DOI: 10.1177/0004563215597943

Annals of Clinical Biochemistry

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Journal:	*Annals of Clinical Biochemistry*
Manuscript ID:	ACB-15-159
Manuscript Type:	Invited Short Report
Date Submitted by the Author:	02-Jul-2015
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Keywords:	Clinical studies < Clinical studies, Endocrinology < Clinical studies, DNA & RNA techniques < Laboratory methods

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Telling biological time from a blood sample — current capabilities and future potential

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Declarations:

Competing interests: D.J.S. is a co-director of Stockgrand Ltd

Funding: This work was supported in part by the Netherlands Forensic Institute, Netherlands Genomics Initiative/Netherlands Organization for Scientific Research within the framework of the

DOI: 10.1177/0004563215597943

Forensic Genomics Consortium Netherlands, BBSRC grants BB/F022883 and BB/I019405/1, and Air Force Office of Scientific Research Grant FA9550-08-1-0080. D.J.S. is a Royal Society Wolfson Research Merit Award holder.

Ethical approval: Ethical approval was granted and disclosed with the original publications referenced herein.

Guarantor: MvS

Contributorship: MvS and DJS researched literature and conceived the study. MvS wrote the first draft of the manuscript. Both authors reviewed and edited the manuscript and approved the final version of the manuscript.

Acknowledgements: This paper is dedicated to Stephen Halloran on the occasion of his retirement.

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Abstract

Circadian rhythms, near-24 h oscillations that reflect homeostatic control by an internal timing system rather than the influence of external factors, are an important and sometimes underappreciated aspect of human physiology and biochemistry. Over the past few decades, the pineal gland hormone melatonin has been established both as a robust marker of circadian phase in plasma or saliva, and as a chronobiotic drug administered to reset the timing of the circadian oscillator. Recent work by our own and other laboratories has sought to systematically investigate whole categories of molecular components in blood samples in a hypothesis-free fashion by employing metabolomic methodologies to study low-molecular-weight compounds and transcriptomic methodologies to study gene expression in white blood cells, respectively. A number of components have been pinpointed that show a rhythmic circadian variation or are affected by imposed factors such as sleep deprivation. Although melatonin, a robust

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and reliable circadian phase marker, will be a hard act to follow, these lines of research suggest numerous potential leads for useful new markers of biological timing.

Key words

Circadian rhythms

Melatonin

Metabolome

Sleep

Sleep deprivation

Transcriptome

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Circadian rhythms govern multiple aspects of human physiology. They allow us to actively anticipate, as opposed to passively react to, the predictable changes that occur across the 24 h cycle of a day and a night. This helps ensure that the body is optimally prepared for timed events such as sleep, activity, and meals.

An understanding of the rhythmic circadian nature of many processes is helpful both in diagnosis and in treatment of disease. A single cortisol reading, for example, is quite meaningless without knowledge of what time of day it was collected, because the concentrations of this hormone exhibit a strong circadian variation across the 24 h. It has been established, given the circadian variability both in drug absorption and metabolism on the one hand and in blood pressure on the other, that intake of multiple antihypertensive medications at bedtime is more efficacious than taking them in the morning [1].

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The pineal gland hormone melatonin plays a unique role in circadian medicine in that it is simultaneously a biological marker and a therapeutic drug. The synthesis of melatonin by the pineal gland essentially follows the biological night, governed by signals from the master circadian oscillator in the paired suprachiasmatic nuclei (SCN) that make its circulating concentration rise 1-2 h before bedtime and peak in the middle of the night. This quality makes dim light melatonin onset (DLMO), determined by measuring half-hourly saliva samples in the evening in dim light conditions [2], a very precise marker of biological timing (phase) that is in routine use for the diagnosis of circadian rhythm sleep disorders [3]. Melatonin is also, however, administered as a chronobiotic drug, and has been used successfully to entrain and reinforce circadian rhythms in a variety of conditions such as circadian rhythm sleep disorders, jet-lag and free-running blind individuals [4].

Forty years after the discovery of melatonin's rhythmic properties, it still reigns supreme as the biochemical phase marker of choice in

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humans, both because of its robust rhythmic properties, being relatively unaffected by confounding external factors such as activity, sleep and stress (although extremely sensitive to ocular light exposure) as well as the relative ease with which it can be measured through immunoassays in blood or saliva. However, the last few years have seen technological developments that have enabled the high-throughput detection of multiple components in a blood sample without any need for a hypothesis based on prior knowledge about a substance, and without the need to develop a specific technology for its detection. Complementing the well-established analytical methodologies for specifically detecting major electrolytes, metabolites, and hormones in a blood sample, *metabolomic* technology has made it possible to simultaneously analyse more than 100 small molecules (<1 kDa) in a biological sample such as bodily fluids (blood, urine, saliva), tissues and breath exhalate, using liquid chromatography (LC) or gas chromatography (GC) coupled with mass spectrometry (MS). It is also possible, using *transcriptomic* technologies such as microarray analysis or high-throughput RNA sequencing, to simultaneously

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profile expression of all known human genes in the white blood cells in a blood sample. In recently published articles, some of them from our own group, these technologies have been employed in search of novel potential markers of biological time, and in search of signatures of sleep deprivation.

Two studies have reported the circadian variability in the human blood plasma metabolome under constant routine conditions (a gold standard protocol designed to minimise effects of external time cues in order to identify endogenously driven circadian rhythms) where volunteers are kept awake in a supine position under constant dim light, and time of feeding effects are minimised by frequent administration of small isocaloric food portions [5] [6]. These studies reported that approximately 15% of the human blood plasma metabolome, most notably fatty acids, is under circadian control. Particularly intriguing is the suggestion that, by determining the concentrations of two of these metabolites, it is possible to infer which time of day the sample has been collected, the so called “metabolite-timetable method” [6]. Our

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laboratory set out to study metabolite rhythms under natural conditions simulating those of real life, with a light-dark cycle and standardised timed meals under which the participants were given a normal sleep opportunity [7]. Under this paradigm, concentrations of 109 out of 171 metabolites varied in a rhythmic fashion, with most metabolites peaking during the day. During a subsequent night of total sleep deprivation, 78 of these maintained their rhythmicity, albeit with a reduced amplitude in the majority of cases, whereas 27 metabolites (including tryptophan, serotonin, and taurine) were increased during sleep deprivation compared with during sleep.

In our study of the human blood transcriptome [8], volunteers were kept under forced desynchrony conditions (where the circadian clock is made to free-run according to its internal properties by imposing an external day length too long or short to adjust to), and blood samples were collected for RNA extraction at 4 hourly intervals during a period when the sleep cycle was in synchrony with the body's circadian timing system, and another period when

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they were 180° out of phase. When circadian phase was in synchrony with sleep phase, 1,502 transcripts (6.4% of all genes that were analysed) were expressed with a rhythmic profile. By contrast, under conditions of asynchrony, only 237 transcripts (1.0%) remained rhythmic. In a separate experiment, our group studied the effect of chronic sleep deprivation (6 h sleep opportunity per night over one week) on white blood cell gene expression during a constant routine for 24 h subsequently [9]. Here, we found downregulation of transcripts related to metabolic processes, and upregulation of transcripts related to nucleic acid binding, as well of transcripts representing a proinflammatory response. We also observed a number of time-awake-dependent transcripts, whose concentrations either increased or decreased over the period of wakefulness. Thus, the expression studies show alterations to the expression of several genes that may correlate to the negative health outcomes reported for circadian asynchrony and sleep deprivation. Furthermore, both the metabolome and transcriptome studies referred to above have identified a large number of molecules that can be measured in a normal blood

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sample and be developed as potential new markers of biological time or sleep deprivation. Only a minority of these are likely to be as robust and versatile markers as melatonin, but after 40 years of hegemony, the useful and interesting melatonin molecule is likely to be seeing some healthy competition in the next few years, as high-throughput biology begins to bring returns in terms of single molecules for which diagnostic assays can be designed.

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References

1 Hermida, R. C., Ayala, D. E., Mojon, A. & Fernandez, J. R. Influence of circadian time of hypertension treatment on cardiovascular risk: results of the MAPEC study. *Chronobiol Int* **27**, 1629-1651, doi:10.3109/07420528.2010.510230 (2010).

2 Lewy, A. J. & Sack, R. L. The dim light melatonin onset as a marker for circadian phase position. *Chronobiol Int* **6**, 93-102 (1989).

3 Nagtegaal, J. E., Kerkhof, G. A., Smits, M. G., Swart, A. C. & Van Der Meer, Y. G. Delayed sleep phase syndrome: A placebo-controlled cross-over study on the effects of melatonin administered five hours before the individual dim light melatonin onset. *J Sleep Res* **7**, 135-143 (1998).

4 Arendt, J. & Skene, D. J. Melatonin as a chronobiotic. *Sleep Med Rev* **9**, 25-39, doi:10.1016/j.smrv.2004.05.002 (2005).

5 Dallmann, R., Viola, A. U., Tarokh, L., Cajochen, C. & Brown, S. A. The human circadian metabolome. *Proc Natl Acad Sci U S A* **109**, 2625-2629, doi:10.1073/pnas.1114410109 (2012).

6 Kasukawa, T. *et al.* Human blood metabolite timetable indicates internal body time. *Proc Natl Acad Sci U S A* **109**, 15036-15041, doi:10.1073/pnas.1207768109 (2012).

DOI: 10.1177/0004563215597943

7 Davies, S. K. *et al.* Effect of sleep deprivation on the human metabolome. *Proc Natl Acad Sci U S A* **111**, 10761-10766, doi:10.1073/pnas.1402663111 (2014).

8 Archer, S. N. *et al.* Mistimed sleep disrupts circadian regulation of the human transcriptome. *Proc Natl Acad Sci U S A*, doi:10.1073/pnas.1316335111 (2014).

9 Moller-Levet, C. S. *et al.* Effects of insufficient sleep on circadian rhythmicity and expression amplitude of the human blood transcriptome. *Proc Natl Acad Sci U S A* **110**, E1132-1141, doi:10.1073/pnas.1217154110 (2013).