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RESEARCH ARTICLE

Acute sleep loss alters circulating fibroblast growth factor 21 levels in humans: A randomised crossover trial

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Summary

The hormone fibroblast growth factor 21 (FGF21) modulates tissue metabolism and circulates at higher levels in metabolic conditions associated with chronic sleep–wake disruption, such as type 2 diabetes and obesity. In the present study, we investigated whether acute sleep loss impacts circulating levels of FGF21 and tissue-specific production, and response pathways linked to FGF21. A total of 15 healthy normal-weight young men participated in a randomised crossover study with two conditions, sleep loss versus an 8.5-hr sleep window. The evening before each intervention, fasting blood was collected. Fasting, post-intervention morning skeletal muscle and adipose tissue samples underwent quantitative polymerase chain reaction and DNA methylation analyses, and serum FGF21 levels were measured before and after an oral glucose tolerance test. Serum levels of FGF21 were higher after sleep loss compared with sleep, both under fasting conditions and following glucose intake (~27%–30%, $p = 0.023$). Fasting circulating levels of fibroblast activation protein, a protein which can degrade circulating FGF21, were not altered by sleep loss, whereas DNA methylation in the *FGF21* promoter region increased only in adipose tissue. However, even

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though specifically the muscle exhibited transcriptional changes indicating adverse alterations to redox and metabolic homeostasis, no tissue-based changes were observed in expression of *FGF21*, its receptors, or selected signalling targets, in response to sleep loss. In summary, we found that acute sleep loss resulted in increased circulating levels of FGF21 in healthy young men, which may occur independent of a tissue-based stress response in metabolic peripheral tissues. Further studies may decipher whether changes in FGF21 signalling after sleep loss modulate metabolic outcomes associated with sleep or circadian disruption.

KEYWORDS
adipose tissue, circadian misalignment, DNA methylation, insulin resistance, skeletal muscle, tissue-specific

1 | INTRODUCTION

The hormone fibroblast growth factor 21 (FGF21) exerts pleiotropic regulation of metabolic homeostasis, including in fasting, browning, and insulin sensitivity, in what has been tied to reciprocal regulation by corticosteroids (Fisher et al., 2012; Fisher & Maratos-Flier, 2016; Liang et al., 2014; Patel et al., 2015; Potthoff et al., 2009). Circulating levels of FGF21 are elevated in obesity and type-2 diabetes (Chavez et al., 2009; Zhang et al., 2008), conditions that are also tied to sleep and circadian disruption. Whole-body overexpression of FGF21 causes resistance to diet-induced obesity in mice (Kharitonenkov et al., 2005), and although the doses required for physiological responses are typically quite high, short-term clinical trials with long-acting FGF21 analogues have demonstrated positive metabolic effects in humans (Angelin, Larsson, & Rudling, 2012; Kliewer & Mangelsdorf, 2019). Elevated blood levels of FGF21 in metabolic disease may thus represent a compensatory increase or resistance to the effects of this hormone (Fisher & Maratos-Flier, 2016).

Animal models have revealed that FGF21 exhibits metabolic effects that are highly dependent on the target tissue. These effects include regulation of insulin sensitivity in adipose tissue, and modulation of brown adipose tissue-mediated thermogenesis, which may also be of metabolic relevance in humans (Kharitonenkov et al., 2005; P. Lee et al., 2014). FGF21 signalling also feeds back centrally by engaging hypothalamic pathways that modulate the function and behavioural circadian output of the central pacemaker, the suprachiasmatic nucleus (Bookout et al., 2013; Fisher et al., 2012; Fisher & Maratos-Flier, 2016), but this role has also not been explored in humans.

Whereas conflicting data exist regarding whether circulating FGF21 levels exhibit diurnal rhythms in humans (Lee et al., 2012; Patel et al., 2015), a consensus has emerged regarding its metabolic role in prolonged nutrient deprivation (Fisher & Maratos-Flier, 2016; Gälman et al., 2008). Circulating FGF21 is primarily thought to be of hepatic origin (Fisher & Maratos-Flier, 2016), but FGF21 can also be produced by other tissues, including adipose tissue and skeletal muscle, to primarily act in an autocrine or paracrine fashion (Kliewer & Mangelsdorf, 2019). For instance, muscle-derived secretion of FGF21 has been noted to increase under conditions of muscle-specific mitochondrial stress in mice (Pereira et al., 2017). Other pathways linked to inflammation and anti-oxidative responses (e.g. the Kelch-like ECH-associated protein 1 [KEAP1]-nuclear factor erythroid 2-related factor 2 [NRF2] axis), may also regulate the production of FGF21 (Chartoumpekis et al., 2011). Similarly, acute hyperinsulinaemia in humans has been demonstrated to increase skeletal muscle expression of the *FGF21* transcript (Hojman et al., 2009). At the same time, FGF21 that is circulating may also be inactivated through C-terminal proteolytical cleavage, mediated by fibroblast activation protein (FAP). As levels of the protease FAP may be regulated by nutrient state (van Baak et al., 2020), this altogether provides multiple mechanisms by which levels of FGF21 may be regulated in response to various physiological stimuli.

Notably, many metabolic functions that are regulated by FGF21, converge on pathways that are disrupted by sleep loss and circadian disruption. For instance, in addition to altered insulin sensitivity (Buxton et al., 2012), recent studies indicate that sleep and circadian disruption may alter metabolic fuel utilisation (Cedernaes et al., 2018; Wefers et al., 2018), in what appears to occur in a highly tissue-specific manner (Cedernaes et al., 2015, 2018). At present, the hormonal regulation of such tissue-specific changes remains largely unknown. This emphasises the need to explore the role of other paracrine signals, such as FGF21, which to our knowledge has not yet been examined under conditions of sleep loss in humans.

In the present study, we investigated whether circulating fasting and glucose-induced levels of FGF21 are altered in healthy individuals exposed to overnight wakefulness. We also explored whether changes in circulating FGF21 levels in response to our intervention were paralleled by alterations in interlinked signalling, production, and degradation pathways, including at the level of peripheral metabolic tissues, i.e. adipose tissue and skeletal muscle.

2 | PATIENTS AND METHODS

2.1 | Participant screening and monitoring

A total of 15 participants (mean [*SEM*] age 22.3 [0.5] years and body mass index 22.6 [0.5] kg/m^2) took part in two conditions of a randomised, within-subject two-session crossover study: overnight

wakefulness, i.e. 1 night of sleep loss versus 1 night of sleep. All participants provided oral and written informed consent. The study was approved by the Ethical Review Board in Uppsala (EPN 2012/477/1) and was conducted in accordance with the Helsinki Declaration (Supplementary Material and Cedernaes et al., 2018 for more details).

Prior to being enrolled by J.C., participants were screened by a medical doctor (J.C.), to verify that they were all of good health (including <5 units of alcohol/week, nicotine-free, normal blood cell counts and normal fasting glucose levels), had no history of shift work within the last 6 months and had not planned to or travelled across one or more time zones within 3 weeks of scheduled participation in the study or between sessions. This interview also verified that all participants were free from chronic medical conditions and medications. The Morningness–Eveningness questionnaire (MEQ) was used to exclude extreme chronotypes (Cedernaes et al., 2018). All participants also passed the criteria for self-reported sleep/night (7–9 hr; verified by 1-week long pre-study sleep, activity, and meal diaries) and adequate sleep quality (Pittsburgh Sleep Quality Index score ≤5).

Prior to participation, participants underwent overnight electroencephalography (EEG) monitoring and habituation to the laboratory environment. Sleep, food intake and activity profiles were assessed by diary during the week prior to each in-laboratory session and were not significantly different between the conditions (participant data and sleep data previously reported in Cedernaes et al., 2018); self-reported total amount of physical activity (mean [*SEM*] sleep: 51.4 [8.2] versus overnight wakefulness: 53.4 [10.1] min; $p = 0.84$) or sweat-inducing activity (mean [*SEM*] 9.5 [3.1] versus 13.2 [5.8] min; $p = 0.78$) were also not significantly different in the week preceding each session.

2.2 | Study protocol

Participants arrived at the sleep laboratory at the biomedical centre (Uppsala) 2 evenings prior to each session's experimental morning, as previously described (Cedernaes et al., 2018).

Low-sugar, low-fat isocaloric meals were provided during pre-intervention days (8:00 a.m., 1:00 p.m. and 7:30 p.m.; energy per meal calculated with the Harris–Benedict equation factored 1.2 for light physical activity) and were kept the same across sessions and participants.

During the first night in the laboratory, participants had a full night's sleep opportunity (8.5 hr long; 10:30 p.m. to 7:00 a.m.). During the following day, participants remained restricted to their in-laboratory rooms under sedentary conditions, except for two supervised and standardised 15-min long walks, and breakfast, lunch and dinner were provided at 8:00 a.m., 1:00 p.m. and 8:00 p.m. In the evening, a blood sample was taken before dinner at 7:30 p.m. Participant blinding to the experimental condition (overnight wakefulness or sleep) during the second night was in effect until 90 min prior to the intervention onset at 10:30 p.m. The ensuing night-time intervention lasted for 8.5 hr, providing a sleep opportunity (10:30 pm. to 7:00 a.m.) in the sleep condition (0 lux), contrasting with continued wakefulness in the overnight wakefulness condition (randomised order, see the Supplementary Material). During the latter condition, participants were also restricted to their beds and room lights were kept at 250 lux at eye-level. No food intake was allowed outside the aforementioned mealtimes, but water was consumed every 90 min during the overnight wakefulness. EEG, electro-oculography, and electromyography were used during each sleep opportunity to record sleep parameters (Embla A10 recorders; Flaga hf, Reykjavik, Iceland; sleep assessed according to standardised criteria; Rechtschaffen & Kales, 1968). As previously reported, the mean sleep duration during the sleep intervention night was 8.0 hr ± 3 min (Cedernaes et al., 2018).

After each overnight condition, i.e. during the second morning of each session, an indwelling venous catheter was placed and subcutaneous (peri-umbilical) adipose tissue and skeletal muscle (vastus lateralis) biopsies were collected as described previously (Cedernaes et al., 2018). Biopsy sampling time-points were kept within ±15 min for any given participant across sessions and were taken ~2–3 hr after the end time-point (7:00 a.m.) of the overnight intervention. Sampling side was randomised in a counterbalanced order, and then alternated for the second session. After the tissue sampling, fasting blood was first obtained, followed by an oral glucose tolerance test (OGTT; Cedernaes et al., 2018).

2.3 | Biochemical analyses

To test the effect of sleep loss on the cortisol awakening response and overnight dynamics on FAP, they were analysed in the fasted state in the evening (7:30 p.m.) and morning (7:30 a.m.), using enzyme-linked immunosorbent assay (ELISA) kits (serum using Cortisol Parameter Assay Kit; plasma using Human FAP DuoSet ELISA [Cat #DF2100], R&D Systems, Minneapolis, MN, USA). Morning plasma glucose and serum insulin (7:30 a.m.) were analysed as described previously, to calculate insulin sensitivity using the Matsuda Index (Cedernaes et al., 2018). Serum FGF21 (human FGF-21 Immunoassay, Cat #DY2539; R&D Systems) was analysed in the morning at the fasting (~10:30 a.m.) and at the 120-min OGTT time-point. ELISA samples were measured in singlets (FAP) or duplicates (for cortisol and FGF21); in the latter case, averages were used for the analyses.

As expected (Kralisch et al., 2013) several FGF21 samples were below the detection limit: out of 60 averaged serum FGF21 values, 14 were below the dynamic range, and out of these, four samples had values too low to be detected. These samples were set to the minimum detectable difference (4.67 pg/ml).

For FAP, a dilution assay was first done to determine the appropriate dilution. Samples were diluted 125-fold prior to running the assay. One sample that still remained above the maximum detection limit was set to the highest detectable level in the assay (corresponding to 500 ng/ml when undiluted).

2.4 | Quantitative polymerase chain reaction (qPCR) and DNA methylation analyses

The RNA was extracted from skeletal muscle and adipose tissue biopsies using the RNeasy Plus Universal Tissue and Lipid Tissue Kits, respectively (Qiagen) with on-column DNase treatment. For more information, see the Supplementary Material and Table S1, which also lists the primers used for the analysis as well as those used as housekeeping genes. For some qPCR targets in adipose tissue, there was not enough complementary DNA for two subjects (resulting in $n = 13$ pairs for the analyses).

The DNA methylation analysis was assessed in skeletal muscle and adipose tissue biopsy samples using the HumanMethylation450 BeadChip (Illumina), as previously described (Cedernaes et al., 2018). Given our targeted interrogation of changes related to FGF21 signalling, in the present study we only assessed DNA methylation within the promoter region of *FGF21*. Briefly, the mean methylation levels of all nine CpG probes in the *FGF21* promoter were considered, comparing overnight wakefulness versus sleep (wake–sleep). DNA methylation data processing and statistical analyses were carried out in R (version 3.1.1; www.r-project.org).

2.5 | Statistical methods

Normally distributed data (Shapiro Wilk's test, $p > 0.05$) were analysed using the paired Student's paired *t* test, or Pearson's correlation. Non-parametric data were analysed with the Wilcoxon matched-pairs signed-rank test, Spearman's rank test, or, in the case of analysis of variance (ANOVA), following $\log_2$ normalisation. Repeatedly measured data variables were analysed with two-way ANOVA, using the main effects *wake* (reflecting sleep condition) and *time* (i.e. fasting [pre] versus 120 min [post] OGTT values). Sidak's multiple comparison test was used for post hoc comparisons when significant main ANOVA effects were observed. Significance was set at $p < 0.05$ and reported as two-sided *p* values unless specified otherwise. Data are presented as mean (± *SEM*).

3 | RESULTS

3.1 | Increased circulating levels of FGF21 after overnight wakefulness in humans

We observed significantly higher serum levels of FGF21 in subjects following their overnight wakefulness versus sleep condition (means were 1.27-fold higher after wakefulness at fasting; 1.30-fold higher post OGTT; ANOVA *wake* effect: $p = 0.023$; Figure 1). While there was no significant *time* × *wake* interaction for serum FGF21 ($p = 0.92$), we did note a significant increase from fasting to 120 min post glucose ingestion (means were 1.63-fold greater when comparing post-120-min to fasting samples; ANOVA *time* effect: $p < 0.001$), supporting previous reports that glucose intake acutely increases

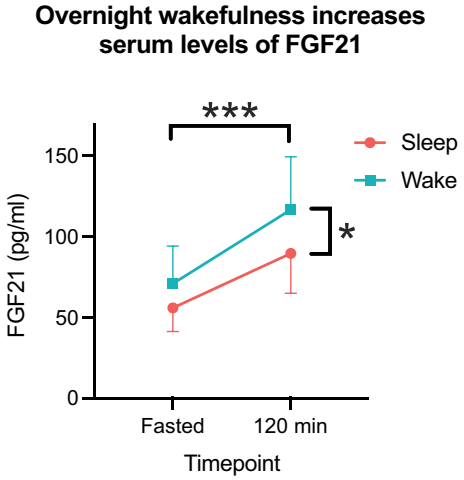

FIGURE 1 Acute sleep loss wakefulness increases circulating levels of fibroblast growth factor 21 (FGF21) in humans. Levels of FGF21 in the normal sleep and overnight wakefulness (acute sleep loss; "wake") condition, in the morning fasted state and glucose stimulated (120 min time-point for oral glucose tolerance test [OGTT]). Vertical lines depict changes between conditions (repeated measures, two-way analysis of variance [ANOVA] $p = 0.023$), whereas horizontal lines indicate changes based on OGTT time-point (ANOVA $p < 0.001$); $n = 15$ pairs and data shown as mean ± *SEM*. Two-way ANOVA, $^{*}p < 0.05$ and $^{***}p < 0.001$

circulating levels of FGF21 (Figure 1; Søberg et al., 2017). Setting the samples that were below the lowest dynamic range (31.3 pg/ml), to this value, did not change these results: by two-way ANOVA, we still observed significant main *wake* ($p = 0.031$) and *time* ($p < 0.001$) effects, such that serum values of FGF21 were significantly higher in response to wakefulness compared with after sleep, as well as FGF21 levels being higher after compared with before the OGTT.

Consistent with our previously reported findings (Cedernaes et al., 2018), we noted that the area under the curve for plasma glucose was significantly higher following wakefulness compared with sleep (mean [*SEM*] 4.87 [0.19] versus 4.41 [0.27] mmol/L/min; $p = 0.018$; data not shown). However, we did not find that the OGTT-mediated induction (i.e. the fold change) in circulating FGF21 levels correlated with the participants' Matsuda Index of insulin sensitivity, in any of the two conditions (sleep: $r = 0.13$, $p = 0.65$; wakefulness: $r = -0.14$, $p = 0.63$; Figure S1a,b). Altogether, this suggests that the increased FGF21 levels occurred without at least acutely impacting systemic glucose tolerance after sleep loss.

Cortisol is involved in circadian tissue responses and has been described as being reciprocally involved in the regulation of FGF21 production (Balsalobre et al., 2000; Bookout et al., 2013). It is also a factor that is well-characterised as being perturbed by sleep–wake disruption in humans. Indeed, in the present study, we observed that from the expected low cortisol levels at the evening time-point (mean [*SEM*] sleep: 51.1 [5.2], wakefulness: 53.8 [5.2] nmol/L; $p > 0.99$), the diurnal (evening to morning) change in circulating serum cortisol levels was significantly blunted in response to sleep loss compared with normal sleep ($p = 0.0381$; Figure S1c). However, we observed

no correlation between the overnight change in cortisol levels and circulating fasting levels of FGF21 in the morning (sleep: $r = 0.39$, $p = 0.15$; wakefulness: $r = -0.36$, $p = 0.19$; *data not shown*).

As FAP can degrade FGF21 (van Baak et al., 2020), we also assessed circulating levels of this protease. There was a statistical trend for a slight evening–morning decrease in FAP levels (ANOVA *time* effect: $p = 0.0633$), that was largely unchanged when excluding one imputed value ($p = 0.0848$). In contrast, there were no changes in FAP levels due to acute sleep loss (ANOVA *wake* effect: $p = 0.27$; *time* × *wake* effect: $p = 0.20$; Figure S1d), possibly suggesting that altered levels of FAP do not contribute to changes in circulating levels of FGF21, in response to sleep loss.

3.2 | Tissue-specific changes in pathways linked to production of FGF21

We next assessed whether systemic changes in FGF21 levels after sleep loss could involve altered regulation of pathways linked to FGF21 production and responsiveness at the peripheral metabolic tissue level.

Especially in skeletal muscle, expression of *FGF21* can increase in response to cellular and mitochondrial stress, reflected and thought to be driven by transcriptional modulation (Pereira et al., 2017). Given that FGF21 is also tied to tissue-specific regulation of insulin sensitivity, we explored a biomarker of cellular stress that is specifically thought to be involved in tissue insulin sensitivity, i.e. thioredoxin-interacting protein (*TXNIP*; Parikh et al., 2007). Using qPCR, we found that expression of both protein-coding isoforms was significantly elevated in skeletal muscle (*TXNIP-201*: +49%, $p = 0.004$; *TXNIP-204*: +56%, $p = 0.003$) in response to sleep loss (Figure 2). In contrast, we only found a trend for a slight decrease in mRNA expression of the main (*TXNIP-204*: −5.2%, $p = 0.071$) but not the secondary (*TXNIP-201*: −4.2%, $p = 0.33$) isoform of *TXNIP* in adipose tissue after wakefulness (Figure 2). These results confirm our prior untargeted RNA-seq approach, in which we observed elevated expression of the transcript *TXNIP* in skeletal muscle in response to overnight wakefulness (Cedernaes et al., 2018).

Consistent with prior findings (Parikh et al., 2007), skeletal muscle expression of *TXNIP* was found to demonstrate an inverse correlation with the Matsuda Index of insulin sensitivity. This was seen regardless of protein-coding isoform but seemed to mainly occur in the sleep condition (*TXNIP-201*, hypothesised contrast, sleep: $r = -0.45$, $p = 0.048$; wakefulness: $r = -0.37$, $p = 0.085$; *TXNIP-204*, sleep: $r = -0.50$, $p = 0.028$; wakefulness: $r = -0.53$, $p = 0.021$; Figure S2a,b).

To further investigate genes specifically linked to regulation of tissue-specific FGF21 production, we examined qPCR-based changes in levels of optic atrophy 1 mitochondrial dynamin like GTPase (*OPA1*) and peroxisome proliferator-activated receptor gamma co-activator 1 alpha (*PGC1α*), but observed no changes in response to sleep loss, neither in skeletal muscle nor adipose tissue (Figure 3a,b; all $p > 0.10$). In contrast, we found that sleep loss resulted in decreased skeletal muscle expression of the transcription factors peroxisome proliferator-activated receptor alpha (*PPARA*; −13%, $p = 0.019$) and transcription factor A, mitochondrial (*TFAM*; −15%, $p = 0.009$; Figure 3a), factors positively associated with skeletal muscle insulin sensitivity, production of *FGF21* and mitochondrial biogenesis (Badman et al., 2007; Pereira et al., 2017). Furthermore, after sleep loss compared with sleep, we observed lower mRNA

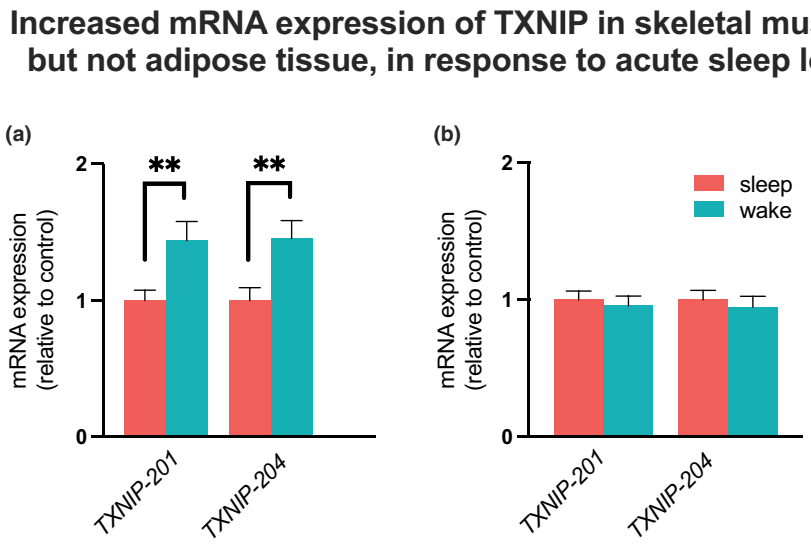

FIGURE 2 Muscle-specific changes in insulin resistance marker following acute sleep loss in humans. quantitative polymerase chain reaction-based mRNA expression analysis of thioredoxin-interacting protein (*TXNIP*) isoforms in skeletal muscle (vastus lateralis muscle [VLM]; left; *TXNIP-201*: $p = 0.004$; *TXNP-204*: $p = 0.003$) and subcutaneous adipose tissue (SAT; right; *TXNIP-201*: $p = 0.33$; *TXNP-204*: $p = 0.071$), in response to sleep and sleep loss in humans that underwent a randomised two-session crossover design. All data were normalised to the mean of the sleep condition arbitrarily set to 1; $n = 13$ (SAT) or 15 (VLM) pairs. Data shown as mean ± *SEM*. Student's paired *t*-test, $^{**}p < 0.01$.

expression in skeletal muscle for *NRF2* (nuclear factor, erythroid 2-like 2 [*NFE2L2*]; −14%, $p = 0.0425$), the protein of which can exert anti-inflammatory activity and suppress production of FGF21. In contrast, no changes were observed for *KEAP1* ($p > 0.10$), which in its protein form can suppress NRF2 activity (Safdar, DeBeer, & Tarnopolsky, 2010). Apart from *KEAP1*, none of these investigated genes exhibited changes in adipose tissue after sleep loss ($p > 0.10$; Figure 3b): there was a statistical trend for a slight downregulation (−6%) of *KEAP1* expression in adipose tissue after sleep loss compared with after sleep ($p = 0.0927$).

Focussing on potential epigenetic regulation of *FGF21* expression, we found that DNA methylation at the *FGF21* promoter region was significantly increased in adipose tissue ($p = 0.033$), but not in skeletal muscle ($p > 0.10$), after wakefulness compared with after sleep (Figure 3c,d). Furthermore, there were no changes in the mRNA expression of *FGF21*, neither in skeletal muscle nor in adipose tissue, in response to wakefulness compared with normal sleep (all $p > 0.10$; Figure S3). Finally, in addition to the lack of an effect on the *FGF21*-responsive gene *PGC1α* (as reported above), there were no changes in mRNA expression of the two FGF21 receptors, *FGFR1* and *Klotho β* (*KLB*), in any of the two tissues (Figure S3). Altogether, these findings indicate that the observed increase in circulating FGF21 levels was not driven by alterations in skeletal muscle or adipose tissue production, nor that the tissue responsiveness to FGF21 was altered following acute sleep loss.

4 | DISCUSSION

In the present study, we observed that acute sleep loss resulted in a modest increase in circulating levels of FGF21 in humans, both under fasting and glucose-stimulated conditions. However, we did not find that the higher circulating levels of FGF21 were reflected by changes in mRNA expression of *FGF21* in peripheral tissues, suggesting that acute sleep loss did not impact pathways regulating FGF21 production in these tissues. We also did not find that sleep loss altered plasma levels of the protease FAP, which can degrade circulating FGF21. Changes in circulating levels of FGF21 in response to sleep loss may therefore primarily be driven by altered production in the liver, which is considered the main origin of FGF21 (Dushay et al., 2010).

Notably, ablation of the core clock component REV-ERBα elevates FGF21 levels in liver and plasma in mice (Chavan et al., 2017). Given that short periods of acute sleep loss alter the function (e.g. its phase and amplitude) of central and tissue-specific peripheral clocks, including in the liver in mice (Barclay et al., 2012; Cedernaes et al., 2015), this may provide a mechanism for how levels of FGF21 can become elevated after sleep loss in humans. However, we found no association between sleep loss-induced changes in circulating cortisol levels, an output of the central pacemaker, and morning levels of circulating FGF21 (Bookout et al., 2013), warranting further studies with greater temporal resolution to examine how such mechanisms may be regulated in humans.

Sleep loss is often considered as a stressor and induces insulin resistance (Buxton et al., 2012). In the present study, we found that mRNA expression of a subset of genes linked to cellular stress and skeletal muscle-specific insulin responsiveness, *TXNIP*, *PPARA*, *TFAM* and *NRF2* (*NFE2L2*; Koh et al., 2019; Safdar et al., 2010) was altered in skeletal muscle in response to sleep loss. Notably, the transcription factor PPARA can induce FGF21 in liver, but whether decreased skeletal muscle expression of *PPARA*, as observed in the present study, may alter local production of FGF21, is unknown. Similarly, lower skeletal muscle expression of *NRF2* after sleep loss could possibly increase local *FGF21* expression (Chartoumpekis et al., 2011), but the tissue-specific role of NRF2 in this context remains unresolved. Decreased expression of *PPARA*, *TFAM* and *NRF2* may impair skeletal muscle mitochondrial function and increase inflammation and oxidative stress, to potentially promote skeletal muscle atrophy (Safdar et al., 2010). Thus, as previously noted by us and others (Cedernaes et al., 2018), sleep loss may, in the acute setting, impact metabolic and redox homeostasis, through pathways that potentially also could alter local FGF21 production. However, the latter mechanistic relationships may possibly only come into play after recurrent or chronic sleep loss.

Other stress-induced transcripts that can induce or respond to FGF21 signalling (such as *PGC1α* and *OPA1*; Fisher et al., 2012; Manoli et al., 2007; Pereira et al., 2017; Potthoff et al., 2009), did not exhibit altered mRNA levels in the studied tissues in response to sleep loss. Nor did we find changes in the tissue mRNA levels of FGF21 receptor subtypes. Together our present findings further highlight how sleep loss may alter insulin sensitivity and metabolic fuel utilisation in a tissue-specific manner (Cedernaes et al., 2018). However, while FGF21 is known to regulate metabolic fuel utilisation, the lack of changes in *FGF21* expression or its response pathways may argue against circulating FGF21 exerting a prominent effect on the studied tissues, under conditions of acute sleep loss. Alternative interpretations could be that the observed changes in serum FGF21 levels may have been too modest to alter the studied tissue pathways in our acute paradigm, or that FGF21 acted on other tissues or pathways, such as via central hypothalamic signalling (Bookout et al., 2013).

Circulating levels of FGF21 are elevated in metabolic disease, such as obesity and type-2 diabetes, possibly due to resistance to the effects of FGF21 (Chavez et al., 2009; Dushay et al., 2010; Fisher & Maratos-Flier, 2016). Increased circulating levels of FGF21 at the systemic level may exert tissue-specific metabolic effects, e.g. increasing glucose uptake in adipose tissue (Kharitonenkov et al., 2005). Our present investigation could not establish whether signalling through this pathway was mechanistically involved, or possibly counteracting, adverse metabolic effects observed following acute or chronic sleep loss. Furthermore, several FGF members, including FGF21, are involved in bone remodelling (Hanks, Casazza, Ashraf, Wallace, & Gutiérrez, 2015; Wei et al., 2012). Given that chronic sleep and circadian disruption may impact the integrity of lean and bone mass (Buchmann et al., 2016; Cedernaes et al., 2018; Chien, Wang, & Chen, 2015), it remains to be investigated whether this may involve disrupted signalling via the FGF21 axis.

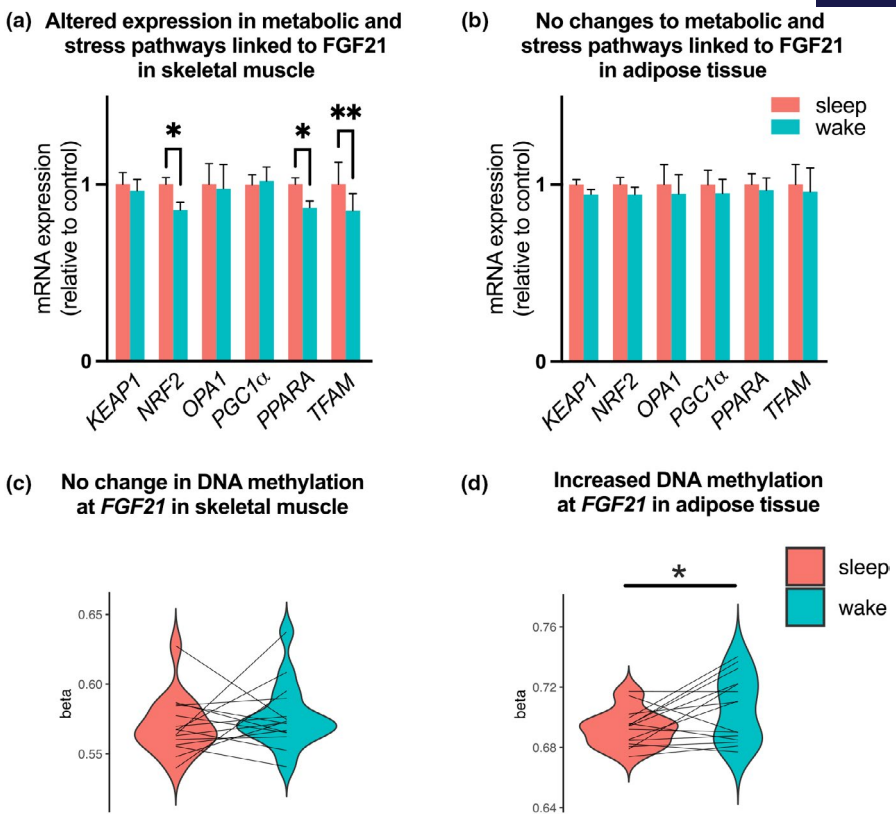

FIGURE 3 Muscle-specific changes in stress genes and altered DNA methylation of *FGF21* in adipose tissue after sleep loss in humans. (a) mRNA expression in skeletal muscle (VLM) and (b) subcutaneous adipose tissue (SAT), across all individuals ($p = 0.0425$ for *NRF2* [*NFE2L2*], $p = 0.0191$ for *PPARA*, $p = 0.0089$ for *TFAM* in VLM; $p = 0.0927$ for *KEAP1* in SAT; all other $p > 0.10$). All data were normalised to the mean of the sleep condition, arbitrarily set to 1 for quantitative polymerase chain reaction analyses. (c) Average change in DNA methylation levels after sleep loss compared with sleep for CpGs in the promoter area of *FGF21* in VLM ($p = 1.0$) and (d) SAT ($p = 0.033$); $n = 15$ pairs for VLM, $n = 13$ pairs for SAT, except for $n = 15$ pairs for *PGC1α*. Wilcoxon matched-pairs signed-rank test and Student's paired *t*-test, $^{*}p < 0.05$ and $^{**}p < 0.01$. FGF21, fibroblast growth factor 21; KEAP1, Kelch-like ECH-associated protein 1; NRF2, nuclear factor erythroid 2-related factor 2; PGC1α, peroxisome proliferator-activated receptor gamma co-activator 1 alpha; PPARA, peroxisome proliferator-activated receptor alpha; SAT, subcutaneous adipose tissue; TFAM, transcription factor A, mitochondrial; VLM, vastus lateralis muscle

We found that the level of DNA methylation in the promoter region of *FGF21* was altered in adipose tissue in response to sleep loss. Given that the effect was modest and that no changes were seen for mRNA levels of *FGF21*, future studies are warranted to investigate whether altered DNA methylation may have a more long-term impact on how peripheral tissues such as adipose tissue regulate local FGF21 production in response to various forms of sleep loss in humans.

Apart from the absence of repeated biopsy sampling, our present study has several limitations. The analyses were done in healthy young, Caucasian men, of normal weight, additional studies are therefore warranted to extend our present findings to other groups such as females, overweight/obese or older individuals. Furthermore, we only observed a modest increase in circulating FGF21 levels in response to our intervention. As such, our present results may involve flooring effects (with regard to overall low FGF21 levels). Our present results may also not apply to individuals with sleep disorders or those with either established (or at risk of) metabolic disease (Darukhanavala et al., 2011; Vasisht, Kessler, Booth, Imperial, & Penev, 2013).

While we did test the dynamic response of circulating FGF21 to acute glucose intake, other interactions between FGF21 and sleep loss likely exist. In the context of peripheral tissue metabolism, exercise can also counteract reduced FGF21 sensitivity at the adipose tissue level due to high fat diet feeding. This occurs via induction of FGF21 receptors but has only been established in mice (Geng et al., 2019). Furthermore, FGF21 is known to modulate food intake and sweet preference, which are adversely impacted by sleep loss (Hill et al., 2019; Nedeltcheva et al., 2009; Søberg et al., 2017). Finally, whereas the observed change in circulating levels of FGF21 in response to sleep loss was modest, they are similar in magnitude to the differences seen between lean and obese subjects (Zhang et al., 2008).

Other forms of sleep restriction (e.g. recurrent partial) and circadian paradigms may reveal whether the mechanisms investigated in the present study modulate the risk of certain individuals to develop metabolic disease due to chronic sleep loss or circadian disruption that mimics social jetlag. Notably, some prior studies have found that circulating levels of FGF21 do not seem to exhibit diurnal rhythms (Foo et al., 2015; Lee et al., 2012). This may indicate that increased

circulating levels of FGF21 in response to overnight wakefulness are not due to acute circadian misalignment per se, as may occur after acute sleep loss (Cedernaes et al., 2015), but rather may be due to the accumulated loss of sleep.

In conclusion, in the present study we found that acute sleep loss resulted in increased circulating levels of FGF21 in healthy young individuals studied under highly standardised conditions. While we found evidence, especially in skeletal muscle, that sleep loss impacts pathways that impinge on metabolic and redox homeostasis and that can modulate FGF21 production, we did not observe any tissue-specific changes in the production of or response to FGF21. Further studies are warranted to investigate what role acute or chronic changes in circulating levels of FGF21 may exert in the context of adverse metabolic health effects attributed to sleep and circadian disruption in humans, such as insulin resistance, gain of fat mass or loss of lean mass.

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CONFLICT OF INTEREST

LEMB reports no conflicts of interest. DE reports no conflicts of interest. JE reports no conflicts of interest. TM reports no conflicts of interest. NW reports no conflicts of interest. LL reports no conflicts of interest. AP reports no conflicts of interest. HV reports no conflicts of interest. AS reports no conflicts of interest. SLD reports no conflicts of interest. CB reports no conflicts of interest. JC reports no conflicts of interest.

AUTHOR CONTRIBUTIONS

JC formulated the study idea and designed the study with input from CB; JC wrote the protocol and conducted the experiments; LEMB, DE, JOW, HV, SLD and JC conducted the analyses; LEMB, DE, JE, TM, NW, LL, AP, HV, AS, SLD, CB and JC interpreted the data; JC wrote the original manuscript draft and all authors contributed to review and editing. JC had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

DATA AVAILABILITY STATEMENT

Anonymous data are available for academic researchers upon request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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