We first conducted a global
358 sensitivity analysis of a previously developed kinetic model of leucine-mediated signaling and
359 muscle protein metabolism [10] to identify mechanisms that most strongly control MPS, MPB,
360 and NB. The analysis revealed that MPS and MPB are primarily controlled by their proximal
361 regulatory mechanisms, whereas NB is largely influenced by MPS regulation. Next, we performed

362 an exploratory analysis simulating an EAA feeding intervention to evaluate mechanisms
363 contributing to anabolic resistance in older adults [31], classifying simulated responses as either
364 anabolic sensitive or resistant. Several parameters and signaling protein concentrations differed
365 significantly between groups, particularly those controlling MPS. We then conducted a targeted
366 analysis to evaluate the extent to which putative mechanisms underlying anabolic resistance could
367 explain the reduced MPS observed with aging. Model simulations showed that, when considered
368 individually, consensus and worst-case estimates of individual mechanisms were unlikely to
369 account for anabolic resistance. However, when these mechanisms were simulated in combination,
370 they produced substantial reductions in MPS consistent with anabolic resistance. Finally, we
371 simulated therapeutic interventions aimed at restoring muscle metabolism in anabolic resistance.
372 While single-target approaches (e.g., enhancing mTOR or p70S6K levels, or increasing mTORC1
373 sensitivity) were generally effective when only individual mechanisms were impaired, they proved
374 insufficient when multiple dysregulated processes were simultaneously present. In contrast,
375 restoring MPS when all dysregulated processes were simultaneously present required a
376 multifactorial therapeutic strategy targeting several pathways. These findings highlight the
377 complex, multifactorial nature of anabolic resistance and motivate novel hypotheses about the
378 most likely dysregulated mechanisms, providing a foundation for future experimental validation
379 and multi-targeted therapeutic development.

380 **Main findings and implications**

381 This study examined strategies to restore the feeding-mediated MPS response in older
382 adults, with a focus on *individual feedings* rather than adjustments to feeding variables such as
383 dose or scheduling. In our simulations, a single 15 g EAA feeding (containing 3.59 g leucine) was
384 modelled. While increasing the simulated dose or changing the scheduling of feeding could be
385 considered as alternatives to improving the MPS responses in older adults, evidence from human
386 studies suggests these strategies provide limited benefits: in older adults, a 10 g EAA feeding
387 maximizes myofibrillar MPS rates with no further increases at 20 g or 40 g EAA [18]. Similarly,
388 studies report that more frequent, smaller feedings (4 × 3.75 g EAA every 45 minutes) [42] or a
389 leucine top-up after an initial EAA bolus (15 g EAA + 3 g leucine at 90 minutes) [31] do not
390 meaningfully enhance MPS rates in older adults compared to a single 15 g EAA bolus. Together,
391 these findings suggest that overcoming anabolic resistance in older adults will require targeting

392 mechanisms that control the response to individual feedings, rather than modifying nutritional
393 feeding strategies alone.

394 In contrast, some studies indicate that increasing protein dose relative to body weight [14]
395 or enriching the EAA dose with leucine [43] can restore MPS in older adults to levels observed in
396 younger adults. While these compensatory strategies may offset the blunted anabolic response,
397 they do not explain *why* higher protein or leucine doses are necessary. The underlying factors
398 driving this reduced sensitivity are likely contributing to muscle loss, and directly addressing them
399 offers a more sustainable solution for overcoming anabolic resistance. Accordingly, this study
400 focused on identifying the mechanisms responsible for the diminished MPS response to individual
401 feedings and proposes therapeutic strategies to restore this response.

402 A well-documented mechanism of anabolic resistance is the diminished sensitivity of
403 mTORC1 signaling to protein or amino acid ingestion [17,18,26]. The findings from our naïve
404 approach support this notion because we observed widespread dysregulation in leucine-mediated
405 signaling, including leucine-mediated mTORC1 activity, mTORC1-mediated p70S6K
406 phosphorylation, p70S6K dephosphorylation, and p70S6K-mediated negative feedback on
407 mTORC1. While experimental studies have yet to pinpoint the specific mechanisms responsible
408 for reduced mTORC1 signaling sensitivity in anabolic resistance [42], our results suggest that a
409 broad dysregulation of the mTORC1 signaling machinery may underlie this phenomenon.

410 One factor that contributes to reduced mTORC1 signaling sensitivity is physical activity.
411 A synergistic relationship exists between nutrient- and exercise-mediated mTORC1 signaling and
412 MPS, in which exercise enhances muscle sensitivity to protein feeding [44] and improves insulin-
413 mediated amino acid delivery to muscle, thereby supporting mTORC1 signaling [45]. Conversely,
414 physical inactivity desensitizes muscle to feeding-mediated MPS responses [46]. While our
415 simulations focused solely on the feeding response, reduced levels of physical activity in older
416 adults may contribute to the diminished mTORC1 signaling sensitivity seen in anabolic resistance
417 [47–49]. This combination contributes to the progressive desensitization of mTORC1 signaling to
418 anabolic stimuli, exacerbating anabolic resistance.

419 Our simulations from the naïve approach revealed that mTORC1 and p70S6K
420 concentrations were significantly reduced in the anabolic-resistant group, suggesting a potential
421 mechanism for the blunted MPS response in aging. Consistent with these findings, previous

research shows that older adults have 50% lower mTOR and p70S6K levels compared to younger adults that was concomitant with a diminished EAA feeding-induced myofibrillar FSR [18]. Despite these reductions in protein content, older adults exhibit increased postabsorptive phospho-p70S6K^{T389}, a potential compensatory mechanism. This increased postabsorptive phospho-p70S6K is seen both in experimental studies [17,18,27,50] and in our previous modeling simulations [10]. This compensatory elevation likely occurs to partially offset the reduced mTORC1 and p70S6K concentrations. Indeed, our targeted analysis found that compensatory increases in postabsorptive phospho-p70S6K can restore a large portion of the MPS and NB response; however, this compensatory signaling response was insufficient to fully restore muscle metabolism in our simulations. This persistent postabsorptive phosphorylation reduces the dynamic range for further postprandial increases [17,18]. This impaired signaling response may contribute to the diminished MPS observed in older adults following amino acid ingestion.

The compensations in muscle metabolism induced by elevated p70S6K phosphorylation in aging could be offset by two negative feedback mechanisms. First, phosphorylated p70S6K inhibits insulin signaling via IRS1 phosphorylation, reducing insulin sensitivity [51–53]. Second, phospho-p70S6K phosphorylates mTORC1 at Ser2448, further suppressing mTORC1 activation [54–57]. These feedback pathways likely act together to decrease the sensitivity of p70S6K to anabolic stimuli and may in part lead to anabolic resistance. Aged rat models similarly exhibit elevated postabsorptive phosphorylation at both p70S6K and its downstream target, ribosomal protein S6 (rpS6), further supporting this mechanism [58]. Interestingly, inhibition of mTORC1 signaling with rapamycin in aged rats reduced phoshpo-p70S6K and phospho-rpS6 levels, resulting in improved muscle mass maintenance and reduced age-related muscle loss [58]. Given that rapalogs inhibiting mTOR activity have been proposed to improve muscle health with age [59], our modeling suggests this could further restore skeletal muscle sensitivity to anabolic stimuli. However, careful titration of doses is needed, particularly in older adults with co-morbidities and/or low physical activity, to avoid excessive suppression of mTOR signaling and anabolism [60]. Nevertheless, our results suggest an alternative intervention to inhibiting mTORC1 – which we hypothesize primarily targets the compensatory response to reduced p70S6K levels – could be increasing absolute mTORC1 and/or p70S6K. Our model suggests that increasing mTOR or p70S6K levels, individually, were two of the most efficacious therapeutic approaches in recovering muscle metabolism. In particular, therapeutic targeting of p70S6K levels

453 was highly effective in restoring MPS and NB in the presence of compensatory signaling. This
454 approach of targeting mTOR or p70S6K protein levels could restore the capacity for
455 phosphorylation increases, reduce postabsorptive mTORC1 signaling, and ultimately mitigate
456 anabolic resistance.

457 While this study has identified key potential therapeutic targets to overcome anabolic
458 resistance, a critical next step is to determine which of these is *druggable*, i.e., can be modified by
459 a pharmacological agent (cf. [37]). Pharmacotherapies for sarcopenia have a long history of
460 targeting cellular signaling pathways by binding to intracellular or membrane receptors [38]. These
461 therapies primarily focus on modulating signaling pathway activity to either increase MPS or
462 decrease MPB [38]. However, our results suggest that restoring mTORC1 sensitivity and
463 recovering mTOR and p70S6K protein levels may represent more efficacious strategies to
464 overcome anabolic resistance – strategies that differ fundamentally from conventional approaches
465 targeting anabolic or catabolic signalling pathways in muscle. Pharmacological agents such as
466 MHY1485 [61] and rapamycin [58] can enhance or repress mTOR activity, respectively, offering
467 opportunities to modulate mTORC1 sensitivity. In contrast, pharmacological strategies aimed at
468 restoring signaling protein levels in older adult muscle represents a novel and promising avenue.

469 Determining effective pharmacological strategies to modulate mTOR or p70S6K protein
470 levels can be informed by bioinformatics-based approaches. Specifically, these proteins are
471 encoded by the *MTOR* and *RPS6KB1* genes, such that plausible strategies include identifying
472 factors that upregulate their expression, such as transcription factors (TFs) and cofactors, and
473 targeting these to increase activity. Conversely, repressors of gene expression, such as microRNAs
474 (miRNA) and long non-coding RNAs (lncRNAs), could be inhibited to relieve suppression. For
475 example, miR-199a-3p is a miRNA known to repress *MTOR* expression [62] and could be
476 inhibited to enhance *MTOR* expression [63]. One issue with targeting miRNAs is that multiple
477 *MTOR*-specific miRNAs have been identified, such that targeting just one might be insufficient.
478 Regarding *RPS6KB1*, estrogen receptor alpha (*ESR1*) is one of the few TFs studied for its role in
479 controlling *RPS6KB1* gene expression [64,65], making it a potential target to increase *RPS6KB1*
480 mRNA levels. Additionally, miR-200b [66] and miR-223-3p [67] represent candidate miRNAs
481 whose inhibition may upregulate *RPS6KB1* expression. While these targets for modulating mTOR
482 and p70S6K protein levels are speculative, pharmacological approaches that inhibit TF-DNA
483 interactions, modulate enhancer activity, or repress miRNAs have been developed and successfully

484 applied in other biological contexts [68–70]. It is important to note that these interventions
485 primarily affect mRNA levels; therefore, proteomic analyses are essential to confirm
486 corresponding changes at the protein level and assess the functional impact of these strategies.

487 Our study features the following noteworthy limitations. First, we evaluated simulated
488 responses and their approximation to anabolic resistance against two studies [30,31]. Given the
489 substantial heterogeneity in anabolic resistance and dysregulated muscle protein metabolism with
490 aging [72,73], future studies should further validate and refine our model using larger, individual-
491 level datasets. Additionally, future human clinical trials could benefit from measuring the critical
492 contributors to anabolic resistance identified here to generate robust datasets. Second, our model
493 was developed using data from males. Some [74], but not all [75], studies suggest that aging-
494 related dysregulation of muscle protein metabolism may exhibit sexual dimorphism. Therefore,
495 future studies should include females to confirm the generalizability of our findings. Finally, our
496 results focus on muscle protein metabolism, which most likely affects muscle mass. While losses
497 of muscle mass, driven by dysregulations in protein turnover, often precedes declines in muscle
498 function [73], the relationship between mass and function is not always direct [75].

499 **Conclusions**

500 We report a novel modeling approach to experimentally manipulate key variables
501 associated with age-related anabolic resistance, without the need for logistically challenging and
502 costly clinical trials. The initial sensitivity analysis revealed widespread dysregulation of reactions
503 controlling mTORC1 activity in the anabolic resistant group, along with reduced levels of
504 mTORC1 and p70S6K. Simulating the putative mechanisms underlying anabolic resistance
505 suggested that these mechanisms do not act in isolation; rather, they act synergistically to suppress
506 MPS. Based on these results, we simulated therapeutic interventions to restore muscle metabolism.
507 Targeting individual mechanisms proximal to mTORC1 signalling (e.g., mTOR or p70S6K levels,
508 mTORC1 sensitivity) effectively restored MPS and NB when only individual impairments were
509 simulated. However, these single-target strategies failed to fully restore MPS when all mechanisms
510 were impaired simultaneously. Under this more realistic condition – reflecting what we suggest is
511 likely occurring in older adults – a multifactorial therapeutic approach was required to recover
512 anabolic responses to feeding. These findings motivate new hypotheses regarding the
513 combinatorial mechanisms underlying anabolic resistance and offer insight for future experimental

514 studies and therapeutic strategies to mitigate feeding-mediated anabolic resistance. Furthermore,
515 our framework could also be applied to other models of muscle loss characterized by similar
516 dysregulations, such as inactivity-induced anabolic resistance [46], or distinct mechanisms, such
517 as increased MPB in cachexia [71].

518

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Conflicts of interest

The authors declare no competing interests.

527 ## Figure titles and legends

528 **Figure 1.** Kinetic model of leucine-mediated signalling and muscle protein metabolism. The
529 topology of the McColl and Clarke kinetic model is shown, with hatched red boxes highlighting
530 the investigated mechanisms of anabolic resistance: 1) increased first-pass splanchnic extraction,
531 2) reduced transport of amino acids into muscle, 3) reduced insulin-mediated signalling due to
532 insulin resistance, 4) reduced mTOR concentration, 5) reduced sensitivity of mTORC1 to leucine
533 signaling, 6) reduced p70S6K concentration, and 7) elevated muscle protein breakdown rates.

534 **Figure 2.** Parameter distributions that significantly differ between the anabolic sensitive and
535 resistant groups. Each panel displays a violin plot showing the parameter distribution density
536 across the range, accompanied by boxplots indicating the median, first and third quartile, and the
537 minimum and maximum values, as well as a scatterplot of the data. The y-axis displays the $\log_{10}$-
538 transformed values of the indicated kinetic model variable.

539 **Figure 3.** Simulation of putative mechanisms underlying anabolic resistance: Impact on muscle
540 protein synthesis and net balance. Muscle protein synthesis (MPS) and net balance (NB) responses
541 were simulated following a 3.59-gram leucine bolus using either consensus (A, B) or worst-case
542 (C, D) estimates for six putative mechanisms of anabolic resistance (Table 1). Each mechanism
543 was individually simulated by multiplying the relevant model parameter by the value estimated
544 from older adult data. In the "Combined" condition, all six putative mechanisms were
545 simultaneously simulated. The "Combined (comp. sig.)" condition additionally includes
546 compensatory signaling (comp. sig.) to simulate elevated postabsorptive phospho-p70S6K levels.
547 The "Healthy" simulation represents a subset of 100 plausible muscle protein metabolism profiles
548 that met a conservative MPSA criterion for a healthy individual. This same subset was used to
549 simulate each anabolic resistance condition, enabling visualization of the potential distribution of
550 responses across mechanisms.
551 Each simulated condition displays a violin plot showing the distribution density, overlaid with a
552 boxplot (median, first and third quartile, and minimum and maximum values), and individual data
553 points. In the MPS panels, the grey dashed line denotes the MPS response in young adults, as
554 calculated from Mitchell et al. (2015) [30]. The red dot-dashed line and surrounding red gradient
555 represent the mean MPS response and 95% confidence interval for older adults, as calculated from

556 Mitchell et al. (2017) [31]. In the NB panels, the grey dashed line indicates the median NB value
557 of the healthy simulation.

558 **Figure 4.** Simulated therapeutic strategies to overcome anabolic resistance and restore muscle
559 protein metabolism. Each therapeutic target was simulated across a subset of 100 model parameter
560 sets by restoring the relevant parameter to its healthy value (i.e., multiplying by the inverse of the
561 pathological estimate from Table 1). Bolded entries in therapeutic targets 1 to 6 indicate cases
562 where restoring a single mechanism, together with the simulation of the respective anabolic
563 resistance mechanism, returned the muscle metabolism response to the healthy level.
564 Combined therapeutic strategies (denoted by "+") reflect the co-simulation of multiple therapeutic
565 targets. All individual and combined therapeutic strategies were simulated against each anabolic
566 resistance mechanism individually and against all six mechanisms combined. The "Pathological
567 baseline" column shows the median muscle metabolism response across the 100 parameter sets
568 for each anabolic resistance condition without intervention (Figure 3).
569 A heatmap displays the simulated median responses for muscle protein synthesis, muscle protein
570 breakdown, and net balance (NB) across conditions, using either consensus (A-C) or worst-case
571 (D-F) estimates. Gold indicates recovery to the healthy value; blue indicates a response that would
572 cause a reduction in NB compared to the healthy value; red indicates a response that cause produce
573 an increase in NB compared to the healthy value. The numerical value in each cell represents the
574 median muscle metabolism response – expressed in units of grams of leucine synthesized (MPS),
575 degraded (MPB), or net incorporated into muscle (NB) – calculated across the subset of 100
576 simulated model parameter sets.

577 **Figure 5.** Simulated therapeutic strategies to overcome anabolic resistance and restore protein
578 metabolism in muscle cells with compensatory signaling. The worst-case estimates for reduced
579 mTOR concentration, reduced p70S6K concentration, and reduced mTORC1 sensitivity were
580 simulated simultaneously ("[mTOR] + [p70S6K] + mTORC1 sensitivity"), along with the
581 combined simulation of all six anabolic resistance mechanisms ("Combined"). These simulations
582 were performed without compensatory signaling ("Pathological baseline"), with compensatory
583 signaling to simulate elevated postabsorptive phospho-p70S6K levels, and with compensatory
584 signaling plus individual or combined therapeutic strategies (combinations denoted by "+").
585 A heatmap displays the simulated median responses for muscle protein synthesis, muscle protein
586 breakdown, and net balance (NB) across conditions. Gold indicates recovery to the healthy value;

587 blue indicates a response that would produce a reduction in NB compared to the healthy value; red
588 indicates a response that would produce an increase in NB compared to the healthy value. The
589 numerical value in each cell represents the median muscle metabolism response – expressed in
590 units of grams of leucine synthesized (MPS), degraded (MPB), or net incorporated into muscle
591 (NB) – calculated across the subset of 100 simulated model parameter sets. For reference, the
592 healthy values for MPS, MPB, and NB were 0.47 g synthesized, 0.154 g degraded, and 0.27 g net
593 incorporated, respectively.

594

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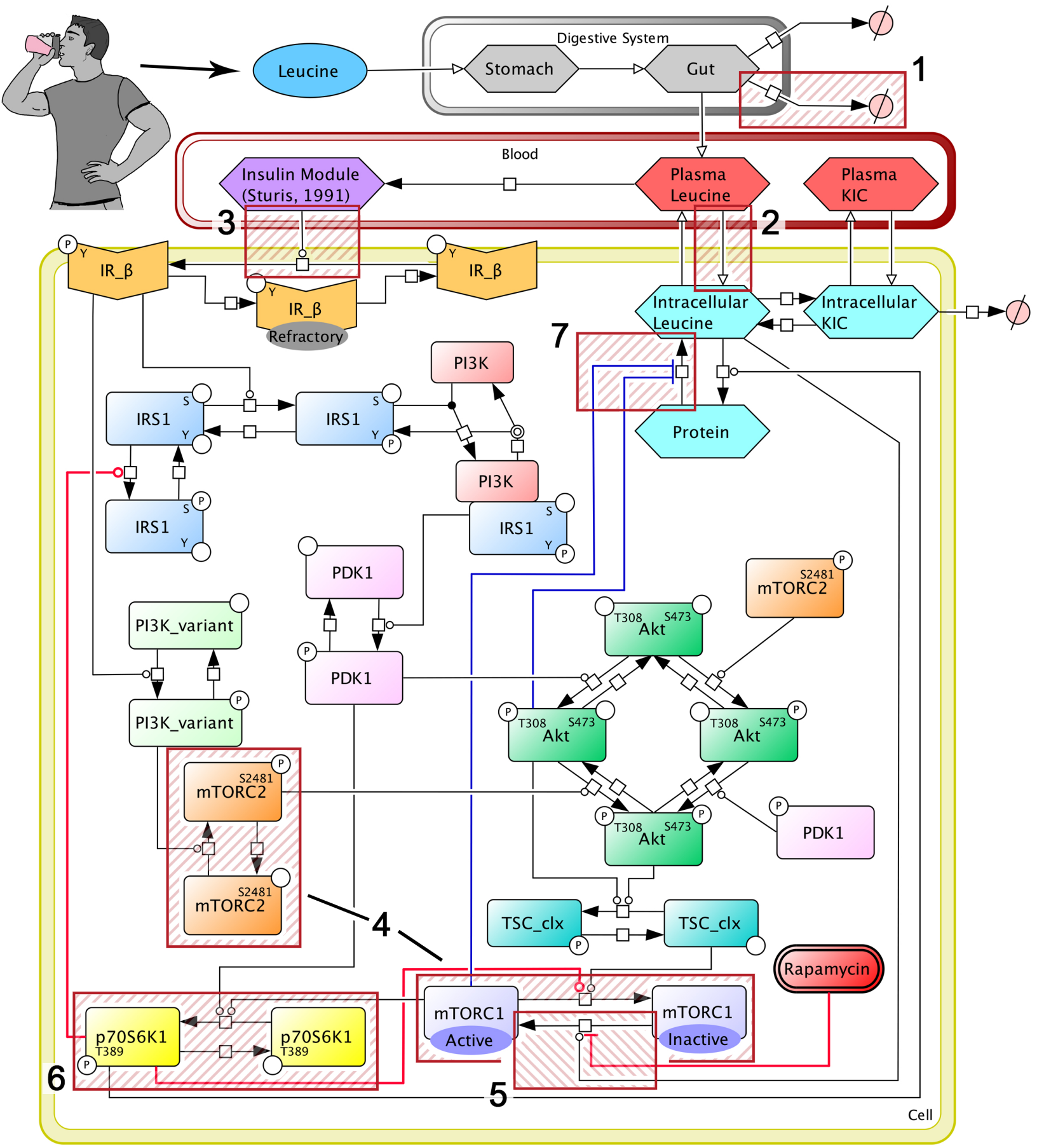
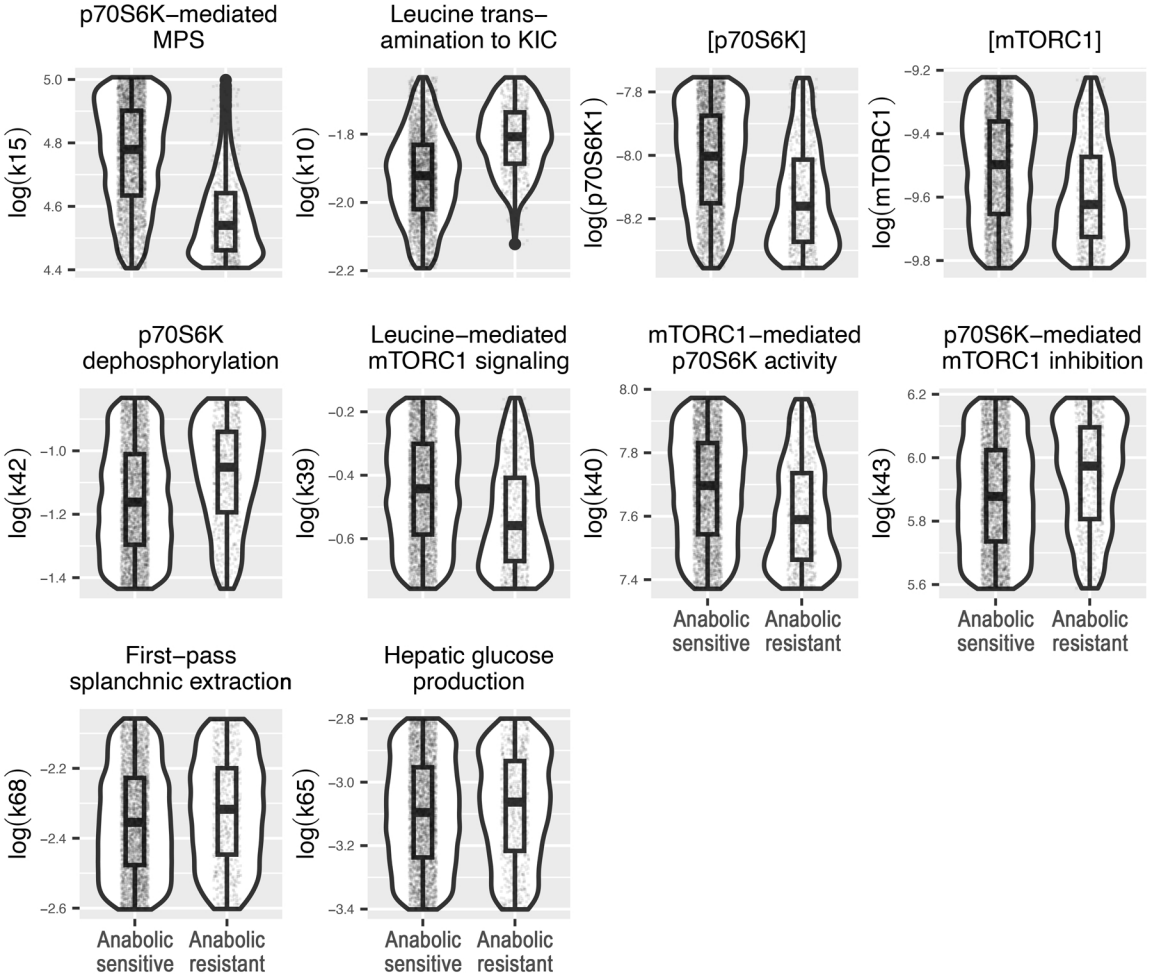
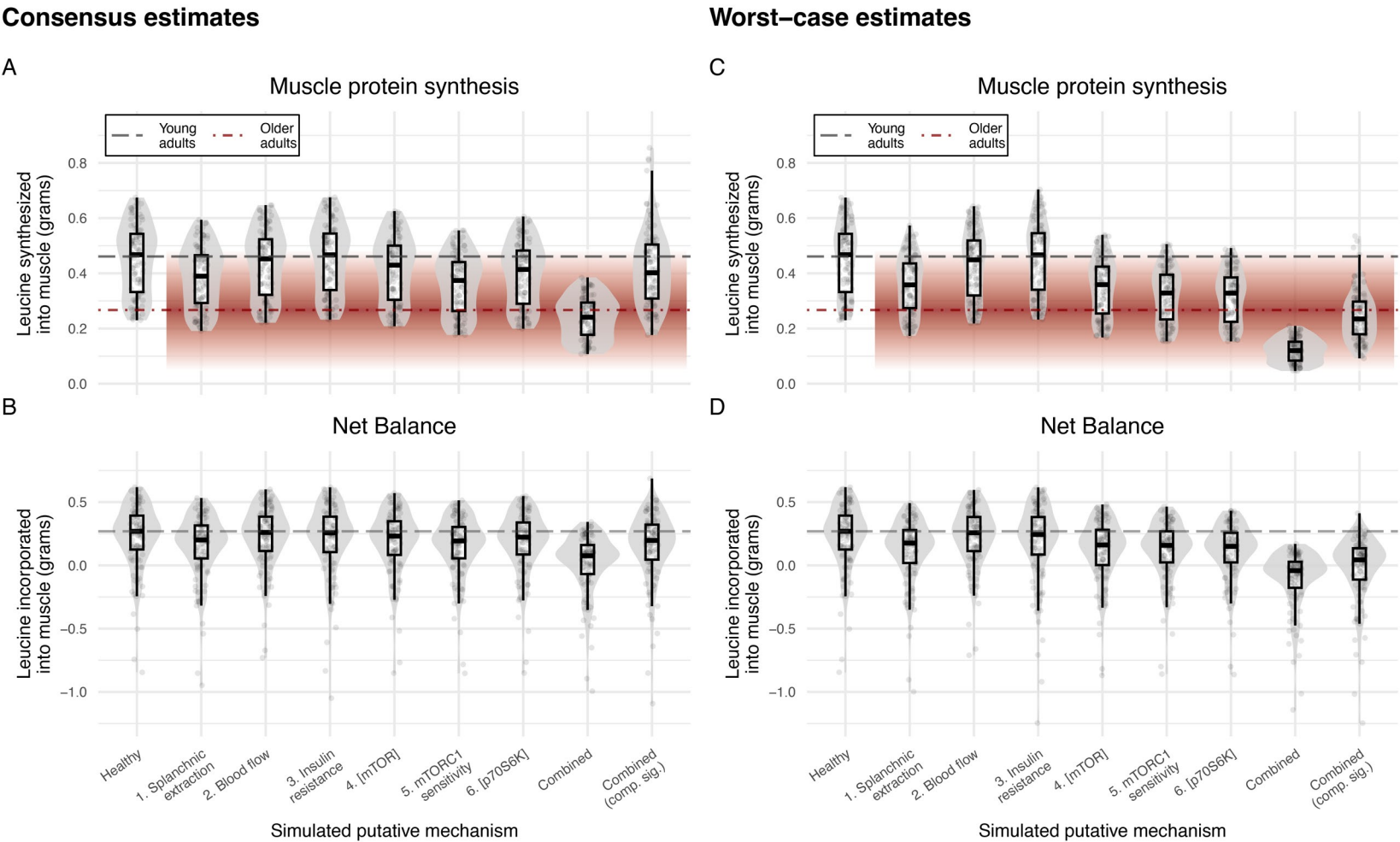

Leucine
Digestive System
Stomach
Gut
1
Blood
Insulin Module
(Sturis, 1991)
Plasma
Leucine
Plasma
KIC
3
2
IR_β
IR_β
IR_β
Refractory
Intracellular
Leucine
Intracellular
KIC
7
PI3K
IRS1
IRS1
Protein
PI3K
IRS1
IRS1
PDK1
mTORC2
S2481
PI3K_variant
T308
S473
Akt
PDK1
PI3K_variant
T308
S473
Akt
T308
S473
Akt
mTORC2
S2481
T308
S473
Akt
PDK1
mTORC2
S2481
4
TSC_clx
TSC_clx
Rapamycin
p70S6K1
T389
p70S6K1
T389
mTORC1
Active
mTORC1
Inactive
6
5
Cell


p70S6K–mediated MPS
log(k15)
5.0
4.8
4.6
4.4
Leucine trans–amination to KIC
log(k10)
−1.8
−2.0
−2.2
[p70S6K]
log(p70S6K1)
−7.8
−8.0
−8.2
[mTORC1]
log(mTORC1)
−9.2
−9.4
−9.6
−9.8
p70S6K dephosphorylation
log(k42)
−1.0
−1.2
−1.4
Leucine–mediated mTORC1 signaling
log(k39)
−0.2
−0.4
−0.6
mTORC1–mediated p70S6K activity
log(k40)
8.0
7.8
7.6
7.4
p70S6K–mediated mTORC1 inhibition
log(k43)
6.2
6.0
5.8
5.6
Anabolic sensitive
Anabolic resistant
First–pass splanchnic extraction
log(k68)
−2.2
−2.4
−2.6
Hepatic glucose production
log(k65)
−2.8
−3.0
−3.2
−3.4
Anabolic sensitive
Anabolic resistant


Consensus estimates
Worst−case estimates
A
Muscle protein synthesis
Young adults
Older adults
Leucine synthesized into muscle (grams)
0.8
0.6
0.4
0.2
0.0
B
Net Balance
Leucine incorporated into muscle (grams)
0.5
0.0
−0.5
−1.0
Healthy
1. Splanchnic extraction
2. Blood flow
3. Insulin resistance
4. [mTOR]
5. mTORC1 sensitivity
6. [p70S6K]
Combined
Combined (comp. sig.)
Simulated putative mechanism
C
Muscle protein synthesis
Young adults
Older adults
Leucine synthesized into muscle (grams)
0.8
0.6
0.4
0.2
0.0
D
Net Balance
Leucine incorporated into muscle (grams)
0.5
0.0
−0.5
−1.0
Healthy
1. Splanchnic extraction
2. Blood flow
3. Insulin resistance
4. [mTOR]
5. mTORC1 sensitivity
6. [p70S6K]
Combined
Combined (comp. sig.)
Simulated putative mechanism

Consensus estimates

A

MPS

Therapeutic targets

Anabolic resistance mechanisms	Pathological baseline	1. Splanchnic extraction	2. Blood flow	3. Insulin resistance	4. [mTOR]	5. mTORC1 sensitivity	6. [p70S6K]	4+5	4+6	5+6	4+5+6	1+4+5+6
Splanchnic extraction	0.39	0.47	0.4	0.39	0.42	0.48	0.44	0.46	0.47	0.54	0.58	0.69
Blood flow	0.45	0.51	0.47	0.45	0.49	0.55	0.51	0.53	0.55	0.62	0.66	0.74
Insulin resistance	0.47	0.53	0.48	0.47	0.51	0.57	0.52	0.56	0.57	0.64	0.69	0.77
[mTOR]	0.43	0.48	0.44	0.43	0.47	0.53	0.48	0.51	0.52	0.59	0.64	0.72
mTORC1 sensitivity	0.37	0.42	0.38	0.37	0.41	0.47	0.43	0.45	0.46	0.52	0.57	0.65
[p70S6K]	0.41	0.46	0.42	0.41	0.45	0.51	0.47	0.49	0.51	0.57	0.62	0.69
Combined	0.24	0.28	0.25	0.24	0.26	0.3	0.27	0.33	0.3	0.35	0.38	0.45

B

MPB

Anabolic resistance mechanisms	Pathological baseline	1. Splanchnic extraction	2. Blood flow	3. Insulin resistance	4. [mTOR]	5. mTORC1 sensitivity	6. [p70S6K]	4+5	4+6	5+6	4+5+6	1+4+5+6
Splanchnic extraction	0.162	0.154	0.166	0.151	0.154	0.176	0.169	0.163	0.166	0.182	0.179	0.173
Blood flow	0.145	0.142	0.154	0.14	0.143	0.158	0.152	0.147	0.149	0.168	0.164	0.159
Insulin resistance	0.161	0.155	0.167	0.154	0.152	0.178	0.173	0.16	0.164	0.186	0.183	0.177
[mTOR]	0.157	0.152	0.165	0.148	0.154	0.172	0.165	0.159	0.161	0.181	0.177	0.171
mTORC1 sensitivity	0.137	0.134	0.142	0.135	0.136	0.154	0.147	0.139	0.14	0.161	0.158	0.152
[p70S6K]	0.143	0.139	0.147	0.139	0.139	0.159	0.154	0.142	0.146	0.167	0.164	0.158
Combined	0.144	0.137	0.149	0.139	0.139	0.16	0.154	0.152	0.145	0.167	0.163	0.154

C

NB

Anabolic resistance mechanisms	Pathological baseline	1. Splanchnic extraction	2. Blood flow	3. Insulin resistance	4. [mTOR]	5. mTORC1 sensitivity	6. [p70S6K]	4+5	4+6	5+6	4+5+6	1+4+5+6
Splanchnic extraction	0.2	0.27	0.21	0.21	0.23	0.27	0.24	0.26	0.28	0.32	0.36	0.45
Blood flow	0.26	0.3	0.27	0.26	0.3	0.34	0.3	0.33	0.34	0.39	0.44	0.5
Insulin resistance	0.26	0.3	0.26	0.27	0.3	0.34	0.3	0.33	0.35	0.39	0.44	0.51
[mTOR]	0.23	0.27	0.23	0.24	0.27	0.31	0.27	0.3	0.31	0.36	0.41	0.47
mTORC1 sensitivity	0.19	0.23	0.19	0.2	0.22	0.27	0.23	0.26	0.27	0.31	0.35	0.42
[p70S6K]	0.22	0.26	0.22	0.23	0.26	0.3	0.27	0.29	0.31	0.35	0.39	0.45
Combined	0.08	0.12	0.07	0.09	0.1	0.13	0.11	0.16	0.13	0.17	0.2	0.25

Worst-case estimates

D

MPS

Therapeutic targets

Anabolic resistance mechanisms	Pathological baseline	1. Splanchnic extraction	2. Blood flow	3. Insulin resistance	4. [mTOR]	5. mTORC1 sensitivity	6. [p70S6K]	4+5	4+6	5+6	4+5+6	1+4+5+6
Splanchnic extraction	0.36	0.47	0.37	0.36	0.45	0.48	0.5	0.55	0.6	0.64	0.76	0.99
Blood flow	0.45	0.52	0.47	0.45	0.57	0.6	0.61	0.69	0.76	0.8	0.95	1.09
Insulin resistance	0.47	0.55	0.48	0.47	0.6	0.63	0.64	0.71	0.8	0.84	0.99	1.13
[mTOR]	0.36	0.42	0.37	0.36	0.47	0.5	0.51	0.6	0.64	0.69	0.84	0.97
mTORC1 sensitivity	0.33	0.38	0.34	0.33	0.44	0.47	0.47	0.56	0.6	0.64	0.79	0.91
[p70S6K]	0.33	0.37	0.34	0.33	0.41	0.44	0.47	0.5	0.59	0.63	0.74	0.85
Combined	0.12	0.15	0.13	0.12	0.17	0.18	0.18	0.24	0.24	0.27	0.35	0.45

E

MPB

Anabolic resistance mechanisms	Pathological baseline	1. Splanchnic extraction	2. Blood flow	3. Insulin resistance	4. [mTOR]	5. mTORC1 sensitivity	6. [p70S6K]	4+5	4+6	5+6	4+5+6	1+4+5+6
Splanchnic extraction	0.165	0.154	0.169	0.147	0.151	0.184	0.182	0.167	0.175	0.216	0.204	0.193
Blood flow	0.143	0.14	0.154	0.136	0.144	0.164	0.166	0.152	0.158	0.19	0.183	0.177
Insulin resistance	0.166	0.158	0.174	0.154	0.157	0.194	0.194	0.173	0.178	0.225	0.211	0.202
[mTOR]	0.168	0.159	0.175	0.15	0.154	0.187	0.187	0.171	0.176	0.218	0.201	0.193
mTORC1 sensitivity	0.133	0.131	0.139	0.129	0.138	0.154	0.153	0.143	0.144	0.176	0.165	0.158
[p70S6K]	0.133	0.13	0.138	0.129	0.138	0.15	0.154	0.141	0.144	0.176	0.159	0.153
Combined	0.148	0.137	0.159	0.138	0.139	0.171	0.17	0.147	0.148	0.186	0.172	0.159

F

NB

Anabolic resistance mechanisms	Pathological baseline	1. Splanchnic extraction	2. Blood flow	3. Insulin resistance	4. [mTOR]	5. mTORC1 sensitivity	6. [p70S6K]	4+5	4+6	5+6	4+5+6	1+4+5+6
Splanchnic extraction	0.18	0.27	0.17	0.19	0.26	0.27	0.28	0.35	0.39	0.4	0.51	0.71
Blood flow	0.26	0.31	0.27	0.26	0.36	0.38	0.39	0.48	0.52	0.54	0.69	0.83
Insulin resistance	0.24	0.31	0.25	0.27	0.36	0.37	0.38	0.48	0.53	0.55	0.7	0.84
[mTOR]	0.16	0.21	0.16	0.17	0.27	0.27	0.28	0.37	0.41	0.42	0.57	0.68
mTORC1 sensitivity	0.16	0.2	0.16	0.17	0.25	0.27	0.27	0.36	0.38	0.41	0.54	0.66
[p70S6K]	0.15	0.2	0.16	0.17	0.23	0.25	0.27	0.32	0.38	0.39	0.52	0.62
Combined	−0.04	0	−0.04	−0.02	0	0.01	0	0.07	0.07	0.08	0.16	0.25

A

MPS

Therapeutic targets

Anabolic resistance mechanisms	Pathological baseline	Compensatory signaling	1. Splanchnic extraction	2. Blood flow	3. Insulin resistance	4. [mTOR]	5. mTORC1 sensitivity	6. [p70S6K]	4+5	4+6	5+6	4+5+6	1+4+5+6
[mTOR] + [p70S6K] + mTORC1 sensitivity	0.16	0.3	0.32	0.3	0.29	0.33	0.35	0.49	0.39	0.53	0.56	0.62	0.69
Combined	0.12	0.23	0.28	0.24	0.23	0.26	0.27	0.4	0.3	0.42	0.45	0.48	0.6

B

MPB

Anabolic resistance mechanisms	Pathological baseline	Compensatory signaling	1. Splanchnic extraction	2. Blood flow	3. Insulin resistance	4. [mTOR]	5. mTORC1 sensitivity	6. [p70S6K]	4+5	4+6	5+6	4+5+6	1+4+5+6
[mTOR] + [p70S6K] + mTORC1 sensitivity	0.132	0.162	0.152	0.173	0.15	0.141	0.169	0.198	0.15	0.171	0.208	0.18	0.173
Combined	0.148	0.189	0.169	0.2	0.167	0.145	0.174	0.203	0.152	0.174	0.215	0.184	0.168

C

NB

Anabolic resistance mechanisms	Pathological baseline	Compensatory signaling	1. Splanchnic extraction	2. Blood flow	3. Insulin resistance	4. [mTOR]	5. mTORC1 sensitivity	6. [p70S6K]	4+5	4+6	5+6	4+5+6	1+4+5+6
[mTOR] + [p70S6K] + mTORC1 sensitivity	0.02	0.11	0.14	0.11	0.12	0.15	0.15	0.25	0.21	0.3	0.3	0.38	0.47
Combined	−0.04	0.04	0.1	0.05	0.07	0.1	0.09	0.18	0.13	0.22	0.2	0.26	0.37