

1 **The effect of acute sleep deprivation on skeletal muscle protein synthesis and the**  
2 **hormonal environment**

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16 **Running title:** Acute sleep deprivation and muscle protein metabolism

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26

27 **Abstract**

28 Chronic sleep loss is a potent catabolic stressor, increasing the risk of metabolic dysfunction  
29 and loss of muscle mass and function. To provide mechanistic insight into these clinical  
30 outcomes, we sought to determine if acute sleep deprivation blunts skeletal muscle protein  
31 synthesis and promotes a catabolic environment. Healthy young adults (N=13; 7 male, 6  
32 female) were subjected to one night of total sleep deprivation (DEP) and normal sleep (CON)  
33 in a randomized cross-over design. Anabolic and catabolic hormonal profiles, skeletal muscle  
34 fractional synthesis rate and markers of muscle protein degradation were assessed across the  
35 following day. Acute sleep deprivation reduced muscle protein synthesis by 18% (CON:  
36  $0.072 \pm 0.015$  vs. DEP:  $0.059 \pm 0.014$  %·h<sup>-1</sup>,  $p=0.040$ ). In addition, it increased plasma  
37 cortisol by 21% ( $p=0.030$ ) and decreased plasma testosterone, but not IGF-1, by 22%  
38 ( $p=0.029$ ). A single night of total sleep deprivation is sufficient to induce anabolic resistance  
39 and a pro-catabolic environment. These acute changes may represent mechanistic precursors  
40 driving the metabolic dysfunction and body composition changes associated with chronic  
41 sleep deprivation.

42

## 43 **Introduction**

44 Acute and chronic sleep loss are linked with a range of negative physiological and  
45 psychological outcomes (22). While complete sleep deprivation rapidly impedes simple and  
46 complex cognitive functions, sleep restriction impairs whole-body homeostasis, leading to  
47 undesirable metabolic consequences in the short- and longer-term (39). Most metabolic  
48 tissues including liver, adipose tissue and skeletal muscle are at risk of developing sleep loss-  
49 associated adverse outcomes.

50 Skeletal muscle is a primary regulator of human metabolism. Sleep deprivation (9, 10) and  
51 restriction (19) have the potential to profoundly affect muscle health by altering gene  
52 regulation and substrate metabolism. Even relatively short periods of sleep restriction (less  
53 than a week) can compromise glucose metabolism, reduce insulin sensitivity and impair  
54 muscle function (5, 7). Skeletal muscle is made up of 80% of proteins and maintaining  
55 optimal muscle protein metabolism is equally critical for muscle health. In situations where  
56 skeletal muscle protein synthesis chronically lags protein degradation, a loss of muscle mass  
57 is inevitable. Low muscle mass is a hallmark of and precursor to a range of chronic health  
58 conditions, including neuromuscular disease, sarcopenia and frailty, obesity and type II  
59 diabetes (41). Population-based studies report that the risk of developing these conditions is  
60 15-30% higher in individuals who regularly experience sleep deprivation, sleep restriction,  
61 and inverted sleep-wake cycles (26, 31, 50). To this end, a growing body of evidence  
62 suggests that a lack of sleep may directly affect muscle protein metabolism (1, 35, 42).

63 Rodent studies first demonstrated a possible causal link between complete sleep deprivation  
64 and disrupted muscle protein metabolism. Rats subjected to 96 h of paradoxical sleep  
65 deprivation, where rapid eye movement sleep is restricted, experienced a decrease in muscle  
66 mass (13) and muscle fibre cross-sectional area (15). In this model, sleep deprivation  
67 negatively impacted the pathways regulating protein synthesis and increased muscle  
68 proteolytic activity (15). These findings were paralleled by a human study reporting a  
69 catabolic gene signature in skeletal muscle following one night of total sleep deprivation in  
70 healthy young males (10). To expand on this acute model, investigators recently  
71 demonstrated that five consecutive nights of sleep restriction (four hours per night) reduced  
72 myofibrillar protein synthesis in healthy young males when compared to normal sleep  
73 patterns (42). The possible mechanisms underlying these effects have not been investigated,  
74 but might involve the hormonal environment.

75 Factors that regulate skeletal muscle protein metabolism at the molecular level are influenced  
76 by mechanical (muscle contraction), nutritional (dietary protein intake) and hormonal inputs  
77 (41). Testosterone and IGF-1 positively regulate muscle protein anabolism by promoting  
78 muscle protein synthesis (43, 46), while repressing the genes that activate muscle protein  
79 degradation (51). In contrast, cortisol drives catabolism by activating key muscle protein  
80 degradation pathways (21). Experimental evidence suggests that acute and chronic sleep loss  
81 alters anabolic (29, 40) and catabolic (10, 14) hormone secretion patterns in humans,  
82 providing a possible mechanism for impaired muscle protein metabolism.

83 While our understanding of the health consequences of sleep deprivation continues to  
84 improve, important gaps and opportunities remain. This includes linking acute mechanistic  
85 changes with clinically observable outcomes and moving towards a more prescriptive,  
86 individualized understanding of sleep deprivation by examining sex-based differences. In this  
87 study, we sought to determine if one night of complete sleep deprivation promotes a catabolic  
88 hormonal environment and compromises post-prandial muscle protein synthesis and markers  
89 of muscle degradation in young, healthy male and female participants.

90

91 **Methods**

92

93 **Participants**

94 Thirteen young (18-35 years old), healthy male and female students gave their informed  
95 consent to participate in this randomized, crossover-designed study. Participants were  
96 excluded if they had a history of recent transmeridian travel (i.e., no travel across multiple  
97 time zones in the previous four weeks), shiftwork (i.e., no involvement in shiftwork over the  
98 previous three months), frequent napping (i.e.,  $\geq 2$  naps per week), or had a diagnosed sleep  
99 disorder. Participants were required to have habitual bed (2200–0000) and wake (0600–0800)  
100 times that were broadly consistent with the experimental protocol and to self-report obtaining  
101 a minimum of 7 hours of sleep (not time in bed) per night. Chronotype was assessed using the  
102 morningness-eveningness (ME) questionnaire (20). Participants exhibiting extreme  
103 morningness (score  $> 70$ ) or eveningness (score  $< 30$ ) were excluded. All participants but  
104 three displayed an ‘intermediate’ ME type. A detailed account of the strategy for female  
105 volunteer recruitment and testing have been comprehensively described by our group (24).  
106 Briefly, effects of female reproductive hormone fluctuations were minimised by testing all  
107 female participants during the same phase of their menstrual cycle in both conditions.  
108 Although it has previously been shown that the menstrual cycle had no effect on female  
109 muscle protein synthesis (34), our primary outcome, the follicular phase was avoided to  
110 ensure the ratio of estrogen to progesterone was at its lowest. The study was approved by the  
111 Deakin University Human Research Ethics Committee (2016-028) and conducted in  
112 accordance to *The Declaration of Helsinki* (1964) and its later amendments. Participants’  
113 physiological characteristics, ME score, and self-reported habitual time asleep are  
114 summarized in Table 1. There were no sex-specific difference in ME score ( $p=0.148$ ) or self-  
115 reported habitual time asleep ( $p=0.401$ ).

116

117 **Table 1.** Participants’ characteristics. Mean  $\pm$  SD; BMI = Body Mass Index; ME =  
118 Morningness Eveningness score

Sex	Age	Mass (kg)	Height (cm)	BMI ( $\text{kg}\cdot\text{m}^{-2}$ )	ME score	Habitual time asleep (self-reported) (h)
Male (N=7)	22 $\pm$ 1.8	71.6 $\pm$ 11.3	173.5 $\pm$ 9.0	22.6 $\pm$ 4.1	48.0 $\pm$ 6.4	7.5 $\pm$ 0.6

Female (N=6)	20 ± 1.3	60.1 ± 10.3	170.5 ± 5.1	20.7 ± 3.2	53.8 ± 6.6	7.8 ± 0.7
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### 119 **Sample size calculation**

120 At the time this study was designed, there was no published study investigating the effect of  
121 sleep deprivation on muscle protein synthesis. Using data from studies investigating the  
122 effects of an anabolic and catabolic stimulus (e.g., immobilization or exercise) on changes in  
123 muscle protein synthesis (28, 36), power analyses conducted on our primary outcome  
124 (fractional synthesis rate) indicated that a sample size of 13 would minimize the risk of type  
125 II error ( $\beta=0.2, \alpha=0.05$ ). Males and females were included as previous work demonstrated that  
126 muscle fractional synthesis rate, our primary outcome, is similar in both sexes (24, 49).

127

### 128 **Pre-study procedure**

129 During the week prior to the study, participants were instructed to maintain their habitual  
130 sleep behaviour. Participants wore an actigraph (Actical MiniMitter/Respironics, Bend, OR)  
131 on their non-dominant wrist, and completed a sleep diary. The diary was used to corroborate  
132 actigraphy data and minimise possibility of incorrectly scoring periods of sedentary  
133 wakefulness as sleep.

134 Participants completed a control (CON) and experimental (DEP; sleep deprivation) trial in a  
135 randomized, crossover design. Trials were separated by at least four weeks to allow for full  
136 recovery. Forty-eight hours prior to each trial, participants were required to refrain from  
137 strenuous exercise, alcohol and caffeine. On the night of the trial (CON or DEP), a  
138 standardized meal containing approximately 20% fat, 14% protein and 66% carbohydrate  
139 (energy intake ranging between 8.4-8.9 kcal/kg) was provided to participants with water ad  
140 libitum.

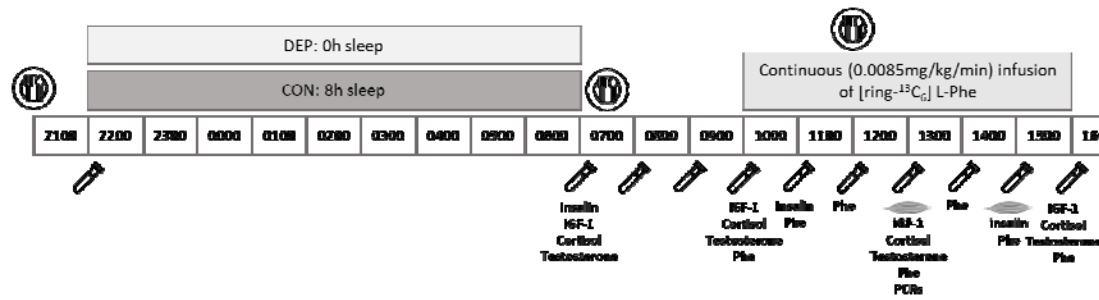
141

### 142 **Study procedure**

143 On the night of the sleep deprivation trial (DEP), participants consumed a standardized meal  
144 at 1900 and reported to the laboratory at 2100 where they were limited to sedentary activities  
145 (i.e., reading a book, watching a movie). Participants were constantly observed by research  
146 personnel and monitored by actigraphy to ensure they did not fall asleep. They remained in a  
147 sound attenuated, light (211±14 lux) and temperature (21±2°C) controlled facility for the

148 entire 30h protocol. Participants were permitted to consume low-protein snacks (i.e., fruits  
149 and vegetables) and water *ad libitum* during the sleep deprivation period. Regardless of  
150 potential differences in insulinemia, adding a non-pharmacological dose of carbohydrates to a  
151 protein synthesis activating dose of proteins (15-30 g) has no additive effect on fractional  
152 synthesis rate (17, 18, 25, 44), our primary outcome. For the control trial (CON), participants  
153 consumed a standardized meal at 1900 and were permitted to sleep from 2200 to 0700 at  
154 home, rather than risking a night of disrupted sleep in an unfamiliar laboratory/clinical  
155 environment. At 0700 the following morning, a researcher and nurse with pre-arranged  
156 access to the participants' home woke the participant and immediately collected a venous  
157 blood sample prior to any physical activity or light exposure. Participants were then  
158 transported to the laboratory to complete the experimental protocol.

159 For both DEP and CON trials, at 0730 participants consumed a standardized breakfast  
160 containing approximately 9% fat, 11% proteins and 80% carbohydrates, and  $20.3 \pm 1.8$  g of  
161 proteins. Rather than fasting our participants, standardized meals were provided as part of the  
162 experimental protocol for DEP and CON. The goal was to: i) reduce participant discomfort  
163 and improve compliance, and ii) model a more realistic and balanced, post-prandial metabolic  
164 environment, rather than the overtly catabolic environment associated with 24h of fasting. At  
165 0800, an 18-gauge cannulae was inserted into the antecubital vein of each arm for blood  
166 sampling and the primed ( $0.34 \text{ mg}\cdot\text{kg}^{-1}$ ), constant infusion ( $0.0085 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) of [ring-  
167  $^{13}\text{C}_6$ ]-L-phenylalanine (Cambridge Isotope Laboratories, Tewksbury, MA) from 1000 to the  
168 end of the protocol. At 1200, participants consumed a standardized lunch containing 12% fat,  
169 21% protein and 67% carbohydrate, and  $20.6 \pm 0.3$  g protein. The slowly digested, whole-  
170 food meals reduced the fluctuations in plasma Phe enrichment, thus avoiding the need to add  
171 tracer to the meals (33). Skeletal muscle samples were obtained at 1300 and 1500 under local  
172 anaesthesia (1% Lidocaine) at separate locations from the belly of the *vastus lateralis* muscle  
173 using a percutaneous needle biopsy technique as previously described by our group (28).  
174 Muscle samples were immediately frozen in liquid nitrogen and used for the measurement of  
175 isotopic enrichment and gene expression analysis. An outline of the experimental protocol is  
176 presented in Figure 1.



177

178 **Figure 1.** Experimental Protocol. blood collection; : muscle collection; : standardized meal. IGF-1, cortisol and testosterone concentrations were measured at the  
179 : standardized meal. IGF-1, cortisol and testosterone concentrations were measured at the  
180 0700, 1000, 1300 and 1600 timepoints. Insulin concentrations were measured at the 0700,  
181 1100 and 1500 timepoints. Phe enrichment (Phe) was measured in both muscle tissue and  
182 blood samples between 1000 and 1600. PCRs were run on muscle tissue collected at 1300.

183

#### 184 **Sleep measures**

185 Sleep was recorded objectively using actigraphy (Actical MiniMitter/Respironics, Bend, OR).  
186 The Actical (28 × 27 × 10 mm, 17 g) device uses a piezoelectric omnidirectional  
187 accelerometer, which is sensitive to movements in all planes in the range of 0.5–3.0 Hz. Data  
188 output from activity monitors (actigraphy) provides an objective, non-invasive, indirect  
189 assessment of sleep and has been validated against polysomnography (2). Primary outcomes  
190 were total sleep time and sleep efficiency (total sleep time/time in bed).

191

#### 192 **Hormone measures**

193 Venous blood samples were collected every hour from 0700 to 1700 in EDTA-tubes,  
194 manually inverted and immediately centrifuged for 15 min at 13,000 rev·min<sup>-1</sup> at 4°C. The  
195 supernatant (plasma) was then isolated and frozen at -80°C for further analysis. Plasma  
196 cortisol, testosterone and insulin growth factor-1 (IGF-1) concentrations were determined  
197 using a high-sensitivity enzyme immunoassay ELISA kit (IBL International, Hamburg,  
198 Germany) according to the manufacturer's instructions. Insulin concentration was determined  
199 using the MILLIPLEX® MAP Human Metabolic Hormone Magnetic Bead Panel (Merck  
200 KGaA, Darmstadt, Germany) according to the manufacturer's instructions.

201



## 202 **Isotopic enrichment in plasma**

203 After thawing, plasma was precipitated using an equal volume of 15% sulfosalicylic acid  
204 (SSA) solution and centrifuged for 20 min at 13,000 rev·min<sup>-1</sup> at 4°C. Blood amino acids  
205 were extracted from 500 uL of supernatant by cation exchange chromatography (Dowex AG  
206 50W-8X, 100–200 mesh H<sup>+</sup> form; Bio-Rad Laboratories). Phenylalanine enrichments were  
207 determined by gas chromatography–mass spectrometry (GC-MS) using the  
208 tertbutyldimethylsilyl derivative with electron impact ionization as described previously (16).  
209 Ions 336 and 342 were monitored.

210

## 211 **Isotopic enrichment in muscle proteins**

212 A 30 mg piece of muscle was used for isolation of mixed muscle bound and intracellular  
213 protein fractions. Briefly, bound muscle proteins were extracted in perchloric acid and  
214 hydrolysed using 6N hydrochloric acid (110°C for 24 h). Isotopic enrichments of [ring-<sup>13</sup>C<sup>6</sup>]-  
215 L-phenylalanine in tissue fluid (intracellular fraction) were used as a precursor pool for the  
216 calculation of the fractional synthesis rate. Total muscle phenylalanine was isolated using  
217 cation exchange chromatography (50W-8X, 200–400 mesh H<sup>+</sup> form; Bio-Rad Laboratories).  
218 Amino acids were eluted in 8 mL of 2N ammonium hydroxide and dried under vacuum.  
219 Muscle intracellular and bound protein [ring-<sup>13</sup>C<sup>6</sup>]-L-phenylalanine enrichments were  
220 determined by GC-MS with electron impact ionization using the tert-butyldimethylsilyl  
221 derivative. Ions 238 and 240 were monitored for bound protein enrichments; ions 336 and  
222 342 were monitored for intracellular enrichments as described previously (16). Mixed muscle  
223 protein FSR (% / hour) was calculated by measuring the direct incorporation of [ring-<sup>13</sup>C<sup>6</sup>]-L-  
224 phenylalanine by using the precursor-product model (36):

$$FSR = \frac{EP2 - EP1}{Em \times t} \times 60 \times 100$$

225 where EP1 and EP2 are the bound enrichments of [ring-<sup>13</sup>C<sup>6</sup>]-L-phenylalanine for the 2  
226 muscle biopsies, Em is the mean enrichment of [ring-<sup>13</sup>C<sup>6</sup>]-L-phenylalanine in the muscle  
227 intracellular pool, and t is the time interval (min) between biopsies.

228

## 229 **RNA extraction and gene expression analysis**

230 Muscle biopsies collected at 1300 were used for gene expression analysis. RNA was  
231 extracted from ~15 mg of skeletal muscle samples using Tri-Reagent© Solution (Ambion  
232 Inc., Austin, TX, USA) according to the manufacturer's protocol. RNA was treated with  
233 DNase I Amplification Grade (Thermo Fisher Scientific, MA) and RNA concentration was  
234 assessed using the Nanodrop 1000 Spectrophotometer (Thermo Fisher Scientific). First-  
235 strand cDNA was generated from 1000 ng RNA using the High Capacity RT-kit (Applied  
236 Biosystems, Carlsbad, CA, USA). cDNA was then treated with RNase H (Thermo Fisher  
237 Scientific) according to the manufacturer protocol. Real-time PCR was carried out using an  
238 AriaMx real-time PCR system (Agilent Technologies, Santa Clara, CA) to measure mRNA  
239 levels. mRNA levels for ARNTL (BMAL1), CRY1, PER1, IGF-1Ea, IGF-1Eb, FBX032  
240 (atrogin-1), TRIM63 (MuRF-1), FOXO1 and FOXO3 were measured using 1 × SYBR©  
241 Green PCR MasterMix (Applied Biosystems) and 5 ng of cDNA. All primers were used at a  
242 final concentration of 300 nM. Primer details are provided in Table 2. Single-strand DNA  
243 was quantified using the Quant it OliGreen ssDNA Assay Kit (Thermo Fisher Scientific)  
244 according to the manufacturer's instruction. ssDNA was used for PCR normalization as  
245 previously validated in (32). No differences in ssDNA concentrations were found between  
246 groups (data can be found at <https://doi.org/10.6084/m9.figshare.12629972.v1>). This  
247 normalization strategy was cross-checked against the common housekeeper gene GAPDH  
248 (data not shown).

249

250 **Table 2.** Primer sequences

Gene	Accession number	Forward	Reverse
ARNTL (BMAL1)	NM_001030272.2	GGCAGCTCCACTGACTACCA	CCCGACGCCGCTTTTCAATC
CRY1	NM_004075.5	CCGTTCCCGTCCTTTC C	CTAAAGACAAAACGGCCCGC
PER1	NM_002616.3	GAGGACACTCCTGCGACCAG	GCCATGGGGAGAACAGAACA
IGF-1Ea	NM_001111283.3 and NM_001111284.2	GACATGCCCAAGACCCAGAAGGA	CGGTGGCATGTCACTCTTCACTC
IGF-1Eb	NM_001111285.3	GCCCCATCTACCAACAAGAACAC	CAGACTTGCTTCTGTCCCCTCCTTC
FBX032 (Atrogin- 1)	NM_001242463.2	AGTTTCGTGAGCGACCTCAG	CTTTGAAGGCAGGCCGGA

TRIM63 (MuRF-1)	NM_032588.3	GGGAGGTGATGTCTTCTCTCTG	CTGACAATCGCAGGTCACCC
FOXO1	NM_002015.4	GCAGCCGCCACATTCAACAG	AGAACTTAACTTCGCGGGGC
FOXO3	NM_001455.4	CCGCACGTCTTCAGGTCCTC	CGACGAACATTTCTCGGCT
GAPDH	XM_006959	CCACCCATGGCAAATTCC	TGGGATTCCATTGATGACAA

251

## 252 **Statistical analysis**

253 Statistical analyses were conducted using SPSS 26.0 (IBM Corp, Armonk, NY). Diagnostic  
254 plots of residuals and fitted values were checked to ensure homogeneity of the variance.  
255 Hormonal levels were analysed using a two-way analysis of variance (ANOVA) with within-  
256 participant factors for time and condition (CON vs DEP) unless specified otherwise. The  
257 Sidak test was used to compare pairs of means when a main effect was identified. For FSR  
258 and hormone concentrations, single-tailed paired t-tests were used to compare group means.  
259 For gene expression data, two-tailed paired t-tests were used to compare group means. Area  
260 under the curve (AUC) was computed on hormone values using the trapezoidal method. The  
261 significance levels for the F-tests in the t-tests and ANOVA and the Sidak tests were set at  
262  $p < 0.05$ . All data are reported as mean  $\pm$  SD.

263

264

## 265 Results

266

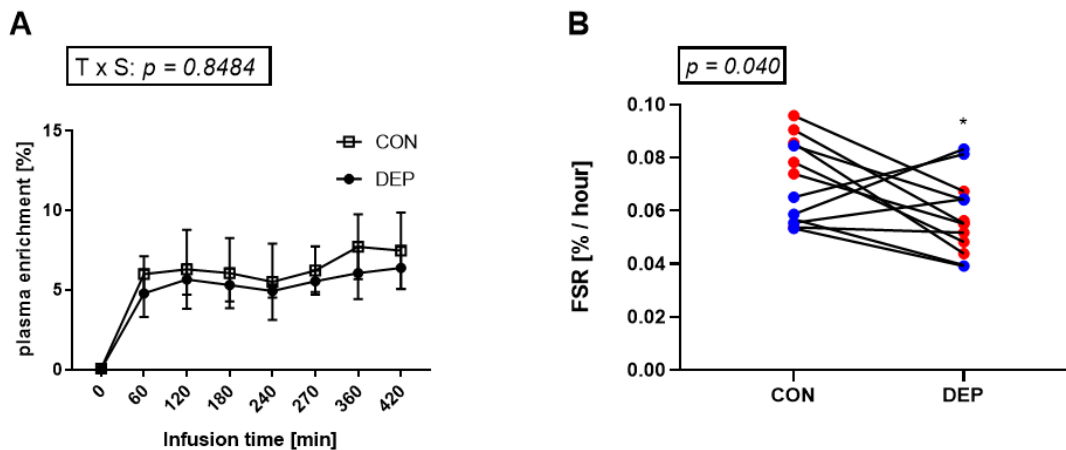
### 267 Sleep

268 During the week prior to the study, there were no differences in total sleep time (CON:  $5.9 \pm$   
269  $0.5$  h, DEP:  $6.1 \pm 1.4$  h,  $p = 0.718$ ) or sleep efficiency (CON:  $78.5 \pm 6.5$  %, DEP:  $79.4 \pm 4.7$   
270 %,  $p = 0.801$ ). Similarly, during the night directly preceding the sleep intervention, there  
271 were no differences in total sleep time (CON:  $6.8 \pm 0.8$  h, DEP:  $7.4 \pm 0.7$  h,  $p = 0.195$ ) or  
272 sleep efficiency (CON:  $77.3 \pm 6.3$  %, DEP:  $81.0 \pm 8.6$  %,  $p = 0.424$ ).

273

### 274 Muscle protein synthesis

275 Subjects remained in isotopic steady state for the duration of the isotope infusion, with no  
276 differences in plasma enrichment between CON and DEP conditions (Figure 2A). Sleep  
277 deprivation reduced post-prandial muscle protein fractional synthesis rate (FSR) by 18%  
278 (CON:  $0.072 \pm 0.015$  vs. DEP:  $0.059 \pm 0.014$  %·h<sup>-1</sup>,  $p=0.040$ ) (Figure 2B).



279

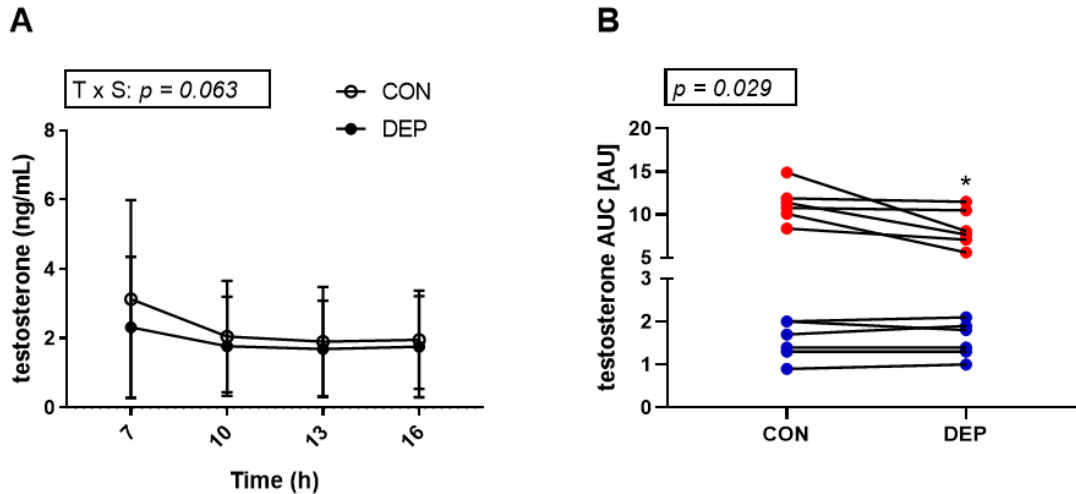
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281 **Figure 2.** Plasma enrichment of [ring-<sup>13</sup>C<sub>6</sub>]-L-phenylalanine during the experimental protocol  
282 (N=4). Data are presented as mean  $\pm$  SD (A). Post-prandial mixed muscle fractional synthesis  
283 rate measured in the control (CON) and sleep-deprived (DEP) conditions. Red dots depict  
284 male subjects. Blue dots depict female subjects (B).

285

## 286 Plasma testosterone levels

287 There was a main effect of time ( $p=0.002$ ) but the interaction effect of sleep  $\times$  time for  
288 plasma testosterone levels did not reach statistical significance ( $p=0.063$ ; Figure 3A). The  
289 area under the curve decreased by 22% in the DEP condition ( $p=0.029$ ; Figure 3B). A male  
290 and a female sub-population group were visually highlighted, where all male subjects had  
291 their testosterone AUC decreasing in the DEP condition (Figure 3B).



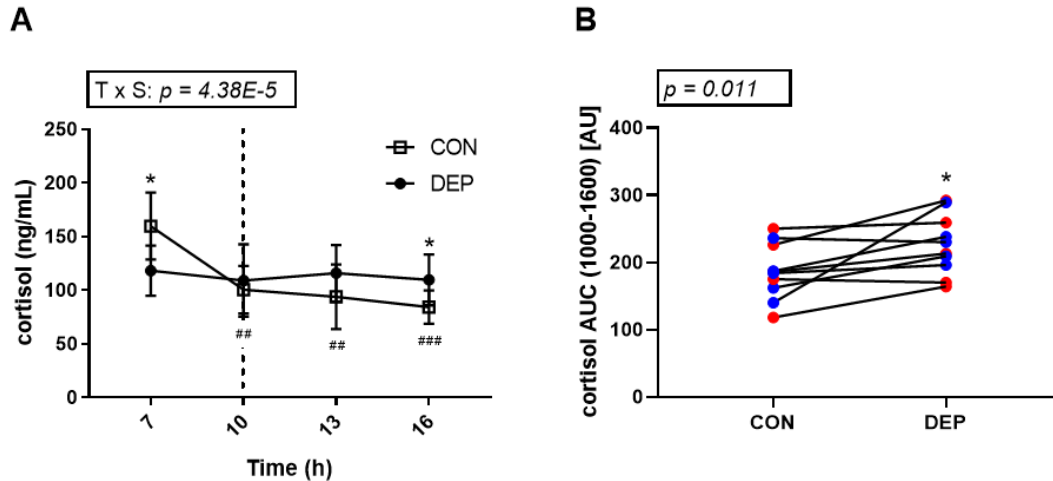
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293 **Figure 3.** Plasma testosterone concentrations in control (CON) and sleep-deprived (DEP)  
294 conditions. Data are presented as mean  $\pm$  SD (A). Area under the curve calculated for plasma  
295 cortisol concentrations. Red dots depict male subjects. Blue dots depict female subjects (B).

296

## 297 Plasma cortisol levels

298 A significant interaction effect of sleep  $\times$  time ( $p=4.38E-5$ ) was observed for plasma cortisol  
299 levels. Consistent with the typical increase in cortisol observed during the later stages of  
300 sleep (47), plasma cortisol levels were significantly higher ( $p=0.014$ ) in the CON condition  
301 than in the DEP condition at 0700 (wake time for the control condition). At 1000, plasma  
302 cortisol was similar in both sleep conditions ( $p=0.940$ ), but by 1600, cortisol was  
303 significantly higher in the DEP condition ( $p=0.048$ ) (Figure 4A). Plasma cortisol area under  
304 the curve (1000-1600), was 21% higher during DEP than CON ( $p=0.011$ ) (Figure 4B).



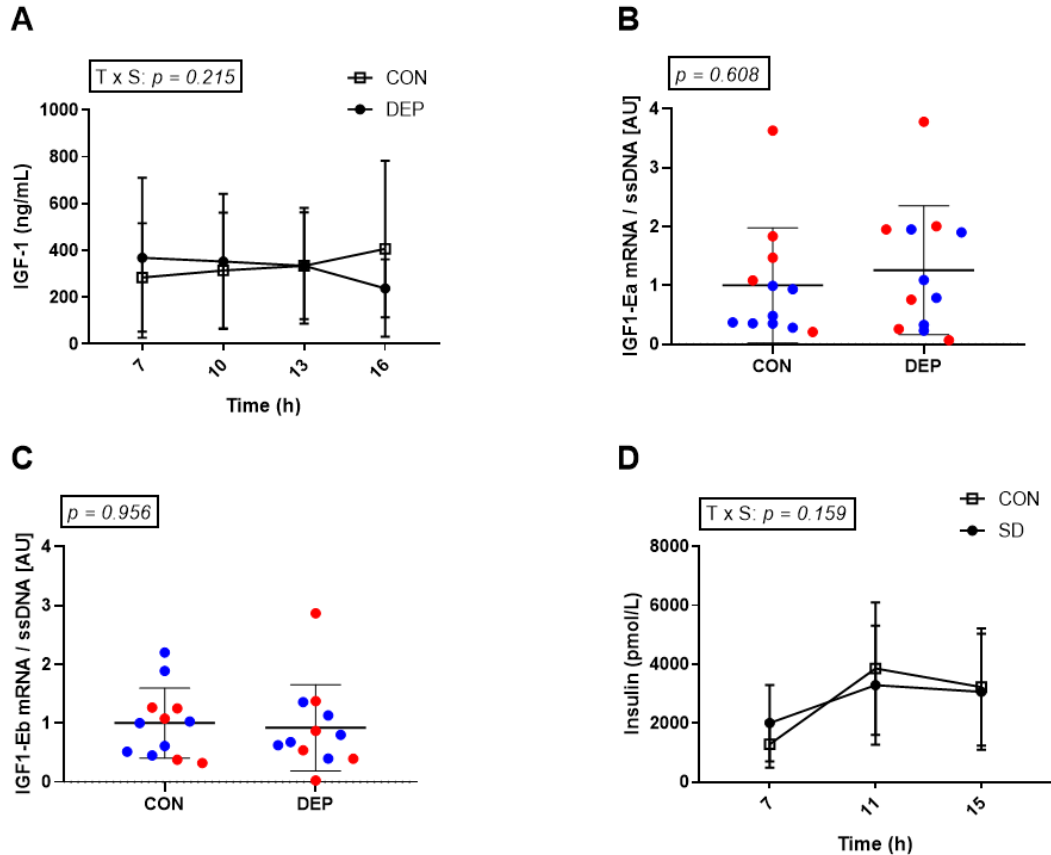
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306 **Figure 4.** Plasma cortisol concentrations in control (CON) and sleep-deprived (DEP)  
307 conditions. \*; significantly different from the CON condition,  $p < 0.05$ . ##; significantly  
308 different from the 700 timepoint in the CON group,  $p < 0.01$ . ###; CON group was significantly  
309 different from the 700 timepoint in the CON group,  $p < 0.001$ . Data are presented as mean  $\pm$   
310 SD (A). Area under the curve calculated for plasma cortisol concentrations from 1000  
311 (dashed line, A) until the end of the protocol. \*; significantly different from the CON  
312 condition,  $p < 0.05$ . Red dots depict male subjects. Blue dots depict female subjects (B).

313

#### 314 **Insulin and IGF-1 levels**

315 Plasma IGF-1 concentrations did not vary with time, sleep, or the combination of both  
316 (Figure 5A). Similarly, sleep deprivation did not influence the muscle expression levels of  
317 IGF1 mRNA isoforms IGF1-Ea and IGF1-Eb when measured in the post-prandial state  
318 (Figure 5B and 5C). Plasma insulin concentrations varied across the day, but there was no  
319 effect of sleep or the combination of sleep and time (Figure 5D).



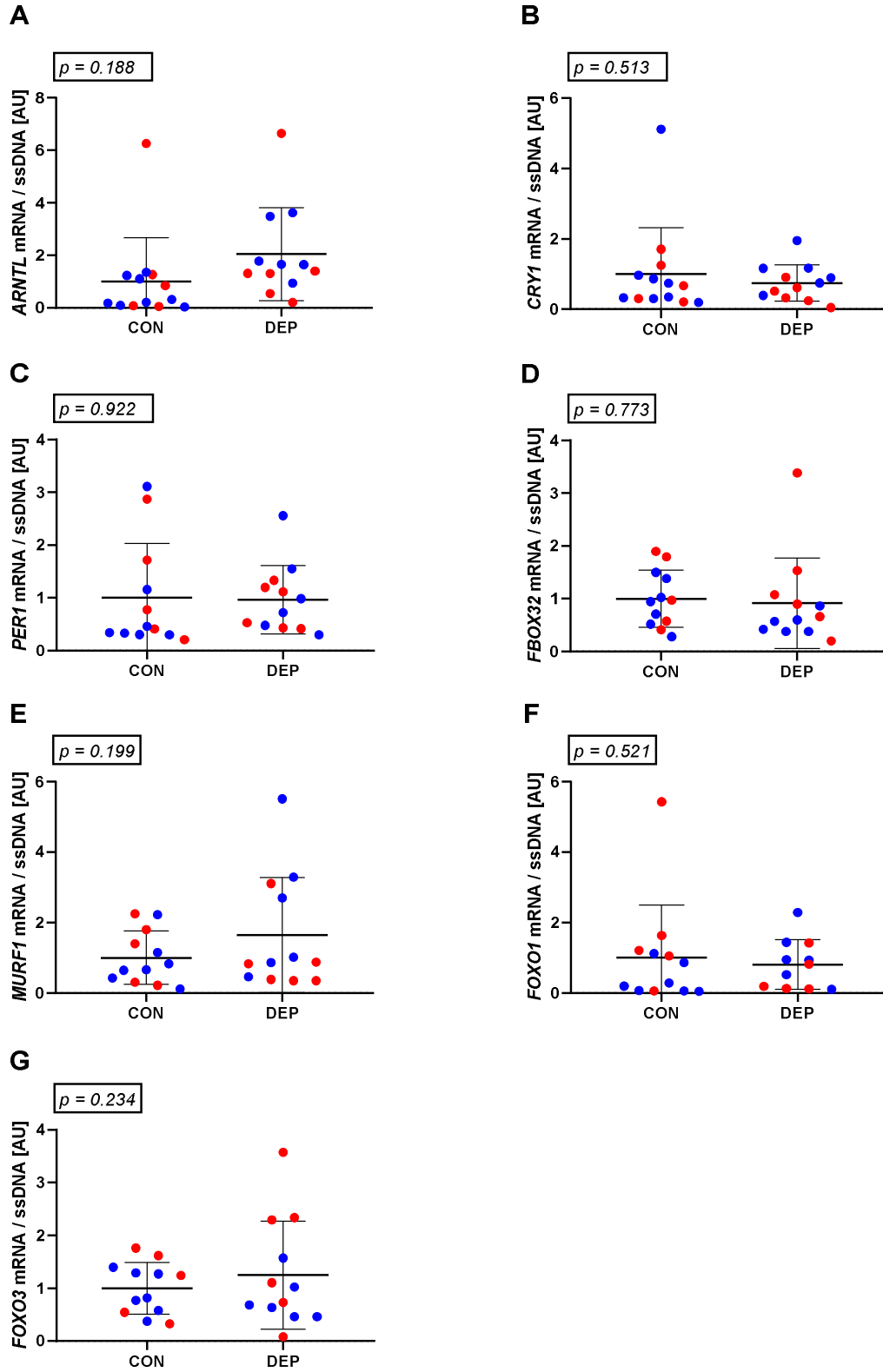
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321 **Figure 5.** Plasma IGF-1 concentrations in control (CON) and sleep-deprived (DEP)  
322 conditions. (A). Muscle mRNA levels of the IGF-1 isoforms IGF1-Ea (B) and IGF1-Eb (C)  
323 in muscle biopsies collected at 1300. Red dots depict male subjects. Blue dots depict female  
324 subjects. Insulin concentrations in control (CON) and sleep-deprived (DEP) conditions (D).  
325 All data are presented as mean  $\pm$  SD

326

### 327 Gene expression

328 The muscle expression levels of core clock genes *ARNTL*, *CRY1* and *PER1* or muscle protein  
329 degradation markers *FBOX-32*, *MURF1*, *FOXO1* and *FOXO3* were assessed in muscle  
330 biopsies collected in the post-prandial state and did not change in response to sleep  
331 deprivation (Figure 6A-6G).





333 **Figure 6.** Muscle mRNA levels of *ARNTL* (A), *CRY1* (B), *PER1* (C), atrogen (*FBOX32*) (D),  
334 *MURF1* (E), *FOXO1* (F) and *FOXO3* (G) in muscle biopsies collected at 1300. Red dots  
335 depict male subjects. Blue dots depict female subjects. All data are presented as mean  $\pm$  SD

336

337

## 338 **Discussion**

339 Chronic sleep loss is a potent catabolic stressor (10, 42) that increases the risk of metabolic  
340 dysfunction (39) and is associated to a loss of muscle mass and function at the population  
341 level (31, 38). In this study, we have demonstrated that a single night of sleep deprivation is  
342 sufficient to induce anabolic resistance, reducing post-prandial skeletal muscle protein  
343 synthesis rates by 18%. This decrease was accompanied by an acute, pro-catabolic increase in  
344 plasma cortisol and a sex-specific reduction in plasma testosterone. Our study is the first to  
345 demonstrate that acute sleep deprivation blunts muscle protein synthesis, a key regulator of  
346 skeletal muscle turnover. It adds to early results reporting a reduction in muscle protein  
347 synthesis in chronic sleep restriction conditions (42) and provides insights into the  
348 mechanisms underlying the suppression of anabolism following an acute or chronic lack of  
349 sleep.

### 350 *Acute sleep deprivation decreases muscle protein synthesis*

351 One night of sleep deprivation significantly reduced post-prandial skeletal muscle protein  
352 synthesis in a population of healthy young adults. In rodents, complete sleep deprivation is  
353 known to decrease muscle mass (13), muscle fibre cross-sectional area (15) and markers of  
354 the protein synthesis pathways. Only one study to date has investigated the effect of poor  
355 sleep on muscle protein synthesis in humans. Using a chronic sleep restriction model, Saner  
356 *et al.* recently reported that five consecutive nights of sleep restriction reduced muscle protein  
357 synthesis rates in healthy young males (42). Despite employing a different stable isotope  
358 (phenylalanine *versus* deuterated water), study design (acute cross-over design *versus* chronic  
359 parallel design) and population (males and females *versus* males only), our findings support  
360 those by Saner *et al.* Negative phenotypic outcomes associated with a period of chronic sleep  
361 deprivation likely reflect a metabolic shift towards catabolism due the accumulation of  
362 blunted anabolic responses to protein containing meals and physical activity. Our group has  
363 further discussed the results and implications of the Saner paper elsewhere (23).

364 A novel, exploratory outcome from our study highlights the potential for sex-specific  
365 responses to sleep deprivation. While the study was not powered to formally compare sexes,  
366 all of our male, but not female, participants experienced a numerical decrease in protein  
367 synthesis in the sleep-deprived *versus* control condition. Since we could not identify  
368 individual characteristics or behaviours consistent with the paradoxical increase in muscle

369 protein synthesis observed in some of our female participants, our data may reflect a broader,  
370 sex-specific physiological response and warrants more focused attention.

371 To balance experimental rigor with subject comfort and improve the potential clinical  
372 translation of our data during both sleep trials, participants consumed a standardized meal  
373 prior to the first muscle biopsy. Dietary protein is a potent activator of muscle protein  
374 synthesis, potentially explaining the slightly higher protein synthesis rates we observed,  
375 compared to typical data obtained from fasted participants (27). By studying participants in  
376 the post-prandial state, we were able to conclude that acute sleep deprivation induces  
377 anabolic resistance, decreasing the capacity of muscle to respond to the typical anabolic  
378 stimulation triggered by dietary protein intake. These results have potential, far-reaching  
379 implications for the musculo-skeletal and metabolic health of populations including  
380 shiftworkers, new parents, students and older adults, who are at increased risk of acute and/or  
381 chronic sleep loss. Future research and clinical interventions prioritizing nutrition and/or  
382 protein-synthesis stimulating exercise (42) is warranted as these may represent practical and  
383 effective means of protecting muscle mass and function in sleep-deprived populations.

#### 384 *Acute sleep deprivation promotes a less anabolic hormonal environment*

385 Testosterone AUC was reduced by 22% following one night of sleep deprivation.  
386 Testosterone is the major androgenic hormone, but is also present in females, albeit in  
387 concentrations that are 10-fold lower than typical male levels (48). In our study, a sex-  
388 specific pattern was visible, where male, but not female, testosterone levels were attenuated  
389 by sleep deprivation. There is limited evidence describing how complete sleep deprivation  
390 alter testosterone daytime secretion patterns. In males, plasma testosterone fluctuates during  
391 the day, with concentrations increasing during sleep and gradually decreasing during waking  
392 periods (3, 45), with marginal circadian effects (3). A minimum of three hours of normal  
393 sleep, including paradoxical sleep opportunities (30), is required to increase testosterone. In  
394 an earlier study in healthy young men, one night of acute sleep deprivation did not alter 24 h  
395 testosterone AUC; however, a pattern similar to ours could be observed across the day (14).  
396 Collectively, these data first suggest that one night of sleep deprivation is sufficient to elicit a  
397 reduction in daytime testosterone concentrations. This effect appears particularly pronounced  
398 or inherent to males, where testosterone is a potent regulator of muscle protein synthesis both  
399 on the short (5 days) (43) and longer term (4 weeks) (46). However, acute exposure to  
400 testosterone is not sufficient to alter post-absorptive muscle protein synthesis or degradation

401 rates over a 5-hour period (12). Whether transiently low testosterone levels can negatively  
402 impact muscle protein synthesis rates in the fasted and/or fed state is unknown and constitutes  
403 a challenge to validate experimentally. However, our results suggest that depressed  
404 testosterone secretion during the sleep deprivation period (30) is followed by another low  
405 testosterone secretion period during the daytime. Whether this phasic response contributes to  
406 anabolic resistance needs to be tested experimentally, but provides a possible mechanism for  
407 our observations.

408 *Acute sleep deprivation promotes a more catabolic hormonal environment but no difference*  
409 *in gene expression*

410 Consistent with previous studies conducted in males, a cortisol response upon awakening was  
411 not observed following one night of acute sleep deprivation (47). This blunted cortisol  
412 response was accompanied by a chronically higher cortisol secretion across the day (10, 14).  
413 While our results are in line with these observations, the participants' night-time calorie  
414 consumption needs to be acknowledged as a potential confounder. Previous studies have  
415 shown that over-night glucose infusion reduces cortisol levels (4). In our study, cortisol AUC  
416 was therefore calculated after the 1000 time point, after nutrient and energy intake was  
417 normalized. Further, post-hoc analyses revealed that, while the control group displayed a  
418 gradual, significant decrease in cortisol across the day, no differences were observed in the  
419 sleep deprived group at any time point, indicating a potential circadian misalignment.  
420 Cortisol has catabolic properties. In rats, corticosterone reduced muscle protein synthesis  
421 while increasing myofibrillar protein breakdown (21). In contrast, in humans, acute  
422 hypercortisolemia did not affect muscle fractional synthesis rates but blunted the net muscle  
423 protein balance (37), suggesting that cortisol preferentially increases muscle protein  
424 breakdown, rather than blunting muscle protein synthesis. Indeed, complete sleep deprivation  
425 can lead to a catabolic gene signature in human skeletal muscle (10), which might be  
426 reflective of an increase in muscle protein degradation. In our acute model, we however  
427 failed to observe any difference in the muscle expression levels of the proteolytic genes  
428 *FOXO1* and *FOXO3*, or in the expression levels of muscle specific atrogenes Atrogin-1  
429 (*FBXO32*) and *MURF1*. This may be explained by the fact that our muscle biopsy was  
430 collected at a later time point (0730 *versus* 1300 in our study), but also by the post-prandial  
431 state of our participants at the time of sample collection. Indeed, consumption of a mixed  
432 meal attenuates ubiquitin-mediated proteolysis when compared to fasted (8). Whilst it should  
433 be kept in mind that acute studies essentially report 'snapshots' of chronic processes, our

434 choice to use a post prandial model has the advantage of providing a better reflection of time  
435 periods where the anabolic flux is greater. Supporting an effect of poor sleep on muscle  
436 protein degradation, some authors also recently suggested that poor sleep-induced  
437 hypercortisolemia might play a role in the development of sarcopenia (38), with potential  
438 sex-specific effects (6); however, further research is required to establish cause-and-effect  
439 relationships.

440 In contrast to others (9), we did not observe a decrease in the muscle expression levels of the  
441 core clock genes following a night of total sleep deprivation. Since the timing of muscle  
442 sample collection was different, it can be hypothesised that the muscle circadian rhythm  
443 might have been able to realign over this time period. It should also be acknowledged that  
444 night time calorie consumption constitutes a potential confounder as food intake can act as a  
445 “Zeitgeber” for peripheral tissues in mammals (11), including skeletal muscle. However,  
446 differences in core clock gene expression might not be reflective of a physiologically  
447 significant change. This warrants the comparison of more functional readouts, such as protein  
448 expression levels, which was not possible in this study due to tissue availability.

#### 449 *Strengths and Limitations*

450 To improve compliance, comfort and retention, we were requested by our human ethics  
451 committee to allow participants to consume low-protein snacks (i.e. fruits and vegetable), and  
452 water *ad libitum* before normalizing calorie consumption at the 0700 time point, six hours  
453 before the first biopsy was collected. This strategy was effective in achieving similar plasma  
454 insulin levels across the two conditions at all time points. Using the home environment  
455 constituted a compromise as it avoids the need for habituation, which is a strength of this  
456 study, but rules out the ability to obtain overnight blood samples. Despite the presumed  
457 familiarity with their home sleep environment, our participants were mildly sleep-restricted in  
458 the week coming into both arms of the study, though total sleep times still fell within the  
459 stated sleep-wake time inclusion criterion. Sleep recorded on the pre-trial nights  
460 approximated their self-reported 7-h typical duration. Whether the mild-sleep restriction state  
461 had any impact on the results remains unclear. Future studies may consider the inclusion of  
462 laboratory-sleep trials and gold-standard polysomnographic sleep measures permitting  
463 analyses of sleep quality. While increasing participant burden, this would allow the  
464 characterisation of relationships between measures of sleep quality and physiological  
465 outcomes, including skeletal muscle protein synthesis and the hormonal environment.

466 Finally, in designing this clinical trial, we did not focus on sex-specific differences, nor did  
467 we power our study in order to detect such differences. Indeed, a wealth of literature indicate  
468 that muscle fractional synthesis rate, our primary outcome, is similar in males and females at  
469 rest and in response to anabolic stimulation (24, 49). However, the potential sex-based  
470 differences observed in this study prompt a dedicated investigation to better examine links  
471 between inadequate sleep and impaired skeletal muscle health in male and female cohorts.

472

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476

### 477 **Author contributions**

478 SL, GV and BA designed the study. SL, AM, OK, DC, SEA, AG collected the data. SL,  
479 EAL, OK, GV, SEA, DPJ processed and analysed the data. SL conducted statistical analyses  
480 and drafted the manuscript. SL and BA supervised the project. All authors commented on  
481 and edited the manuscript drafts.

482

### 483 **Additional Information**

484 The authors have no conflict of interest to declare.

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## 488 **References**

489

- 490 1. **Aisbett B, Condo D, Zacharewicz E, and Lamon S.** The Impact of Shiftwork on Skeletal  
491 Muscle Health. *Nutrients* 9, 2017.
- 492 2. **Ancoli-Israel S, Cole R, Alessi C, Chambers M, Moorcroft W, and Pollak CP.** The role of  
493 actigraphy in the study of sleep and circadian rhythms. *Sleep* 26: 342-392, 2003.
- 494 3. **Axelsson J, Ingre M, Akerstedt T, and Holmback U.** Effects of acutely displaced sleep on  
495 testosterone. *J Clin Endocrinol Metab* 90: 4530-4535, 2005.
- 496 4. **Benedict C, Kern W, Schmid SM, Schultes B, Born J, and Hallschmid M.** Early morning rise in  
497 hypothalamic-pituitary-adrenal activity: a role for maintaining the brain's energy balance.  
498 *Psychoneuroendocrinology* 34: 455-462, 2009.
- 499 5. **Bescos R, Boden MJ, Jackson ML, Trewin AJ, Marin EC, Levinger I, Garnham A, Hiam DS,  
500 Falcao-Tebas F, Conte F, Owens JA, Kennaway DJ, and McConell GK.** Four days of simulated shift  
501 work reduces insulin sensitivity in humans. *Acta physiologica (Oxford, England)* 223: e13039, 2018.
- 502 6. **Buchmann N, Spira D, Norman K, Demuth I, Eckardt R, and Steinhagen-Thiessen E.** Sleep,  
503 Muscle Mass and Muscle Function in Older People. *Dtsch Arztebl Int* 113: 253-260, 2016.
- 504 7. **Buxton OM, Pavlova M, Reid EW, Wang W, Simonson DC, and Adler GK.** Sleep restriction  
505 for 1 week reduces insulin sensitivity in healthy men. *Diabetes* 59: 2126-2133, 2010.
- 506 8. **Carbone JW, Margolis LM, McClung JP, Cao JJ, Murphy NE, Sauter ER, Combs GF, Jr., Young  
507 AJ, and Pasiakos SM.** Effects of energy deficit, dietary protein, and feeding on intracellular  
508 regulators of skeletal muscle proteolysis. *Faseb j* 27: 5104-5111, 2013.
- 509 9. **Cedernaes J, Osler ME, Voisin S, Broman JE, Vogel H, Dickson SL, Zierath JR, Schiøth HB,  
510 and Benedict C.** Acute Sleep Loss Induces Tissue-Specific Epigenetic and Transcriptional Alterations  
511 to Circadian Clock Genes in Men. *J Clin Endocrinol Metab* 100: E1255-1261, 2015.
- 512 10. **Cedernaes J, Schonke M, Westholm JO, Mi J, Chibalin A, Voisin S, Osler M, Vogel H,  
513 Hornaeus K, Dickson SL, Lind SB, Bergquist J, Schiøth HB, Zierath JR, and Benedict C.** Acute sleep  
514 loss results in tissue-specific alterations in genome-wide DNA methylation state and metabolic fuel  
515 utilization in humans. *Science advances* 4: eaar8590, 2018.
- 516 11. **Challet E.** The circadian regulation of food intake. *Nat Rev Endocrinol* 15: 393-405, 2019.
- 517 12. **Church DD, Pasiakos SM, Wolfe RR, and Ferrando AA.** Acute testosterone administration  
518 does not affect muscle anabolism. *Nutrition & metabolism* 16: 56, 2019.
- 519 13. **Dattilo M, Antunes HK, Medeiros A, Monico-Neto M, Souza Hde S, Lee KS, Tufik S, and de  
520 Mello MT.** Paradoxical sleep deprivation induces muscle atrophy. *Muscle Nerve* 45: 431-433, 2012.
- 521 14. **Dattilo M, Antunes HKM, Galbes NMN, Monico-Neto M, H DESS, Dos Santos Quaresma  
522 MVL, Lee KS, Ugrinowitsch C, Tufik S, and MT DEM.** Effects of Sleep Deprivation on Acute Skeletal  
523 Muscle Recovery after Exercise. *Med Sci Sports Exerc* 52: 507-514, 2020.
- 524 15. **de Sa Souza H, Antunes HK, Dattilo M, Lee KS, Monico-Neto M, de Campos Giampa SQ,  
525 Phillips SM, Tufik S, and de Mello MT.** Leucine supplementation is anti-atrophic during paradoxical  
526 sleep deprivation in rats. *Amino Acids* 48: 949-957, 2016.
- 527 16. **English KL, Mettler JA, Ellison JB, Mamerow MM, Arentson-Lantz E, Pattarini JM, Ploutz-  
528 Snyder R, Sheffield-Moore M, and Paddon-Jones D.** Leucine partially protects muscle mass and  
529 function during bed rest in middle-aged adults. *Am J Clin Nutr* 103: 465-473, 2016.
- 530 17. **Glynn EL, Fry CS, Timmerman KL, Drummond MJ, Volpi E, and Rasmussen BB.** Addition of  
531 carbohydrate or alanine to an essential amino acid mixture does not enhance human skeletal muscle  
532 protein anabolism. *The Journal of nutrition* 143: 307-314, 2013.
- 533 18. **Hamer HM, Wall BT, Kiskini A, de Lange A, Groen BB, Bakker JA, Gijsen AP, Verdijk LB, and  
534 van Loon LJ.** Carbohydrate co-ingestion with protein does not further augment post-prandial muscle  
535 protein accretion in older men. *Nutrition & metabolism* 10: 15, 2013.
- 536 19. **Harfmann BD, Schroder EA, and Esser KA.** Circadian rhythms, the molecular clock, and  
537 skeletal muscle. *Journal of biological rhythms* 30: 84-94, 2015.

- 538 20. **Horne JA and Ostberg O.** A self-assessment questionnaire to determine morningness-  
539 eveningness in human circadian rhythms. *Int J Chronobiol* 4: 97-110, 1976.
- 540 21. **Kayali AG, Young VR, and Goodman MN.** Sensitivity of myofibrillar proteins to  
541 glucocorticoid-induced muscle proteolysis. *Am J Physiol* 252: E621-626, 1987.
- 542 22. **Kecklund G and Axelsson J.** Health consequences of shift work and insufficient sleep. *BMJ*  
543 355: i5210, 2016.
- 544 23. **Knowles OE.** No time to sleep on it - start exercising! *J Physiol* 598: 2059-2060, 2020.
- 545 24. **Knowles OE, Aisbett B, Main LC, Drinkwater EJ, Orellana L, and Lamon S.** Resistance  
546 Training and Skeletal Muscle Protein Metabolism in Eumenorrheic Females: Implications for  
547 Researchers and Practitioners. *Sports Med*, 2019.
- 548 25. **Koopman R, Beelen M, Stellingwerff T, Pennings B, Saris WH, Kies AK, Kuipers H, and van  
549 Loon LJ.** Coingestion of carbohydrate with protein does not further augment postexercise muscle  
550 protein synthesis. *American journal of physiology Endocrinology and metabolism* 293: E833-842,  
551 2007.
- 552 26. **Kowall B, Lehnich AT, Strucksberg KH, Fuhrer D, Erbel R, Jankovic N, Moebus S, Jockel KH,  
553 and Stang A.** Associations among sleep disturbances, nocturnal sleep duration, daytime napping,  
554 and incident prediabetes and type 2 diabetes: the Heinz Nixdorf Recall Study. *Sleep Med* 21: 35-41,  
555 2016.
- 556 27. **Kumar V, Selby A, Rankin D, Patel R, Atherton P, Hildebrandt W, Williams J, Smith K,  
557 Seynnes O, Hiscock N, and Rennie MJ.** Age-related differences in the dose-response relationship of  
558 muscle protein synthesis to resistance exercise in young and old men. *J Physiol* 587: 211-217, 2009.
- 559 28. **Lamon S, Zacharewicz E, Arentson-Lantz E, Gatta PA, Ghobrial L, Gerlinger-Romero F,  
560 Garnham A, Paddon-Jones D, and Russell AP.** Erythropoietin Does Not Enhance Skeletal Muscle  
561 Protein Synthesis Following Exercise in Young and Older Adults. *Front Physiol* 7: 292, 2016.
- 562 29. **Leprout R and Van Cauter E.** Effect of 1 week of sleep restriction on testosterone levels in  
563 young healthy men. *JAMA* 305: 2173-2174, 2011.
- 564 30. **Luboshitzky R, Zabari Z, Shen-Orr Z, Herer P, and Lavie P.** Disruption of the nocturnal  
565 testosterone rhythm by sleep fragmentation in normal men. *J Clin Endocrinol Metab* 86: 1134-1139,  
566 2001.
- 567 31. **Lucassen EA, de Mutsert R, le Cessie S, Appelman-Dijkstra NM, Rosendaal FR, van Heemst  
568 D, den Heijer M, Biermasz NR, and group NEOs.** Poor sleep quality and later sleep timing are risk  
569 factors for osteopenia and sarcopenia in middle-aged men and women: The NEO study. *PLoS One* 12:  
570 e0176685, 2017.
- 571 32. **Lundby C, Nordsborg N, Kusuhara K, Kristensen KM, Neuffer PD, and Pilegaard H.** Gene  
572 expression in human skeletal muscle: alternative normalization method and effect of repeated  
573 biopsies. *Eur J Appl Physiol* 95: 351-360, 2005.
- 574 33. **Mamerow MM, Mettler JA, English KL, Casperson SL, Arentson-Lantz E, Sheffield-Moore  
575 M, Layman DK, and Paddon-Jones D.** Dietary protein distribution positively influences 24-h muscle  
576 protein synthesis in healthy adults. *The Journal of nutrition* 144: 876-880, 2014.
- 577 34. **Miller BF, Hansen M, Olesen JL, Flyvbjerg A, Schwarz P, Babraj JA, Smith K, Rennie MJ, and  
578 Kjaer M.** No effect of menstrual cycle on myofibrillar and connective tissue protein synthesis in  
579 contracting skeletal muscle. *American journal of physiology Endocrinology and metabolism* 290:  
580 E163-e168, 2006.
- 581 35. **Monico-Neto M, Antunes HK, Dattilo M, Medeiros A, Souza HS, Lee KS, de Melo CM, Tufik  
582 S, and de Mello MT.** Resistance exercise: a non-pharmacological strategy to minimize or reverse  
583 sleep deprivation-induced muscle atrophy. *Medical hypotheses* 80: 701-705, 2013.
- 584 36. **Paddon-Jones D, Sheffield-Moore M, Cree MG, Hewlings SJ, Aarsland A, Wolfe RR, and  
585 Ferrando AA.** Atrophy and impaired muscle protein synthesis during prolonged inactivity and stress.  
586 *J Clin Endocrinol Metab* 91: 4836-4841, 2006.



- 587 37. **Paddon-Jones D, Sheffield-Moore M, Creson DL, Sanford AP, Wolf SE, Wolfe RR, and**  
588 **Ferrando AA.** Hypercortisolemia alters muscle protein anabolism following ingestion of essential  
589 amino acids. *American journal of physiology Endocrinology and metabolism* 284: E946-953, 2003.
- 590 38. **Piovezan RD, Abucham J, Dos Santos RV, Mello MT, Tufik S, and Poyares D.** The impact of  
591 sleep on age-related sarcopenia: Possible connections and clinical implications. *Ageing Res Rev* 23:  
592 210-220, 2015.
- 593 39. **Reutrakul S and Van Cauter E.** Sleep influences on obesity, insulin resistance, and risk of  
594 type 2 diabetes. *Metabolism* 84: 56-66, 2018.
- 595 40. **Reynolds AC, Dorrian J, Liu PY, Van Dongen HP, Wittert GA, Harmer LJ, and Banks S.** Impact  
596 of five nights of sleep restriction on glucose metabolism, leptin and testosterone in young adult  
597 men. *PLoS One* 7: e41218, 2012.
- 598 41. **Russell AP.** Molecular regulation of skeletal muscle mass. *Clinical and experimental*  
599 *pharmacology & physiology* 37: 378-384, 2010.
- 600 42. **Saner NJ, Lee MJ, Pitchford NW, Kuang J, Roach GD, Garnham A, Stokes T, Phillips SM,**  
601 **Bishop DJ, and Bartlett JD.** The effect of sleep restriction, with or without high-intensity interval  
602 exercise, on myofibrillar protein synthesis in healthy young men. *J Physiol*, 2020.
- 603 43. **Sheffield-Moore M, Urban RJ, Wolf SE, Jiang J, Catlin DH, Herndon DN, Wolfe RR, and**  
604 **Ferrando AA.** Short-term oxandrolone administration stimulates net muscle protein synthesis in  
605 young men. *J Clin Endocrinol Metab* 84: 2705-2711, 1999.
- 606 44. **Staples AW, Burd NA, West DW, Currie KD, Atherton PJ, Moore DR, Rennie MJ, Macdonald**  
607 **MJ, Baker SK, and Phillips SM.** Carbohydrate does not augment exercise-induced protein accretion  
608 versus protein alone. *Med Sci Sports Exerc* 43: 1154-1161, 2011.
- 609 45. **Touitou Y, Motohashi Y, Reinberg A, Touitou C, Bourdeleau P, Bogdan A, and Auzéby A.**  
610 Effect of shift work on the night-time secretory patterns of melatonin, prolactin, cortisol and  
611 testosterone. *Eur J Appl Physiol Occup Physiol* 60: 288-292, 1990.
- 612 46. **Urban RJ, Bodenbun YH, Gilkison C, Foxworth J, Coggan AR, Wolfe RR, and Ferrando A.**  
613 Testosterone administration to elderly men increases skeletal muscle strength and protein synthesis.  
614 *Am J Physiol* 269: E820-826, 1995.
- 615 47. **Vargas I and Lopez-Duran N.** The cortisol awakening response after sleep deprivation: Is the  
616 cortisol awakening response a "response" to awakening or a circadian process? *Journal of health*  
617 *psychology*: 1359105317738323, 2017.
- 618 48. **Vingren JL, Kraemer WJ, Ratamess NA, Anderson JM, Volek JS, and Maresh CM.**  
619 Testosterone Physiology in Resistance Exercise and Training. *Sports Medicine* 40: 1037-1053, 2010.
- 620 49. **West DW, Burd NA, Churchward-Venne TA, Camera DM, Mitchell CJ, Baker SK, Hawley JA,**  
621 **Coffey VG, and Phillips SM.** Sex-based comparisons of myofibrillar protein synthesis after resistance  
622 exercise in the fed state. *J Appl Physiol (1985)* 112: 1805-1813, 2012.
- 623 50. **Wu Y, Zhai L, and Zhang D.** Sleep duration and obesity among adults: a meta-analysis of  
624 prospective studies. *Sleep Med* 15: 1456-1462, 2014.
- 625 51. **Zhao W, Pan J, Wang X, Wu Y, Bauman WA, and Cardozo CP.** Expression of the muscle  
626 atrophy factor muscle atrophy F-box is suppressed by testosterone. *Endocrinology* 149: 5449-5460,  
627 2008.

628

629