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

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Running title: Acute sleep deprivation and muscle protein metabolism

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Abstract

Sleep loss has profound, negative consequences on human tissues including skeletal muscle. Animal and human models suggest that acute and chronic sleep loss may directly impair muscle protein metabolism, which is critical to maintain muscle mass and function over the lifespan. This study aimed at providing a proof-of-concept that sleep deprivation acutely decreases muscle protein synthesis in humans. To this end, thirteen young males and females experienced one night of total sleep deprivation or slept normally at home. Anabolic and catabolic hormonal profiles, muscle fractional synthesis rate and markers of muscle protein degradation were assessed across the following day. In the sleep-deprived condition, cortisol release was 21% higher over a six-hour period ($p=0.030$). One night of complete sleep deprivation was however not sufficient to elicit significant changes in testosterone release ($p=0.090$) and muscle protein synthesis rates ($p=0.081$). Apparent sex-specific differences prompt the need for further dedicated investigations into the specific effects of total sleep deprivation on muscle protein metabolism in males and females.

Introduction

Acute and chronic sleep loss is linked with a range of negative physiological and psychological outcomes (22). While complete sleep deprivation rapidly impedes simple and complex cognitive functions, chronic sleep restriction impairs whole-body homeostasis, leading to undesirable physiological consequences in the short- and longer-term (34). Most metabolic tissues including liver, adipose tissue and skeletal muscle are at risk of developing sleep-associated adverse outcomes.

Skeletal muscle is a primary regulator of human metabolism. It acts as a major storage site for energy substrates and accounts for a large proportion of whole-body energy expenditure at rest and during exercise (19). By disrupting the body's circadian rhythm, acute and chronic sleep loss have the potential to profoundly affect muscle health. In the muscle, sleep deprivation (8) and restriction (18) significantly alter the regulation of numerous genes fluctuating in a near-daily manner and their associated phenotype. Sleep loss also affects substrate metabolism in the muscle (9). Even relatively short periods of sleep restriction (less than a week) can compromise glucose metabolism, reduce insulin sensitivity and impair muscle function (5, 7).

Skeletal muscle is made up of 80% of proteins and maintaining optimal muscle protein metabolism is critical for muscle health. Situations where muscle protein synthesis rates chronically lags protein degradation rates can result in an accelerated loss of muscle mass. Low muscle mass is both a hallmark and a precursor to a range of chronic health conditions including neuromuscular diseases, sarcopenia and frailty, obesity and type II diabetes (36). Population-based studies report that the risk of developing these conditions is 15-30% higher in individuals who regularly experience sleep deprivation, sleep restriction, and inverted sleep-wake cycles (25, 29, 45). To this end, a growing body of evidence suggests that a lack of sleep may directly affect muscle protein metabolism (1, 30, 37).

Rodent studies first demonstrated a possible causal link between complete sleep deprivation and disrupted muscle protein metabolism. Rats subjected to 96 h of sleep deprivation (specifically paradoxical sleep deprivation, i.e., where rapid eye movement sleep is restricted) experienced a decrease in muscle mass (12) and muscle fibre cross-sectional area (14). In this model, sleep deprivation attenuated markers of the protein synthesis pathways and increased muscle proteolytic activity (14). These effects were partially restored following a 96-h recovery period. These findings were paralleled by a human study reporting a catabolic gene signature in skeletal muscle following one night of total sleep deprivation (9). Such effects might even

be cumulative in a chronic setting. Indeed, a new human study demonstrated that five consecutive nights of sleep restriction (four hours per night) induced a decrease in myofibrillar protein synthesis when compared to normal sleep (37). The possible mechanisms underlying these effects are currently unknown but might involve the hormonal environment.

Factors that regulate skeletal muscle protein metabolism at the molecular level are influenced by mechanical (muscle contraction), nutritional (dietary protein intake) and hormonal inputs (36). Testosterone and IGF-1 positively regulate muscle protein anabolism by promoting muscle protein synthesis (38, 41), while repressing the genes that activate muscle protein degradation (46). In contrast, cortisol drives catabolism by activating key muscle protein degradation pathways (21). Experimental evidence suggests that acute and chronic sleep loss alters anabolic (27, 35) and catabolic (9, 13) hormone secretion patterns in humans, providing a possible mechanism for impaired muscle protein metabolism. However, human studies looking into the mechanisms that would establish a cause-and-effect relationship between acute and chronic sleep loss and clinically observable changes in body composition, muscle function and metabolism are lacking. This proof-of-concept study sought to determine if one night of complete sleep deprivation promotes a catabolic hormonal environment and compromises muscle protein synthesis and markers of muscle degradation in young, healthy male and female participants.

Methods

Participants

Thirteen young (18-35 years old), healthy male and female students gave their informed consent to participate in this randomized, crossover-designed study. Participants were excluded if they had a history of recent transmeridian travel (i.e., no travel across multiple time zones in the previous four weeks), shiftwork (i.e., no involvement in shiftwork over the previous three months), frequent napping (i.e., ≥ 2 naps per week), or had a diagnosed sleep disorder. Participants were required to have habitual bed (2200–0000) and wake (0600–0800) times that were broadly consistent with the experimental protocol and to self-report obtaining a minimum of 7 hours of sleep (not time in bed) per night. Chronotype was assessed using the morningness-eveningness (ME) adapted from (20). Participants exhibiting extreme morningness (score > 70) or eveningness (score < 30) were excluded. All participants but three displayed an 'intermediate' ME type. Effects of female reproductive hormone fluctuations were minimised by testing female participants during the same phase of the menstrual cycle, and avoiding the follicular phase. A detailed account of the strategy for female volunteer recruitment and testing have been comprehensively described elsewhere (23). The study was approved by the Deakin University Human Research Ethics Committee (2016-028) and conducted in accordance to *The Declaration of Helsinki* (1964) and its later amendments. Participants' physiological characteristics, ME score, and self-reported habitual time asleep are summarized in Table 1. No sex-specific difference was reported in ME score ($p=0.148$) and self-reported habitual time asleep ($p=0.401$).

Table 1. Participants' characteristics

Sex	Age	Mass (kg)	Height (cm)	BMI ($kg \cdot m^{-2}$)	ME score	Habitual time asleep (self-reported) (hrs)
Male (N=7)	22 ± 1.8	71.6 ± 11.3	173.5 ± 9.0	22.6 ± 4.1	48.0 ± 6.4	7.5 ± 0.63
Female (N=6)	20 ± 1.3	60.1 ± 10.3	170.5 ± 5.1	20.7 ± 3.2	53.8 ± 6.6	7.8 ± 0.68

Mean ± SD; BMI = Body Mass Index; ME = Morningness Eveningness score

Sample size calculation

At the time this study was designed, there was no published study investigating the effect of sleep deprivation on muscle protein synthesis. Using data obtained for previous work conducted by our group investigating the effects of a metabolic stimulus (immobilization or acute exercise) on changes in muscle protein synthesis rates in humans (26, 31), power analyses conducted on the expected primary outcome (fractional synthesis rate) indicated that a sample size of 13 is sufficient to minimize the risk of type II error (β=0.2,α=0.05). Males and females were included as previous work demonstrated that muscle fractional synthesis rate, our primary outcome, is similar in both sexes (23, 44).

Pre-study procedure

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

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

Study procedure

On the night of the sleep deprivation trial, the participants received their standardized meal and then reported to the laboratory at 2100 where they were limited to sedentary activities (i.e., reading a book, watching a movie). Participants were constantly observed by research personnel and monitored by actigraphy (Actical MiniMitter/Respironics, Bend, OR) to ensure they did not fall asleep. They remained in a sound attenuated, light (211±14 lux) and temperature (21±2°C) controlled facility for the entire protocol. To comply with ethical

requirements, participants were permitted to consume low-protein snacks (i.e., crude fruits and vegetable) and water *ad libitum* during the sleep deprivation period. Regardless of potential differences in insulinemia, adding a non-pharmacological dose of carbohydrates to a protein synthesis activating dose of proteins (15-30 g) has no additive effect on fractional synthesis rate (16, 17, 24, 39), our primary outcome. The same protocol was repeated for the control trial. Participants consumed their standardized meal and were permitted to sleep from 2200 to 0700 at home. Spending the night of the control condition at home prevented the need to habituate participants to another sleeping environment.

The following day, a researcher and a nurse drove the participants' home. Access to the home has been pre-organised. At 0700, they woke up the participant and immediately collected a venous blood sample before driving them to the laboratory. The participants sleeping at home had not undergone any physical activity and had not been exposed to light. Upon arrival at the laboratory, participants then consumed a standardized breakfast containing approximately 9% fat, 11% proteins and 80% carbohydrates, and a total of 20.3 ± 1.8 g of proteins. At 0800, an 18-gauge cannulae was inserted into the antecubital vein of each arm and blood was sampled every hour for the duration of the protocol. In the other arm, another cannulae was inserted for the continuous infusion of [ring-$^{13}C_6$]-L-phenylalanine (0.34 mg·kg^{-1} bolus followed by a constant infusion rate of 0.0085 mg·kg^{-1}·min^{-1}) (Cambridge Isotope Laboratories, Tewksbury, MA) from 1000 to the end of the protocol. At 1200, participants consumed a standardized lunch containing 12% fat, 21% protein and 67% carbohydrate, and a total of 20.6 ± 0.3 g protein. Skeletal muscle samples were obtained at 1300 and 1500 under local anaesthesia (1% Lidocaine) at separate locations from the belly of the *vastus lateralis* muscle using a percutaneous needle biopsy technique as previously described by our group (26). Muscle samples were immediately frozen in liquid nitrogen and used for the measurement of isotopic enrichment and gene expression analysis. An outline of the experimental protocol is presented in Figure 1.

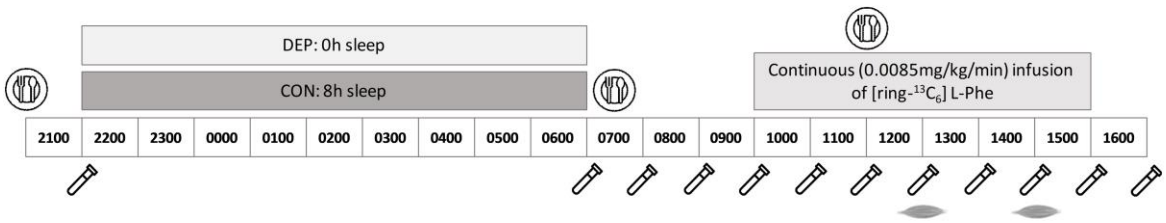

Figure 1. Experimental Protocol. : blood collection; : muscle collection

Sleep measures

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

Hormone measures

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

Isotopic enrichment in plasma

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

Isotopic enrichment in muscle proteins

A 30 mg piece of muscle was used for isolation of mixed muscle bound and intracellular protein fractions. Briefly, bound muscle proteins were extracted in perchloric acid and hydrolysed using 6N hydrochloric acid (110°C for 24 h). Isotopic enrichments of [ring-$_{13}C^6$]-L-phenylalanine in tissue fluid (intracellular fraction) were used as a precursor pool for the calculation of the fractional synthesis rate. Total muscle phenylalanine was isolated using cation exchange chromatography (50W-8X, 200–400 mesh H+ form; Bio-Rad Laboratories). Amino acids were eluted in 8 mL of 2N ammonium hydroxide and dried under vacuum. Muscle intracellular and bound protein [ring-$_{13}C^6$]-L-phenylalanine enrichments were determined by GC-MS with electron impact ionization using the tert-butyldimethylsilyl derivative. Ions 238 and 240 were monitored for bound protein enrichments; ions 336 and 342 were monitored for intracellular enrichments as described previously (15). Mixed muscle protein FSR (% / hour) was calculated by measuring the direct incorporation of [ring-$_{13}C^6$]-L-phenylalanine by using the precursor-product model (31):

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

where EP1 and EP2 are the bound enrichments of [ring-$_{13}C^6$]-L-phenylalanine for the 2 muscle biopsies, Em is the mean enrichment of [ring-$_{13}C^6$]-L-phenylalanine in the muscle intracellular pool, and t is the time interval (min) between biopsies.

RNA extraction and gene expression analysis

Muscle biopsies collected at 1300 were used for gene expression analysis. RNA was extracted from ~15 mg of skeletal muscle samples using Tri-Reagent© Solution (Ambion Inc., Austin, TX, USA) according to the manufacturer's protocol. RNA was treated with DNase I Amplification Grade (Thermo Fisher Scientific, MA) and RNA concentration was assessed using the Nanodrop 1000 Spectrophotometer (Thermo Fisher Scientific). First-strand cDNA was generated from 1000 ng RNA using the High Capacity RT-kit (Applied Biosystems, Carlsbad, CA, USA). cDNA was then treated with RNase H (Thermo Fisher Scientific) according to the manufacturer protocol. Real-time PCR was carried out using an AriaMx real-time PCR system (Agilent Technologies, Santa Clara, CA) to measure mRNA levels. mRNA levels for ARNTL (BMAL1), CRY1, PER1, IGF-1Ea, IGF-1Eb, FBX032 (atrogin-1), TRIM63

(MuRF-1), FOXO1 and FOXO3 were measured using 1 × SYBR© Green PCR MasterMix (Applied Biosystems) and 5 ng of cDNA. All primers were used at a final concentration of 300 nM. Primer details are provided in Table 2. Single-strand DNA was quantified using the Quant it OliGreen ssDNAAssay Kit (Thermo Fisher Scientific) according to the manufacturer's instruction and used for PCR normalization. No differences in ssDNA concentrations were found between groups (data can be found at https://doi.org/10.6084/m9.figshare.12629972.v1).

Table 2. Primer sequences

Gene	Accession number	Forward	Reverse
ARNTL (BMAL1)	NM_001030272.2	GGCAGCTCCACTGACTACCA	CCCGACGCCGCTTTTCAATC
CRY1	NM_004075.5	CCGTTCCCGGTCCTTTC C	CTAAAGACAAAACGGCCCGC
PER1	NM_002616.3	GAGGACACTCCTGCGACCAG	GCCATGGGGAGAACAGAACA
IGF-1Ea	NM_001111283.3 and NM_001111284.2	GACATGCCCAAGACCCAGAAGGA	CGGTGGCATGTCACTCTTCACTC
IGF-1Eb	NM_001111285.3	GCCCCCATCTACCAACAAGAACAC	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	CGACGAACATTTCCTCGGCT

Statistical analysis

Statistical analyses were conducted using SPSS 26.0 (IBM Corp, Armonk, NY). Diagnostic plots of residuals and fitted values were checked to ensure homogeneity of the variance. Hormonal levels were analysed using a two-way analysis of variance (ANOVA) with within-participant factors for time and condition (CON vs DEP) unless specified otherwise. The Sidak test was used to compare pairs of means when a main effect was identified. Paired t-tests were used to compare group means where there were single variables. Area under the curve (AUC) was computed on hormone values using the trapezoidal method. The significance levels for the F-tests in the t-tests and ANOVA and the Sidak tests were set at $p<0.05$. All data are reported as mean ± SD.

Results

Sleep

In the week prior to the study, there was no difference in total sleep time (CON: 5.9 ± 0.5 h, DEP: 6.1 ± 1.4 h, $p = 0.718$) or sleep efficiency (CON: 78.5 ± 6.5 %, DEP: 79.4 ± 4.7 %, $p = 0.801$) between conditions. In the night directly preceding trial participation, there was no difference in total sleep time (CON: 6.8 ± 0.8 h, DEP: 7.4 ± 0.7 h, $p = 0.195$) or sleep efficiency (CON: 77.3 ± 6.3 %, DEP: 81.0 ± 8.6 %, $p = 0.424$).

Muscle protein synthesis

Subjects remained in isotopic steady state during the duration of the isotope infusion, with no differences in plasma enrichment between the CON and DEP condition (Figure 2A). The average fractional synthesis rate (FSR) was 0.072%·h^{-1} at rest and 0.059%·h^{-1} following sleep deprivation. Despite this numerical difference, this effect did not reach statistical significance ($p=0.081$). All male subjects had their FSR decreasing in the DEP condition when compared to the CON condition (Figure 2B).

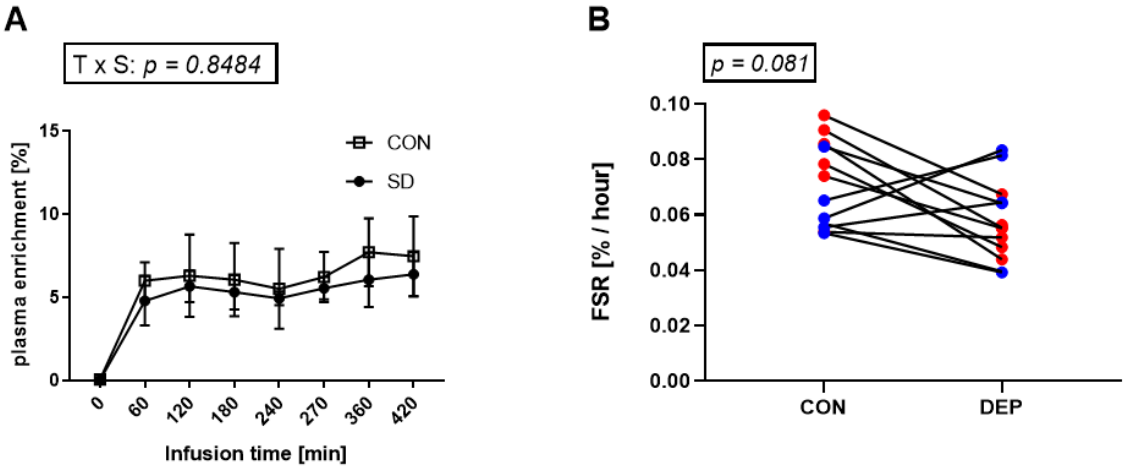

Figure 2. Plasma enrichment of [ring-$^{13}C_6$]-L-phenylalanine during the experimental protocol (N=4) (A). Mixed muscle fractional synthesis rate measured in the control (CON) and sleep-deprived (DEP) conditions at rest in the fed state. Data are presented as mean ± SD (A). Red dots depict male subjects. Blue dots depict female subjects. (B).

Plasma cortisol levels

A significant interaction effect of sleep × time (*p=4.38E-5*) was observed for plasma cortisol levels. Consistent with the typical increase in cortisol observed during the later stages of sleep (42), plasma cortisol levels were significantly higher (*p=0.014*) in the CON condition than in the DEP condition at 0700 (wake time for the control condition) (Figure 3A). At 1000, plasma cortisol was similar in both sleep conditions (*p=0.940)*, but by 1600, cortisol was significantly lower in the CON condition (*p=0.048)* (Figure 3A). When the area under the curve was calculated from 1000, the cortisol levels 21% higher during the DEP than during the CON condition over this 6-h period (*p=0.023)* (Figure 3B).

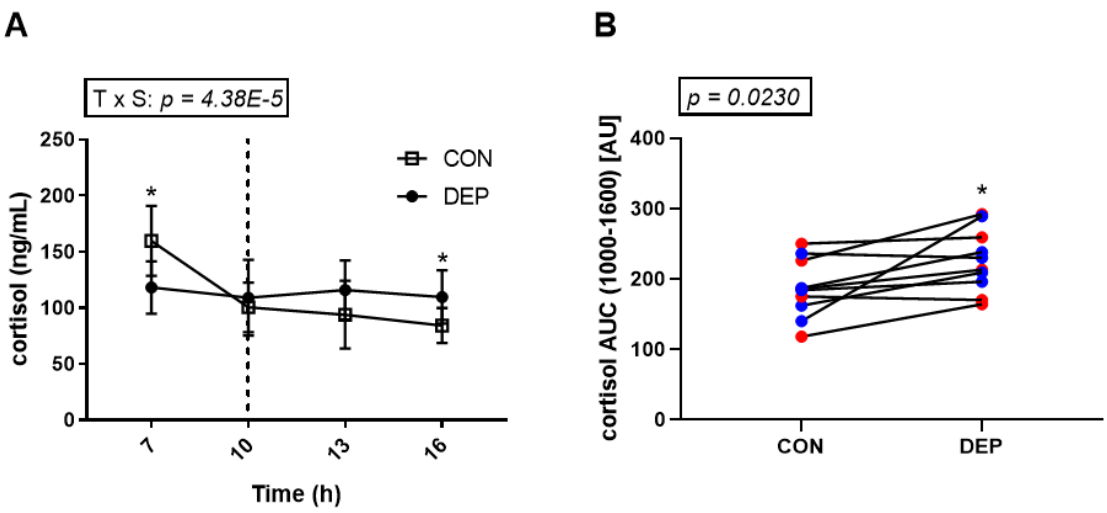

Figure 3. Plasma cortisol concentrations in control (CON) and sleep-deprived (DEP) conditions. *; significantly different from the CON condition, *p<0.05*. Data are presented as mean ± SD (A). Area under the curve calculated for plasma cortisol concentrations from 1000 (dashed line, A) until the end of the protocol. *; significantly different from the CON condition, *p<0.05*. Red dots depict male subjects. Blue dots depict female subjects (B).

Insulin and IGF-1 levels

Plasma insulin concentrations varied across the day, but there was no effect of sleep or the combination of sleep and time (Supplementary figure 1; https://doi.org/10.6084/m9.figshare.12630095.v3). Plasma IGF-1 concentrations did not vary with time, sleep, or the combination of both factors (Figure 4B). In line with these results, one

night of total sleep deprivation did not influence the muscle expression levels of IGF1 mRNA isoforms IGF1-Ea and IGF1-Eb when measured at 1300 (Figure 4B and 4C).

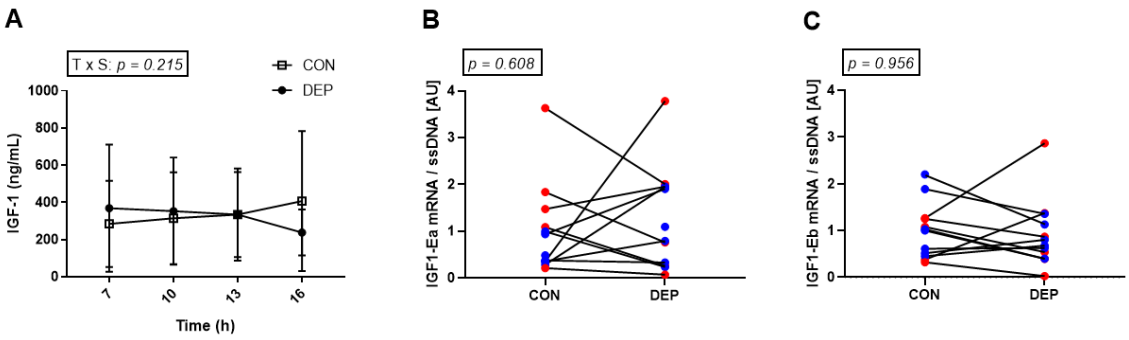

Figure 4. Plasma IGF-1 concentrations in control (CON) and sleep-deprived (DEP) conditions. Data are presented as mean ± SD (A). Muscle mRNA levels of the IGF-1 isoforms IGF1-Ea (B) and IGF1-Eb (C). Red dots depict male subjects. Blue dots depict female subjects.

Plasma testosterone levels

There was a main effect of time (*p=0.002*) but the interaction effect of sleep × time for plasma testosterone levels did not reach statistical significance (*p=0.063*; Figure 5A). The area under the curve visually highlighted the existence of a male and a female sub-population group but the overall difference between the control and sleep-deprived condition did not reach statistical significance (*p=0.090*). All male subjects had their testosterone AUC decreasing in the DEP condition (Figure 5B).

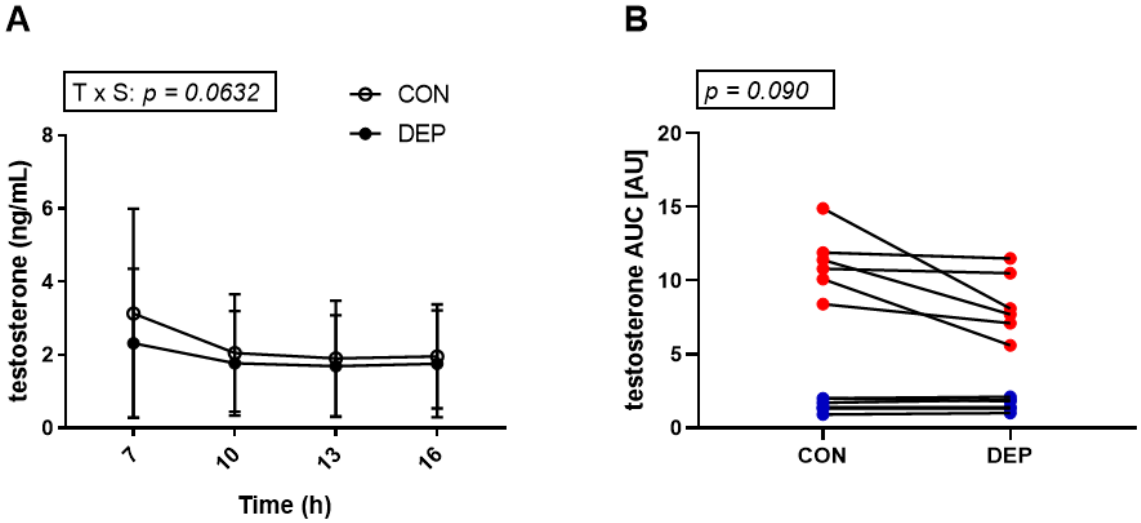

Figure 5. Plasma testosterone concentrations in control (CON) and sleep-deprived (DEP) conditions. Data are presented as mean ± SD (A). Area under the curve calculated for plasma cortisol concentrations. Red dots depict male subjects. Blue dots depict female subjects (B).

Gene expression

The muscle expression levels of the core clock genes *ARNTL*, *CRY1* and *PER1* (Supplementary Fig 2; https://doi.org/10.6084/m9.figshare.12629912.v2) or of the muscle protein degradation markers *FBOX-32*, *MURF1*, *FOXO1* and *FOXO3* (Supplementary Fig 3; https://doi.org/10.6084/m9.figshare.12629933.v2) did not change in response to one night of total sleep deprivation.

Discussion

The primary aim of this study was to determine whether a 30-h complete sleep deprivation period would impair muscle protein synthesis in young, healthy males and females. In this population, total sleep deprivation was not sufficient to significantly impair muscle protein synthesis rates, despite numerical decreases. Sleep deprivation elicited a higher cortisol AUC but no significant change in testosterone AUC. This lack of statistical significance may be due to sex-specific differences that will be discussed below.

In contrast to our hypothesis, one night of complete sleep deprivation was not sufficient to elicit a significant decrease in muscle protein synthesis, even if our results were close to statistical significance. Interestingly, all males displayed a numerical decrease in protein synthesis rate in the sleep-deprived when compared to the control condition. In rodents, complete sleep deprivation caused a decrease in muscle mass (12), muscle fibre cross-sectional area (14) and markers of the protein synthesis pathways and an increase in proteolytic activity (14). In a human model similar to ours, complete sleep deprivation lead to a a catabolic gene signature in skeletal muscle (9), which might be reflective of an increase in muscle protein degradation. It is possible that, in humans, several nights of sleep restriction are needed to observe a chronic effect on muscle protein synthesis (37). Possibly supporting a more important role for muscle protein degradation, some authors recently suggested that poor sleep-induced hypercortisolemia might play a role in the development of sarcopenia (33), with potential sex-specific effects (6); however, further research is required to establish cause-and-effect relationships.

Consistent with previous studies (42), the cortisol response upon awakening was supressed following one night of acute sleep deprivation in both males and females. As previously shown in males (9, 13), this blunted cortisol response was accompanied by a chronically higher cortisol release across the day in the sleep-deprived cohort. While our results are in line with observations from other sleep deprivation studies, the participants' night-time calorie consumption needs to be acknowledged as a potential confounder. Previous studies have shown that glucose infusion reduces cortisol levels during night-time (4), and it is possible that limited carbohydrate consumption might have further decreased cortisol concentration at the 0700 time point. Differences in AUC were however calculated after the 1000 time point, that is after nutrient and energy intake was normalized and at the start of the tracer infusion period. Cortisol has catabolic properties. In rats, corticosterone reduced muscle protein synthesis while

increasing myofibrillar protein breakdown (21). In contrast, acute hypercortisolemia in humans did not affect muscle fractional synthesis rates but blunted the net muscle protein balance (32), suggesting that, in humans, cortisol makes a greater contribution to increased muscle protein breakdown than to decreased muscle protein synthesis. While others have reported a catabolic signature in skeletal muscle following a night of total sleep deprivation (8), we did not observe any difference in the muscle expression levels of the proteolytic genes *FOXO1* and *FOXO3*, or in the expression levels of muscle specific atrogenes Atrogin-1 (*FBXO32*) and *MURF1*. Our results suggest that, if sleep deprivation induces protein breakdown, it might not be facilitated by activation of the muscle-specific E3 ubiquitin ligases, but instead originate from other proteolytic pathways potentially including lysosome dependant mechanisms.

Testosterone, the major androgenic hormone, activates muscle protein synthesis via the non-DNA binding-dependent activation of the Akt/mTOR pathway; the latter being the central regulator of protein synthesis in the muscle (36). Testosterone is also present in females, albeit in concentrations that are 10-fold lower than typical male levels (43). We hypothesized that testosterone AUC would be reduced following one night of sleep deprivation. While our results did not reach statistical significance, a visible sex-specific pattern, where male but not female testosterone levels were attenuated, may be influential. Limited evidence describes how complete sleep deprivation may alter testosterone daytime secretion patterns in males. Plasma testosterone fluctuate during the day, where concentrations rise during sleep periods and gradually decrease during wake periods (3, 40), with marginal circadian effects (3). A minimum of three hours of normal sleep including paradoxical sleep opportunities (28) is required to observe such increase. In healthy young men, one night of acute sleep deprivation did not significantly decrease testosterone AUC when calculated over a 24-hour period; however, a pattern similar to ours could be observed across the day (13). While further evidence about complete sleep deprivation is lacking, sustained sleep restriction (five hours of sleep per night during one week) lowered testosterone levels during waking hours (27) and five nights of sleep restriction (four hours of sleep per night) led to a trend towards reduced total daytime testosterone (35). Our study supports these findings and suggests that, in males, one night of sleep deprivation may be sufficient to elicit a reduction in daytime testosterone concentrations. Testosterone is a potent regulator of muscle protein synthesis in males, both on the short (5 days) (38) and longer term (4 weeks) (41). However, acute exposure to testosterone is not sufficient to influence muscle protein synthesis or degradation rates over a 5-hour period (11). In addition, high levels of testosterone are not needed to induce muscle protein synthesis in

females, which typically display similar protein synthesis rates as their male counterparts (44). Whether transiently low testosterone levels can negatively impact muscle protein synthesis rates is unknown and constitutes a challenge to validate experimentally. However, our results suggest that depressed testosterone secretion during the sleep deprivation period (28) is followed by another low testosterone secretion period during the daytime. Whether this accumulation may promote a catabolic environement that may affect muscle protein synthesis remains to tested experimentally.

In contrast to others (8), we did not observe a decrease in the muscle expression levels of the core clock genes following a night of total sleep deprivation. Since the timing of muscle sample collection was different (0730 *versus* 1300 in our study), it can be hypothesised that the muscle circadian rhythm might have been able to realign over this time period. It should also be acknowledged that night time calorie consumption constitutes a potential confounder as food intake can act as a "Zeitgeber" for peripheral tissues in mammals (10), including skeletal muscle. However, differences in core clock gene expression might not be reflective of a physiologically significant change. This warrant the comparison of more functional readouts, such as protein expression levels, which was not possible in this study due to tissue availability.

Ethical limitations of this study included the decision to not completely fast participants during the night of total sleep deprivation. Instead, to improve compliance, comfort and retention, we were requested by our human ethics committee to allow participants to consume low-protein snacks (i.e. crude fruits and vegetable), and water *ad libitum* before normalizing calorie consumption at the 0700 time point, that is six hours before the first biopsy was collected. This strategy was effective in achieving similar plasma insulin levels across the two conditions at all time points. Using the home environment constituted a compromise as it avoids the need for habituation, which is strength of this study, but rules out the ability to obtain overnight blood samples. Despite the presumed familiarity with their home sleep environment, our participants were mildly sleep-restricted in the week coming into both arms of the study, though total sleep times still fell within the stated sleep-wake time inclusion criterion. Sleep recorded on the pre-trial nights approximated their self-reported 7-h typical duration. Whether the mild-sleep restriction state had any impact on the results remains unclear. Future studies may consider the inclusion of laboratory-sleep trials and gold-standard polysnographic sleep measures permitting analyses of sleep quality. While increasing participant burden, this would allow the characterisation of relationships between measures of sleep quality and physiological outcomes, including skeletal muscle protein synthesis and the hormonal environment.

Finally, in designing this clinical trial, we did not focus on sex-specific differences, nor did we power our study in order to detect such differences. Indeed, a wealth of literature indicate that muscle fractional synthesis rate, our primary outcome, is similar in males and females at rest and in response to anabolic stimulation (23, 44). The apparent sex-based differences observed in our result prompt a dedicated investigation to establish the causal links between inadequate sleep and impaired skeletal muscle health in male and female cohorts.

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Author contributions

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

Additional Information

The authors have no conflict of interest to declare.

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