Commentary

MOLECULAR METABOLISM

Sleepy, circadian disrupted and sick: Could intestinal microbiota play an important role in shift worker health?

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A sizable percentage of the population stands to benefit from elucidating mechanisms linking sleep loss, circadian misalignment, and metabolic disease. In particular, shift work is associated with increased risk for metabolic diseases, including type 2 diabetes, obesity, and metabolic syndrome [1]. These workers also report less sleep per day, and often outside of the biological night. While a reflexive instinct to these discoveries is to encourage more sleep, this may not always be practical for shift working individuals. In light of inevitable sleep loss and circadian misalignment associated with these work patterns comes a need for suitable therapeutic targets to support better long-term health outcomes. In a recent issue of *Molecular Metabolism*, Benedict and colleagues [2] provide the first published insights into the relationship between sleep and gut microbiota in human subjects. Their study provides a novel consideration of acute sleep restriction and the gut microbiota and opens an important discussion of future investigations of the gut microbiota in human sleep research.

The last twenty years have seen a proliferation of rodent and human sleep studies investigating health consequences associated with sleep loss and circadian misalignment. However, the foundations for the more recent research investigating sleep, gut microbiota, and health can be traced to seminal work in the sleep field by Rechtschaffen and colleagues [3] who identified systemic demise in rats exposed to prolonged sleep deprivation. The possibility that this was at least in part a function of negative impacts on the community of microbes in the intestinal tract (collectively known as the intestinal microbiota) and subsequent bacterial translocation was then reflected in the work of Everson and Toth [4]. Proliferation of intestinal bacteria and movement of bacteria into what was thought to be previously sterile tissue (particularly in mesenteric lymph nodes, where bacteria correlated with those identified in intestinal overgrowth) and negative energy balance are facilitated by immune suppression in rats. More recent research has shown that sleep and circadian disruption, via clock gene mutation or weekly shifts of the light-dark cycle, can negatively impact gastrointestinal tract function and produce dysbiosis, especially when combined with alcohol induced colitis or a high-fat high-sugar diet (see [5] for review). With the increasing accessibility of high-throughput sequencing technology for quantifying microbiota, support for the importance of sufficient, consolidated sleep to maintain a diverse and functional gut microbiota is growing.

There are a number of methodological considerations for future investigations of gut microbiota in a human sleep laboratory context. With the transition from rodent to human models comes the additional complexity of obtaining routine, non-invasive samples for analysis. Unlike blood measures, which can be obtained at prescribed intervals via indwelling catheters in sleep laboratories, measurement of intestinal microbiota is reliant on fecal samples, and, by extension, a subject's habitual bowel motions. One approach, as reflected in the work of Benedict et al. [2], is to collect ad libitum fecal samples within a controlled laboratory protocol. Another is to request a routine sample at a prescribed time of day — an option which preliminary work in our laboratory indicates is feasible [*unpublished data*]. The frequency and timing of collection is especially important in light of recent findings suggesting diurnal rhythms in certain gut microbes, and sensitivity to melatonin [6]. Future studies will need to determine the sensitivity of samples to time of day in order to establish best practice in timing and frequency of sample collection.

The work of Benedict and colleagues [3] utilizes one of many potential manipulations of sleep — an acute curtailment of sleep opportunity for two nights. Within this brief protocol, there was not an association between microbial diversity and the acute reduction in insulin sensitivity seen in this study. This finding in a small number of subjects using common sequencing technology suggests that changes to the overall diversity of microbiota may not be a primary mechanism linking acute sleep loss with insulin sensitivity. It will also be important to ascertain whether the sleep, gut microbiota, health relationship is differentially characterized by acute and chronic sleep loss, as the authors highlight. Indeed, the recent work of Poroyko and colleagues [7] in mice suggests that chronic models of disrupted sleep are associated with increased food intake, disruption of gut microbiota known to contribute to intestinal epithelial barrier integrity, and promotion of systemic and adipose tissue inflammatory response, as well as insulin resistance. This same inflammatory response is present in germ-free mice when they are exposed to fecal water from mice who experienced sleep fragmentation. This work suggests that an additional measure in future human investigations of gut microbiota should

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be inflammatory markers, as this may reflect an important component of the pathway [5]. This is particularly important in light of an absence of weight gain but presence of an inflammatory response in conventionalized, germ-free mice fed water primed with fecal matter from mice exposed to sleep disruption. Indeed, it would appear that a complex relationship exists between sleep loss, food energy intake and the gut microbiota.

Controlled experiments in human sleep laboratories afford a unique opportunity to characterize gut microbiota response to sleep loss with a range of sleep doses and circadian timings. Quantifying the stability of human gut microbiota in the laboratory environment will be essential to ensure meaningful comparisons are made between baseline and experimental sleep conditions. Protocols such as the within-subjects design presented by Benedict et al. [2] represent an important component of this approach, as there are marked individual differences in intestinal microbiota [8]. The role of food intake will need to be carefully considered within experimental paradigms, particularly in light of the recent work of Poroyko et al. [7] as altered food intake likely influences the relationship between sleep fragmentation and changes in intestinal microbiota [9]. Further, mice fed Western (high-fat) diets exhibit altered eating patterns, disrupted metabolic signals from microbes, altered diurnal variation in microbial metabolites (for example, loss of short chain fatty acids), and changes in hepatic circadian clock gene expression and hepatic metabolome [10]. With these considerations in mind, identifying any differences between controlled intake and ad libitum feeding will be important, particularly when sleep and wake opportunities are altered with shift working conditions. The potential for complex interactions between sleep loss, circadian misalignment, energy intake, and metabolic disturbance will need to be carefully considered. Simulated shift work studies of humans in a controlled environment can be used to begin exploring these relationships. Cohort studies will also play an important role in determining the impact of ongoing shift work in real world settings, providing the larger sample sizes needed to detect changes when energy intake and lifestyle are not held constant [8]. With these considerations in mind, characterizing the impact of sleep on gut microbiota in controlled human studies may provide important insights into therapeutic potential for shift workers who often experience the intrinsically interwoven burden of sleep loss and circadian misalignment and suffer the health consequences.

REFERENCES

[1] Kecklund, G., Axelsson, J., 2016. Health consequences of shift work and insufficient sleep. BMJ 355:i5210. http://dx.doi.org/10.1136/bmj.i5210.

[2] Benedict, C., Vogel, H., Jonas, W., Woting, A., Blaut, M., Schurmann, A., et al., 2016. Gut microbiota and glucometabolic alterations in response to recurrent partial sleep deprivation in normal-weight young individuals. Molecular Metabolism 5:1175—1186.

[3] Rechtschaffen, A., Gilliland, M.A., Bergmann, B.M., Winter, J.B., 1983. Physiological correlates of prolonged sleep deprivation in rats. Science 221: 182—184.

[4] Everson, C.A., Toth, L.A., 2000. Systemic bacterial invasion induced by sleep deprivation. American Journal of Physiology — Regulatory, Integrative and Comparative Physiology 278:R905—R916.

[5] Reynolds, A.C., Paterson, J.L., Ferguson, S.A., Stanley, D., Wright Jr., K.P., Dawson, D., 2016. The shift work and health research agenda: considering changes in gut microbiota as a pathway linking shift work, sleep loss and circadian misalignment, and metabolic disease. Sleep Medicine Reviews. http://dx.doi.org/10.1016/j.smrv.2016.06.009.

[6] Paulose, J.K., Wright, J.M., Patel, A.G., Casson, V.M., 2016. Human gut bacteria are sensitive to melatonin and express endogenous circadian rhythmicity. Plos One, e0146643.

[7] Poroyko, V.A., Carreras, A., Khalyfa, A., Khalyfa, A.A., Leone, V., Peris, E., et al., 2016. Chronic sleep disruption alters gut microbiota, induces systemic and adipose tissue inflammation and insulin resistance in mice. Scientific Reports 6:35405.

[8] Caporaso, J.G., Lauber, C.L., Costello, E.K., Berg-Lyons, D., Gonzalez, A., Stombaugh, J., et al., 2011. Moving pictures of the human microbiome. Genome Biology 12:R50.

[9] Zarrinpar, A., Chaix, A., Yooseph, S., Panda, S., 2014. Diet and feeding pattern affect the diurnal dynamics of the gut microbiome. Cell Metabolism 20:1009—1017.

[10] Leone, V., Gibbons, S.M., Martinez, K., Hutchison, A.L., Huang, E.Y., Cham, C.M., et al., 2015. Effects of diurnal variation of gut microbes and high-fat feeding on host circadian clock function and metabolism. Cell Host & Microbe 17:681—689.

