

**1      Chronovaccination: the competitive edge in vaccine  
2      effectiveness?**

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5      **Abstract**

6      Although chronovaccination - controlling the time of day of vaccination (TODV) to  
7      enhance protective immunity - has been described as a potential paradigm shift in  
8      vaccine immunology<sup>1</sup>, unequivocal evidence for clinical benefit outweighing other  
9      societal and practical considerations has yet to be established<sup>2</sup>. The current mixed  
10     evidence for chronovaccination providing an additional competitive edge to vaccine  
11     effectiveness is likely due to complex population level interactions between various  
12     circadian, environmental, and sociodemographic factors influencing antibody responses  
13     to vaccines. Nonetheless, TODV shows the greatest potential for sub-cohorts of popula-  
14     tions, particularly older age, and immunocompromised groups. In these contexts,  
15     this review provides a Person, Place and Time consideration of the factors influencing  
16     the outcomes of chronovaccination studies.

17     **Plain Language Summary**

18     Researchers have asked whether there is a ‘sweet spot’ in the day where vaccination  
19     is most effective in terms of providing long-term protection. So far, their answers  
20     have not been crystal clear, probably due to the different ways in which studies are  
21     performed and the daily variability of our immune system responses for populations in  
22     our modern 24/7 society. However, scheduling vaccinations focussed on distinct phases  
23     of the day, mornings, afternoons, or evenings for example, might be beneficial for  
24     groups where a boost in vaccine effectiveness may be especially welcome. This review  
25     sets out the key concepts when thinking about how our internal timing mechanism  
26     – our circadian clock – might be used to get the most out of vaccinations, similar to  
27     how our clocks are harnessed in other areas of society.

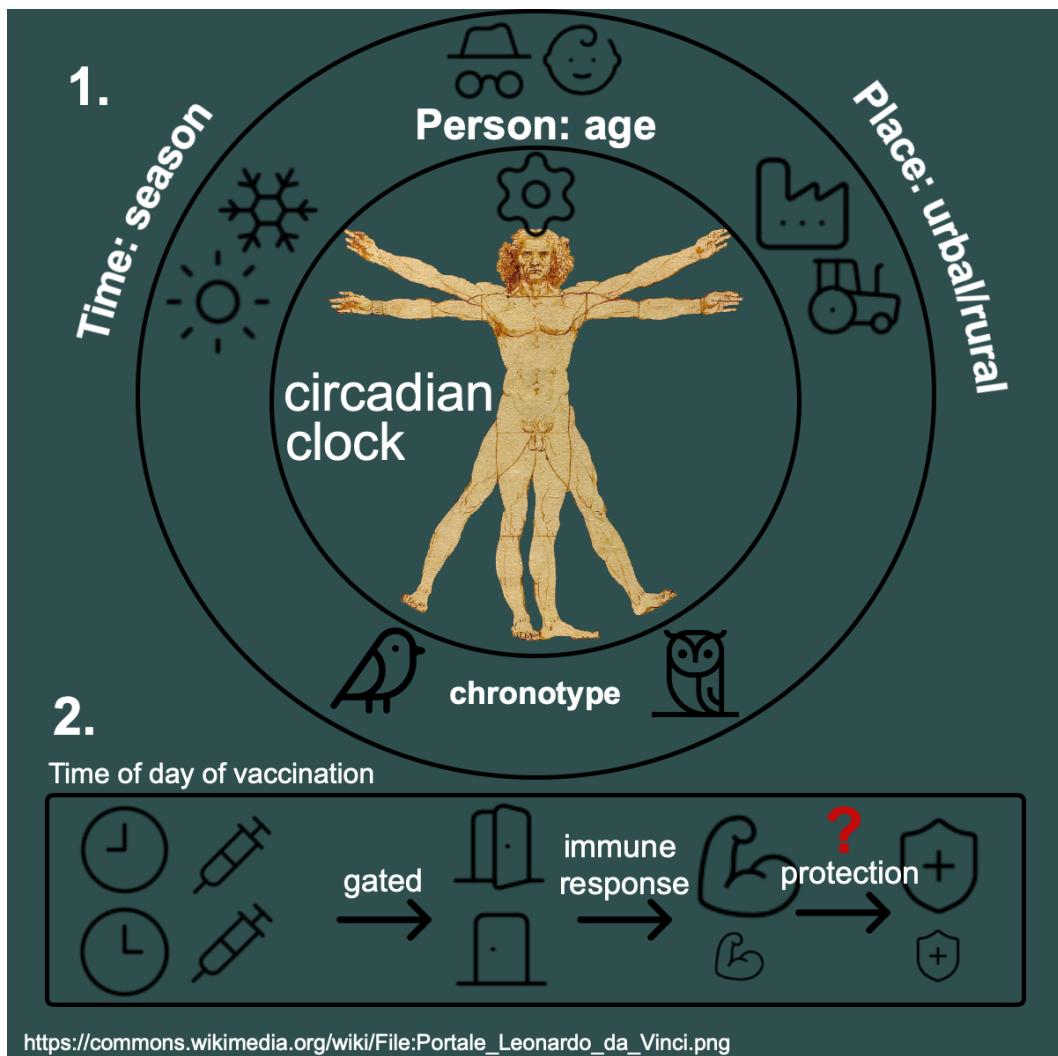


Figure 1: Graphical abstract

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## 1 Introduction

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Researchers in epidemiology and public health commonly use three descriptive variables - Person, Place and Time - to look for associations and health determinants explaining health phenomena. This review highlights the key features of the mammalian circadian clock and its role in the daily gating of our immune function, and uses Person, Place and Time to describe the features that impact the close relationship between circadian clocks, their environment, and our immune system. An understanding of these relationships is crucial to our awareness of confounding factors in epidemiological studies addressing the question as to whether daily variations in our immune response extends to our antibody response to vaccination.

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### 1.1 The circadian clock

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Whether we realise it or not, our internal timing mechanism, or clock, dictates our daily lives by harmonising our physiology with alterations in our external environment, principally the predictable alterations in light and dark that are hallmarks of life on a revolving planet. Simplistically, the clock is a network of clock genes whose sequential transcription and translation progress over a near 24-hour period.

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44 These daily cycles, often referred to as circadian rhythms (derived from the Latin  
 45 ‘circa diem’ or ‘about a day’), orchestrate virtually all aspects of our physiology,  
 46 including sleep-wake cycles, behaviour and locomotor activity, body temperature  
 47 cycles, cardiovascular and digestive processes, endocrine systems and metabolic and  
 48 immune functions<sup>3–8</sup>. A copy of the clock is found in most cell types including cells of  
 49 the immune system<sup>8,9</sup>, but precise and robust coordination of circadian rhythmicity  
 50 is the product of a network of tissue and organ clocks, including a ‘master’ clock –  
 51 primarily influenced by light - situated in the suprachiasmatic nucleus (SCN) of the  
 52 hypothalamus brain region that dictates the overall pace for synchronising ‘slave’  
 53 clocks located in virtually all tissues and organs of the body<sup>10</sup>.

### 54 **1.2 Clock plasticity permits entrainment**

55 Clocks exist in Nature where the environment is in a constant state of flux. Over  
 56 the course of evolution, clocks have developed the capacity to adjust their internal  
 57 rhythms based on fluctuating environmental variables, and a key consequence of  
 58 this plasticity is the ability for circadian clocks to be reset daily, or entrained, by  
 59 these signals, or zeitgebers ('time givers') - notably light, but can be other cues,  
 60 for example temperature, food availability, hormones<sup>11,12</sup> and even social signals<sup>13</sup>.  
 61 Entrainment allows the clock to readapt upon temporary desynchronisation, whether  
 62 this is due to misalignment between an individual's internal circadian phase and their  
 63 environment, for example when crossing multiple Time Zones ('jetlag'), as a result of  
 64 abrupt changes to work patterns, such as shift work, or due to social jetlag<sup>14</sup> - the  
 65 discrepancy between work and free days, between social and biological time. Daylight  
 66 saving is another example of temporary desynchronization in which misalignment  
 67 occurs between endogenous circadian phase and shifted clock time. While the SCN  
 68 master pacemaker itself adjusts to changes in the environment relatively quickly,  
 69 the slave clocks can take longer to adjust<sup>15,16</sup>. Chronic circadian disruption has  
 70 serious implications for human health and can increase the risk for the expression and  
 71 development of neurologic, psychiatric, cardiometabolic, and immune disorders<sup>17,18</sup>.

### 72 **1.3 Clocks, what are they good for?**

73 Broadly speaking biological clocks allow us to adapt to and anticipate temporal  
 74 changes in the environment. Clocks have evolved over billions of years, possibly in  
 75 response to the need for single celled eukaryotes to ‘escape from light’ to avoid the  
 76 damaging effects of ionising radiation and oxidative stress during cell division<sup>19,20</sup>. At  
 77 the core of mammalian clockwork are a set of conserved cellular transcription factor  
 78 proteins operating as interlocked rhythmic transcription-translation feedback loops.  
 79 The formation, cellular trafficking and degradation of different clock protein complexes  
 80 throughout this transcription-translation cycle generates the intrinsic nature and  
 81 stability of the clock, ultimately driving the rhythmic expression of the clock genes  
 82 themselves as well as ‘outputs of the clock’ such as clock-controlled genes<sup>9</sup>. Essentially  
 83 then, the rhythmicity of the core clock radiates out to the physiological outputs of  
 84 the clock via interaction with clock-controlled genes. The clock therefore does not  
 85 orchestrate a single output, but rather partitions physiological outputs to appropriate  
 86 phases of the day. This is beneficial because by timing physiological processes  
 87 appropriately – or ‘gating’ these processes – cellular resources can be effectively and  
 88 efficiently allocated.

89 Clocks provide a competitive advantage for the organisms they serve. The best  
 90 demonstration of this is for experiments in plant circadian biology where for plants  
 91 genetically compromised in the pacing of their clock - and therefore unable to match  
 92 the light and dark conditions of their environment – survived more poorly than  
 93 plants able to correctly match the external light-dark cycle<sup>21</sup>. Clocks anticipate  
 94 predictable changes in the environment (light & dark, warm & cool, for example),  
 95 rather than reacting to change. For example, in plants again, it has been shown that  
 96 photosynthetic machinery is assembled daily before the onset of dawn in anticipation  
 97 of the availability of light so that energy harvesting is optimised<sup>22</sup>. In mammalian

systems the liver and intestine clocks anticipate, rather than react to, mealtimes to coordinate food availability with nutrient processing and energy demand<sup>4,23</sup>.

The ‘clock as a tool’ for optimising performance is increasingly being harnessed in modern life. A good example of this is in the arena of elite sports, often occurring across multiple Time Zones, where adapting training strategies to align with circadian regulation is now viewed as essential. Marginal, yet meaningful, advantages over competition can be gained when clock factors are keyed into training regimes<sup>24</sup>. ‘Tools for the clock’ are also increasingly apparent, for example the development of non-invasive wearable smart devices for characterising the clock, viewed as crucial for advancing personalised medicine – developments that can be viewed as providing an extra edge or dimension via the design of individualised treatment plans tailored to the specific needs of patients<sup>25,26</sup>.

Therefore, clocks themselves inherently provide a competitive edge and, thanks to decades of fundamental research into circadian clock function, we increasingly see clock knowledge harnessed for gaining competitive edges in various societal contexts, many of which are referred to as chronotherapies. Chronovaccination – a vaccination chronotherapy – has the potential to provide a competitive edge in vaccine effectiveness.

#### **1.4 Circadian gating**

A useful concept for the utility of circadian clocks is the idea that they gate biological events to particular times of the day<sup>27</sup>. While circadian gating has different meanings depending on the types of physiology or behaviour under investigation, for example whether entrainment to zeitgebers is gated or the amplitude of rhythms are modulated, gating can be simply thought of as time periods when the clock allows or forbids a particular biological process. Chronotherapy harnesses the principles of circadian gating and has been, for some time now, regarded as a beneficial and wide-ranging socio-economic tool, from the timing of the application of agrochemicals<sup>28</sup>, to the administration of anti-malaria drugs at specific times of the day<sup>29</sup>, to the rhythmic responses to drugs in mammals<sup>30,31</sup>. In the latter case drug timing is optimised to enhance drug efficacy, reduce toxicity and adverse side effects and improve patient outcomes in, for example, cancer treatments<sup>31,32</sup>. A seminal study in 2014 by Zhang *et al.*<sup>30</sup> found that 119 of the WHO’s list of essential medicines target a circadian gene, and since many of these drugs have short half-lives (<6 h), the impact of the time of administration on their action is significant<sup>30</sup>. For example, statins are a class of drug that lower cholesterol levels by inhibiting HMGCR (HMG-CoA reductase)<sup>33</sup>. HMGCR is the rate-limiting enzyme in cholesterol biosynthesis and its activity peaks during the night. Statins with short half-lives showed maximal efficacy when taken in the evening (when their target gene was most active)<sup>30</sup>. There are now numerous examples of health conditions where timed drug treatments align with the circadian gated biological target, so called ‘clocking the drugs’<sup>31</sup>, examples include hypertension, diabetes and anti-inflammation<sup>31,34,35</sup>. Despite this, many in the scientific community still consider ‘time-of-day’ a neglected variable in fundamental biological and clinical research<sup>35–37</sup>. The lack of circadian enlightenment extends to clinical practice where only 4 of the 50 most prescribed drugs in the United States in 2019 had time-of-day dosing recommendations from the Food and Drug Administration, and the 20th World Health Organization’s Model List of Essential Medicines makes no mention of dosing time<sup>38</sup>. There have therefore been calls for time-of-day to be recognised as a key biological variable, similar in status to the ubiquitous sex and age variables, in both clinical studies and clinical practice<sup>35,38,39</sup>. Indeed, chronotherapies that consider time of day may significantly improve clinical trial success and therefore ultimately patient care<sup>39</sup>.

**149 1.5 Clock gating of the immune response**

Innate and adaptive defences constitute two functionally distinct, yet intertwined, arms of defence against pathogenic invasion or insult, and while the former arm is regarded as a first line of defence with no memory of the challenge, adaptive immunity provides more finely-tuned, specific recognition of foreign particles and the selective expansion of cells prepared to target specific pathogens and the development of immunological memory<sup>40</sup>. Vaccinations utilise the adaptive immune system to generate antibodies towards a target antigen as well as the development of ‘immune memory’ - the capacity to respond quicker and more effectively when challenged after an initial exposure<sup>41</sup>. The function of the adaptive immune system is regulated by the circadian clock, with the clock contributing to, for example, the 24-h regulation of homeostatic processes underlying maintenance of adaptive immunity, such as lymphocyte (white blood cell) trafficking<sup>40,42,43</sup>, where the clock essentially modulates leukocyte migration across the body, effectively gating the number of leukocytes at specific sites throughout the body across the day<sup>44</sup>.

It is therefore generally regarded that the circadian clock is a potent regulator of immune function<sup>6–8,40,45</sup>, however less clear is why our immunity is gated by the clock? One idea is that since metabolic and immune processes are intricately linked, over the course of evolution these processes have been partitioned<sup>46</sup>. This is evidenced by disruption of clock gene function resulting in a host of metabolic disorders, which are often associated with immune observable traits, or phenotypes<sup>47</sup>. Another idea is that heightened immune sensitivity is an adaptation to recurring environmental challenges (such as time-of-day prominence of pathogens), with the additional benefit of dimming immune responses at times when they are not needed<sup>9</sup>, chiming with the idea that the metabolic costs of immune activation are monitored closely by the clock so that immunity-associated metabolic costs are kept in check, in turn, enhancing organismal fitness<sup>45</sup>.

There are many examples of the detrimental effects of a disrupted clock on aggravating disease pathology related to our immune system. Employment in rotating shift work is a prime example and has been associated with an increased risk of developing inflammatory diseases such as psoriasis and irritable bowel syndrome<sup>48,49</sup>. It is also well known that chronic inflammatory disease symptoms can show time-of-day variation; for example, asthma, whereby symptoms are exacerbated in the early morning<sup>50</sup>, or increased joint stiffness and pain in the early morning with rheumatoid arthritis sufferers<sup>51</sup>. Other studies have linked social jetlag to higher levels of inflammatory markers, and a study of the effects of circadian and sleep rhythm disruptions on immune biomarkers among hospital healthcare professionals working night shifts and rotating day shifts showed that night workers altered pattern expressions of immune cells biomarkers<sup>52</sup> may increase vulnerability to infections and reduce vaccination efficiency. Increased inflammation can weaken the body’s defences and make individuals more susceptible to infections and reduce vaccine efficiency<sup>52</sup>.

However, while this discussion has focussed on the role of the clock in gating immune function, the influence of sleep on immune function cannot be ignored. Traditionally, clocks, sleep and the immune system have been regarded as separate subjects, but it is increasingly clear the extent of the interconnectivity between circadian rhythms, sleep and immune function, and their implications for public health. A key concept worth considering is that, both circadian rhythm and sleep disruption happen concurrently every day, and this is especially true in modern life through the widespread experiences of shift work, light at night, and social jet lag<sup>53</sup>.

**198 2 Person - Chronotype**

Timekeeping is not universally the same in populations, and we all possess the potential for different ‘settings’ of the clock. These versions – or chronotypes - fall on a continuum, at the either end of which are individuals colloquially referred to

as morning larks and night owls. Larks have a morning type ('morningness') and prefer to awaken early in the morning and feel most active during the earlier parts of the day, while owls have an evening type ('eveningness'), preferring to awaken later in the day and typically feel most active and motivated in the late evening or at night. Individuals who fall between the ends of the continuum are referred to as intermediate types<sup>54</sup>. While chronotype is largely imprinted in small differences in our clock genes, changes in morning/evening predisposition adapts with life span with morning preference during childhood development, evening type in adolescence and early adulthood, then progressively morning preference with age advancement<sup>55,56</sup>. Chronotype is a risk factor for cardiometabolic and mental health, with evening types being more susceptible to conditions such as obesity and bipolar disease<sup>57,58</sup>. A systematic review of evidence has also shown a trend for evening chronotypes to experience more serious symptoms for immune-mediated inflammatory diseases<sup>59</sup>. There is an appreciation that sex as a biological variable might impact immune responses. For example, adult females it seems produce higher antibody responses to diverse vaccines against, influenza, hepatitis B, yellow fever, rabies, herpes, and smallpox viruses<sup>60,61</sup>. However, females may experience greater vulnerability to circadian misalignment from factors like shift work, possibly because females are more likely to be classified as morning types as compared to males meaning that there is potentially more severe misalignment with the 24-hour day-night cycle imposed by shift work<sup>62</sup>.

### 222 3 Place - Circadian clocks and our modern lifestyles

223 Modern life and our environment also shape chronotype. Roenneberg & Merrow<sup>18</sup>  
 224 describe two distinct environmental scenarios influencing clock entrainment; modern/  
 225 industrial and ancestral/rural entrainment of the clock by weak and strong  
 226 zeitgebers, respectively. Modern/industrial entrainment features indoor light and  
 227 artificial light at night and a concomitant reduction in the difference between the  
 228 maximum and minimum amount of light exposure during the day and the level of  
 229 darkness at night. This environment predisposes individuals to later chronotypes  
 230 because of the blurring of the alignment of internal clock rhythms with 24-hour  
 231 day-night patterns. This contrasts with ancestral/rural entrainment featuring bright  
 232 daylight and no artificial light at night, where clock rhythms are effectively synchronised  
 233 to the 24-hour day-night pattern. Seasons also modulate chronotype; in winter,  
 234 chronotype is also later, probably due to a combination of later sunrise and lower  
 235 light levels<sup>63,64</sup>. Therefore, a wide range of factors inherent in modern life and our  
 236 environment influence chronotype – although genetics and age play roles, external  
 237 factors such as light, social activities and school or work schedules can mask our  
 238 endogenous chronotype.

239 Light at night is a hallmark for our modern lifestyle and is thought to be a key driver  
 240 of social jetlag – a condition resembling a mild but chronic form of shift work and  
 241 epidemiological studies show that this correlates with health problems, including  
 242 the tendency towards being overweight. Modern life can therefore be thought of as  
 243 living against the circadian clock in that it induces a sub-optimal form of entrainment,  
 244 a mis-match between the natural rhythm of our light:dark environment and our  
 245 physiology driven by our internal, or endogenous, circadian clock<sup>18</sup>. The evening, or  
 246 'owl' chronotype – typical of teenage years – appears to be particularly at odds with  
 247 our modern society that is skewed towards morning-orientated activities such that  
 248 individuals are forced to live in a state of circadian misalignment, the schism being  
 249 their innate tendency to wake late and the societal demands of attending school or  
 250 work in the morning<sup>65</sup>. A study of chronotypes in school age children (8-12 years)  
 251 showed that evening types have altered sleep patterns, social jet lag, higher BMI and  
 252 higher metabolic risk (higher values of insulin, glucose, triglycerides and cholesterol)<sup>66</sup>.  
 253 Also, while light at night not only shifts populations towards later chronotypes, mod-  
 254 elling studies suggest that it also has the tendency to reinforce evening preference and  
 255 exacerbate circadian and sleep-related problems associated with the owl chronotype<sup>67</sup>.

256 In 2022, around 27% of the UK workforce were employed in the evening or during the  
 257 night<sup>1</sup>, compared to a reported 16% of adults who usually work a non-daytime schedule  
 258 in 2018 in the US<sup>17</sup>. Working indoors, in low-light or artificial light conditions  
 259 with relatively little blue light, does not provide the same light exposure as natural  
 260 daylight, and is a risk factor for disruption to the harmonious relationship between  
 261 our endogenous clock rhythms and the external environment. Natural daylight at high  
 262 intensities has been shown to be particularly beneficial for advancing the timing of  
 263 sleep to earlier hours thereby lengthening the duration of sleep, and for improving  
 264 sleep quality<sup>68</sup>. A 2002 publication estimated that around 70% of US workers are  
 265 employed in indoor work environments<sup>69</sup>.

266 Outside our homes and work environments, the biological effects of light pollution on  
 267 human wellbeing and natural ecosystems are significant, exemplified by a UK Royal  
 268 Commission on Artificial Light in the Environment<sup>2</sup> that recognises the explosive  
 269 growth in outdoor lighting in the UK since the 1940's – and the growing sense of loss  
 270 of 'dark skies' together with the resultant cultural disconnect<sup>70</sup>. The Royal Commis-  
 271 sion comparison of light at night light from 1993 to 2000 shows that almost every area  
 272 in the UK has become brighter, particularly rural areas such that in 2003 only 22%  
 273 of England possessed pristine night skies compared to 77% for Scotland, although  
 274 in Scotland the main populated areas stretching from Glasgow to Edinburgh – the  
 275 'central belt' - shows almost unbroken levels of light pollution, creeping out from the  
 276 cities and towns to blur any distinction between urban and rural areas<sup>3</sup>. The Royal  
 277 Commission also recognised that since light is perceived as a natural and benign  
 278 phenomenon, the insidious and negative issues of 'light blight' on our natural world  
 279 are overlooked when any other anthropogenic impact would naturally provoke stronger  
 280 interogation, although artificial light as a neglected pollutant was recognised as a  
 281 public health issue in a 2023 UK House of Lords Science and Technology Committee  
 282 investigation<sup>4</sup>.

283 Place – rurality or urbanity for example – influences the daily synchronisation of  
 284 our internal endogenous clock with the light:dark environment, conceptually em-  
 285 boldening a harmonised, ancient, form of entrainment or a mis-matched, modern,  
 286 socially jetlagged, form of entrainment, respectively. Although light is thought of  
 287 as the principal zeitgeber, clock epidemiologists have wondered whether social cues  
 288 – hardwired into the fabric of our lives by social constructs such as school or work  
 289 times and governed by digital clock time – can counter natural light:dark cues; after  
 290 all rurality does not preclude a modern lifestyle. Roenneberg *et al.*<sup>13</sup> teased apart  
 291 this question of the relative balance of the coupling of the circadian clock to natural  
 292 'sun time' or to constructed 'social time' in a population sized study – disentangling  
 293 the cues using the principle that within a Time Zone people and live and work to a  
 294 common social time – constant over multiple longitudes – that is at odds with sun  
 295 time that continually changes across longitude. The population study used individuals  
 296 in towns and cities across Germany and measured their responses to a questionnaire  
 297 based around their estimated chronotypes, finding that although the clock was princi-  
 298 pally entrained by sun time, individuals in lightly populated areas were more tightly

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<sup>1</sup> Office for National Statistics (ONS) The Night-Time Economy, UK: 2022 <https://www.ons.gov.uk/businessindustryandtrade/business/activitysizeandlocation/articles/thenightimeconomyuk/2022>

<sup>2</sup> The Royal Commision on Environmental Pollution. Artificial Light in the Environment (2009) <https://www.gov.uk/government/publications/artificial-light-in-the-environment>

<sup>3</sup> Campaign to Protect Rural England. Night Blight: Mapping England's light pollution and dark skies (2016) <https://www.cpre.org.uk/resources/night-blight-2016-mapping-england-s-light-pollution-and-dark-skies/>

<sup>4</sup> House of Lords Science and Technology Committee 2nd Report of Session 2022–23. The neglected pollu-  
 tants: the effects of artificial light and noise on human health (2023) <https://committees.parliament.uk/publications/40937/documents/199438/default/>

299 coupled to sun time than those living in densely populated areas, leading the authors  
 300 to conclude that the uncoupling was a reflection of the emboldening of social cues  
 301 versus nature's light–dark cycles. Tipping this balance from environmental towards  
 302 behavioural light–dark cycles is predicted to lead to later chronotypes, a characteristic  
 303 increased population size<sup>13</sup>. Our preference for aligning our clocks to sun time rather  
 304 than clock time is seen with the effects of travel across Time Zones, where the clock  
 305 dynamically re-entrains to external light/dark cycles and environmental cues in their  
 306 new environment, with faster recovery from jetlag after westward, as opposed to  
 307 eastward, travel, possibly as a result of a preference for the clock to delay, rather  
 308 than advance, its entrainment<sup>71</sup>. Where we reside in a Time Zone contributes to  
 309 our harmony with sun time – living on the western edge of a Time Zone where light  
 310 later in the morning extends to later in the evening because of delayed solar time  
 311 can result in circadian misalignment resulting in significant deleterious effects on  
 312 human health outcomes<sup>72,73</sup>. For example, cancer-specific mortality has shown to be a  
 313 function of the distance from the eastern border of a Time Zone, with risks increasing  
 314 and longevity decreasing from east to west<sup>74</sup>. Reductions in sleep duration due to  
 315 extra natural light at night because of the ‘western edge effect’ was also found to  
 316 have significant adverse effects on human health and on economic performance (per  
 317 capita income)<sup>75</sup>. Spain, geographically aligned with Greenwich Mean Time (GMT),  
 318 operates on Central European Time (CET), while neighbouring Portugal operates on  
 319 GMT. This discrepancy between social time and sun time leads to later bedtimes and  
 320 meal schedules in regions northwestern Spain, potentially affecting sleep and overall  
 321 circadian rhythmicity<sup>75</sup>.

#### 322 **4 Time – Immune function**

323 Despite adaptive immunity occurring over a long period of time compared to the  
 324 innate ‘first line of defence’ response – weeks compared to days<sup>76</sup> – studies have  
 325 suggested that the timing of the initial immune stimulus can have lasting effects on  
 326 immune (patho)physiology<sup>76–79</sup>. This has led to the question as to whether daily  
 327 immune rhythms could be used to target the adaptive immune system at the time of  
 328 highest sensitivity to bring about enhanced vaccination responses<sup>80</sup>. Experimental  
 329 evidence for variation in antibody production after vaccination has been obtained<sup>81–85</sup>,  
 330 for example a large, randomised trial of different times of vaccination showed that  
 331 morning vaccination enhanced the antibody response to the influenza vaccine<sup>84</sup>. There  
 332 has been some scepticism about the role of the clock in the development of adaptive  
 333 immunity<sup>86</sup> – after all, how can a 24-h rhythm influence adaptive immunity responses  
 334 forming over days and weeks? However, the step-by-step generation of the adaptive  
 335 immune response over several weeks has been traced, where it was found that the  
 336 timing of the initial immune challenge was imprinted on all subsequent downstream  
 337 processes<sup>76</sup>. Taken together these studies suggest a ‘sweet spot’ at or just before  
 338 the behavioural activity phase for optimal vaccination – correlating with morning  
 339 vaccination timing for human subjects.

340 While daily timing is the focus of TODV, it seems that immune function is also sen-  
 341 sitive to another predictable timing dimension – seasonality – the result of the axial  
 342 tilt of the Earth combined with its orbital path around the Sun. A large population  
 343 study of UK Biobank participants not only showed daytime variation in immune  
 344 parameters, but also a seasonal variation, with lymphocyte numbers showing the  
 345 greatest contrast<sup>64</sup>. Daylength modulates with season and both the intensity and  
 346 spectral composition of light to which people are exposed vary with season<sup>87</sup>, and  
 347 while direct evidence for seasonality in humans is limited<sup>88</sup>, in animals the seasonal  
 348 clock is generated by changes in thyroid hormones in the brain that respond to day  
 349 length as signalled by the pineal hormone melatonin. A study collecting light quality  
 350 across seasons using wearable colour-sensitive ‘actiwatch’ devices in human subjects  
 351 showed that summer subjects were exposed to twice as much blue light than winter  
 352 subjects<sup>87</sup>. The spectral sensitivity of the photopigment melanopsin, located in the

353 human inner retina is known to be greatest for blue light and has been shown to  
 354 mediate many aspects of non-vision related human behaviour and physiology such  
 355 as sleep timing and daily melatonin rhythms<sup>87</sup>. The relative contribution of blue  
 356 light is therefore greater in summer compared to winter, and while blue-enriched light  
 357 has positive effects on sleep quality, alertness and performance<sup>89</sup>, the timing of blue  
 358 light is important such that exposure at night with the overuse of electronic devices  
 359 rich in LED exacerbates social jetlag<sup>90,91</sup>, the resultant disrupted clock affecting the  
 360 timing of hormone secretion, the activity of immune cells, and body temperature, and  
 361 changes in mood at different times of day and night<sup>92</sup>, and has been shown to be a  
 362 risk factor for psychological disorders<sup>93</sup>, and obesity<sup>94</sup>.

363 In comparison to the well characterised circadian clocks, other biological timers –  
 364 circatidal clocks, linked to the ~12.4-h tidal cycle, and circalunar clocks, cycling every  
 365 ~29.5 days – are less well understood and hotly debated to the present day<sup>95</sup>. Both  
 366 circalunar rhythms and circatidal rhythms are widespread in marine environments,  
 367 but recent evidence suggests their potential influence on human health<sup>96</sup>. Experiments  
 368 in sleep laboratories, in which external light sources such as moonlight were systemati-  
 369 cally excluded, show the effect of the lunar cycle on sleep, with deep sleep patterns  
 370 and total sleep time negatively and significantly reduced around full moon<sup>97</sup>. Circati-  
 371 dal clocks are used by marine organisms to anticipate changes linked with the ebb and  
 372 flow of tides, such as water levels, food availability, currents and temperature<sup>95</sup> and  
 373 a recent seminal study in humans intriguingly demonstrated 12-h rhythms in gene  
 374 transcript levels<sup>98</sup>. However there remains a debate as to the potential function of a  
 375 12-h mammalian clock - after all why would terrestrial animals need a ~12 h rhythm  
 376 generator if they are not subjected to tides. One line of thinking is that a mammalian  
 377 12-h clock is either an evolutionary remnant of the marine circatidal clock or has  
 378 evolved convergently, and that it facilitates the ‘rush hour’ processing of elevated  
 379 gene expression and processing around dawn and dusk<sup>99</sup>. The clear characterisation  
 380 of circatidal and circalunar rhythmicity in humans is challenging at the practical  
 381 level, requiring strict control of light sources, as well as meal and sleep timing, and  
 382 there remains the problem of elucidating if 12-h rhythms are mechanistically driven  
 383 by the circadian clock, rather than by a dedicated oscillator, or simply driven by  
 384 environmental cues such as light<sup>95</sup>. A link between immune function and the potential  
 385 twice daily circatidal rhythm anticipation of metabolic stress or circalunar rhythm  
 386 sleep pattern modulation has not been made. Immunity, sleep, and metabolism are  
 387 however intricately intertwined and understanding the influence of ultradian rhythms  
 388 (period shorter than a day) and infradian rhythms (period longer than a day) on  
 389 immune function homeostasis will pave the way to appreciating immune processes  
 390 across different timescales.

## 391 5 Person, Place & Time

392 Populations therefore feature a rich mixture of chronotypes, young/old clocks with  
 393 natural variation in our genetically predetermined lark and owl propensities in-  
 394 teracting with, and ultimately being shaped by, their surroundings; for example,  
 395 rural/urban settings and summer/winter light environments. Generally, less is known  
 396 about how ‘slave’ clocks, in peripheral organs (brain, heart, liver, gut, pancreas,  
 397 adipose tissue, adrenal glands, lungs, and skeletal muscle<sup>12</sup>) entrain with the SCN  
 398 ‘master’ clock and how potential misalignment mechanistically contributes to human  
 399 health. Roenneberg & Merrow<sup>18</sup> point out, however, that there is likely to be a con-  
 400 stellation of possible phase entrainments, or ‘phase maps’, describing the entrainment  
 401 between the master and slave clocks; reflecting the idea that we entrain differently  
 402 at different times of our lives, at different times of the year and amongst each other,  
 403 and whether we live in cities or in the country<sup>18</sup>. Table 1 summarises Person, Place &  
 404 Time variables relevant to chronovaccination studies.

Table 1: Person, Place &amp; Time variables for chronovaccination studies

## 405 6 Chronovaccination

406 Naturally, the question as to whether daily variations in our immune response extends  
 407 to our antibody response to vaccination has been posed. Early work showed mixed ev-  
 408 idence for effects of time of vaccination on antibody response, possibly as a reflection  
 409 of low sample sizes, variable design, short-term follow-up, and differences in vaccine  
 410 types. However, a large-scale cluster-randomised trial examined the influence of the  
 411 timing of vaccination on influenza antibody titres and found that morning vaccination  
 412 enhanced the antibody response to the influenza vaccine<sup>84</sup>. Using a prospective  
 413 cohort study design Zhang *et al.*<sup>100</sup> showed that vaccination in the morning lead to a  
 414 stronger immune response to SARS-CoV-2 vaccine. In their 2022 review, Otasowie *et*  
 415 *al.*<sup>1</sup> coined the term ‘chronovaccination’ – a term encapsulating the evidence of clock  
 416 gating of the immune response and the potential of TODV for maximising vaccine  
 417 immunogenicity. The authors highlighted four clinical studies demonstrating TODV,  
 418 including the aforementioned first large-scale randomised controlled trial assessing  
 419 the effect of different vaccination times for the influenza vaccine<sup>84</sup>, an assessment  
 420 of the effect of TODV on specific and non-specific immunity induced by the TB  
 421 vaccine, Bacillus Calmette-Guérin (BCG)<sup>81</sup>, and for two prospective cohort studies,  
 422 including the aforementioned Zhang *et al.* study, assessing antibody responses after  
 423 administration of SARS-CoV-2 vaccines at different phases of the day<sup>100,101</sup>.

424 The Otasowie *et al.*<sup>1</sup> review, however, sets out conflicting evidence for the optimum  
 425 TODV. The work by Zhang *et al.*<sup>100</sup> demonstrated that vaccination in the morning  
 426 (9 am - 11 am) compared to vaccination in the afternoon (3 pm - 5 pm) lead to a  
 427 stronger immune response to an inactivated SARS-CoV-2 vaccine (BBIBP-CorV,  
 428 Sinopharm), while in contrast Wang *et al.*<sup>101</sup> showed, with vaccinating mRNA or  
 429 Adenovirus based SARS-CoV-2 vaccines (Pfizer, mRNA bnt162b2 or AstraZeneca,  
 430 Adenoviral AZD1222) in a larger cohort, that antibody responses were higher in those  
 431 who were vaccinated later in the day (11 am - 10 pm) compared to those vaccinated  
 432 in the morning (7 am - 11 am). Wang *et al.*<sup>101</sup> speculated that the use of the inac-  
 433 tivated whole virus in the Zhang *et al.*<sup>100</sup> study might explain the difference in the  
 434 TODV outcomes, and note several other potential factors complicating interpretation  
 435 of the outcomes including altered sleep and shift-work patterns of the healthcare  
 436 worker participants of the study potentially influencing vaccine responses.

437 Recently Hazan *et al.*<sup>102</sup> carried out a retrospective large population cohort analy-  
 438 sis of timestamped COVID-19 vaccinations (1.5M individuals, age > 12 yo, 99.2%  
 439 receiving Pfizer-produced BNT162b2), using COVID-19 breakthrough infection and  
 440 COVID-19-associated emergency department visits and hospitalisations as endpoints  
 441 for cohorts split by vaccination timing (morning, afternoon, or evening). This study  
 442 found lowest rates of breakthrough infection with late morning to early afternoon  
 443 vaccination and highest rates associated with evening vaccination. Vaccination timing  
 444 remained significant after adjustment for patient age, sex, and comorbidities. The ben-  
 445 efits of daytime vaccination were concentrated in younger (<20 years old) and older  
 446 patients (>50 years old)<sup>102</sup>. Although vaccinations were not performed across 24-h  
 447 cycles, the authors were able to demonstrate that the risk of COVID-19 breakthrough  
 448 infection as a function of vaccination timing was sinusoidal in shape, displaying a  
 449 periodicity of between 7.4 and 16.7 hours, considerably shorter than the classical 24-h  
 450 periodicity of circadian rhythms, suggesting that rhythms in vaccine effectiveness are  
 451 potentially ultradian in nature, for example driven by a mammalian 12-h clock<sup>102</sup>.  
 452 Another retrospective large population cohort study (0.25M individuals, age < 6  
 453 yo), this time for the varicella vaccine, showed that vaccination in the morning and

454 afternoon was associated with lower infection rates than evening vaccination<sup>103</sup>. Like  
 455 that for the risk of COVID-19 breakthrough infection, varicella infection risk, after  
 456 adjusting for ethnicity, sex, immunodeficiency, and obesity, followed a sinusoidal  
 457 pattern consistent with a diurnal rhythm in vaccine effectiveness.

458 Two independent systematic reviews<sup>2,104</sup> have surveyed published studies on the  
 459 human immune response to vaccination at different times of day in order to gauge the  
 460 evidence supporting diurnal variation in the effectiveness of vaccination. Wyse *et al.*<sup>2</sup>  
 461 noted the challenge in generalising the findings based on the heterogeneity of the stud-  
 462 ies surveyed, however the authors concluded that overall there was insufficient overall  
 463 evidence that administration of vaccines at different times of day affected immune  
 464 outcomes. On the other hand, Vink *et al.*<sup>104</sup> concluded that that morning vaccination  
 465 enhanced antibody responses in adults aged 60 years and older, noting that this age  
 466 group is a key demographic for influenza and COVID-19 vaccination. Studies qualified  
 467 for inclusion in the Vink *et al.* review if they measured antigen-specific antibody or  
 468 T-cell responses following vaccination, and if these immune responses were compared  
 469 between participants vaccinated at different time points during the day, whereas for  
 470 Wyse *et al.* studies were included if they reported any immune or clinical outcome  
 471 following vaccination at a defined time of day.

472 The Wyse *et al.*<sup>2</sup> review assessed over 3,100 studies, 23 of which met their inclusion  
 473 criteria. These studies were published between 1967 and 2024 and almost half of them  
 474 included reported data collected for SARS-CoV-2 vaccination programs during the  
 475 COVID-19 pandemic. The review authors found that most studies were biased by  
 476 failing to account for immune status prior to vaccination, self-selection of vaccination  
 477 time, or due to the presence of confounding factors such as sleep, chronotype, and  
 478 shift work. Of these 23 studies, the optimum TODV was concluded to be afternoon  
 479 (5 studies), morning (5 studies), morning and afternoon (1 study), midday (1 study),  
 480 and morning or late afternoon (1 study), with the remaining 10 studies reporting  
 481 no effect. Of the studies that reported an association between TODV and outcome  
 482 of vaccination, 3 presented data that could be used to estimate the size of this  
 483 effect<sup>100,102,105</sup>. For example, Hazan *et al.*<sup>102</sup> estimated that optimising the time of  
 484 vaccination might improve vaccine effectiveness by 8.6–25%.

485 Vink *et al.*<sup>104</sup> identified 860 records through their literature search, 17 of which  
 486 met their eligibility criteria; five investigated the effect of vaccination timing on  
 487 immune responses to influenza vaccines<sup>84,85,106–108</sup> and nine explored how time of  
 488 day influences immune responses to SARS-CoV-2 vaccines<sup>100,101,105,109–114</sup>. Eleven  
 489 out of the 17 studies reported statistically significant effects of vaccination timing,  
 490 with ten reporting stronger antibody responses following morning vaccination, while  
 491 one study favoured vaccination later in the day. The strongest evidence for diurnal  
 492 variation was found for influenza vaccines in older adults; sub-group analysis for age  
 493 stratification in the included studies consistently showed stronger antibody responses  
 494 for 60-year-olds or older for morning vs afternoon vaccination. Pooled results from  
 495 two randomised controlled trials<sup>84,106</sup> for influenza vaccination showed a statistically  
 496 significant small-to-medium standardised mean difference (SMD) in antibody titers  
 497 for adults aged 65 and older (effect size; SMD = 0.32, 95% CI = 0.21–0.43), with  
 498 morning vaccination consistently yielding higher titers 1 month post-vaccination.  
 499 However, Vink *et al.*<sup>104</sup> caution over-interpretation of this reported effect size due to  
 500 the limited number of randomised controlled trials used in the meta-analysis.

## 501 7 Discussion

502 The role of circadian rhythms in many diverse strands of modern society are increas-  
 503 ingly apparent, in areas such as health and well-being - from increasing understanding  
 504 of chronic clock disruption (e.g., shift work, social jet lag) through to optimal living  
 505 – the integration of personal circadian rhythms into daily schedules for eating, exer-  
 506 cising, and sleeping to maximise health and performance, increasingly regarded as a

‘game changer’ in elite sports regimes<sup>24</sup>. Another example, in agriculture – harnessing knowledge of crop clocks to increase yields and resilience, more and more topical with the gathering storm of climate change and food security challenges<sup>28</sup>. Both examples under the cloak of cultural disconnect from natural cycles of light and dark and the insidious creep of artificial light, including blue light at inappropriate phases of our day. Modern lifestyles embolden living against our natural clock settings, yet decades of fundamental scientific research in biological clockwork, rewarded with the 2017 Nobel prize in Physiology or Medicine<sup>5</sup>, are offering opportunities to harness aspects of clock function as chronotherapies. One such therapy – chronovaccination - might offer an extra edge in vaccine effectiveness. However, is the current evidence strong enough to support the idea that it can be a low-cost, low-risk strategy for boosting vaccine effectiveness? The case for chronovaccination – surveyed in systematic reviews<sup>2,104</sup> – is somewhat patchy. While the influence of TODV is compelling in animal studies where environmental conditions are strictly controlled<sup>76,80</sup>, human populations feature a panoply of individual clock types set in a multitude of environments. Young clocks, teenage clocks, old clocks, with their spectrum of lark and owl clock ‘settings’. Clocks in rural and modern industrial environments, seasonal clocks, socially jetlagged clocks - all muddling interpretation of human circadian studies assessing the effect of TODV. Large population cohort epidemiological studies have been successful in teasing apart relationships between our endogenous clock function and the environment – for example revealing the association between where we live in a Time Zone and chrono-disruption<sup>13</sup>. Population-level approaches have also been used in chronovaccination studies<sup>102,103</sup>, but report modest effect sizes and suffer from potential biases due to non-randomisation of cohorts. Vink *et al.*<sup>104</sup> demonstrated that the strongest evidence for diurnal variation in vaccination effectiveness was found for influenza vaccines using randomised control trials and Wyse *et al.*<sup>2</sup> argue that large population randomised trials are the ideal, with the power of thousands of participants enabling adjustment for multiple demographic and lifestyle factors that might otherwise confound detection of an effect of TODV. These would be costly enterprises, but Wyse *et al.*<sup>2</sup> reason that the onus of determining whether effectiveness is a function of the TODV should fall to the vaccine producers during clinical trials.

Even if there was a compelling advantage of TODV, would it be readily integrated into vaccination programmes? One only has to look at the debate around Daylight Saving Time (DST)<sup>115</sup> to understand the sensitive nature of policies that have disruptive effects on social routines. Another relevant example is the idea of later school start times for adolescents – designed to align better with their clock driven ‘owl’ behaviours – that illustrates how interventions, however well intended, can shift the problem onto other groups of individuals, in this case later scheduling working against teachers and parents own chronotypes and social schedules. Would the potential benefit of TODV justify its disruption to well-established vaccination programming? On the one hand boosted vaccine effectiveness can help improve vaccine hesitancy, with hesitancy identified by the WHO as one of the 10 threats to global health<sup>6</sup>. On the other hand, the convenience of accessing vaccinations is an important factor for vaccine uptake<sup>2</sup>, and programs scheduled across much of the day offer individual choice and convenience that may be expected in a 24-7 society. Conceivably immunisation initiatives more narrowly aligned to an optimum TODV may be detrimental to vaccine uptake, although nudging based interventions have shown potential in bolstering intention to vaccinate<sup>116</sup>, and may therefore be a useful strategy for fostering confidence in time-of-day vaccinations.

<sup>5</sup> Nobel Foundation, The Nobel Prize in Physiology or Medicine 2017 - Press Release <https://www.nobelprize.org/prizes/medicine/2017/summary/>

<sup>6</sup> Understanding the Behavioural and Social Drivers of Vaccine Uptake, World Health Organization (2022) <https://www.who.int/publications/i/item/who-wer9720-209-224>

556 While the benefits of TODV across whole populations remains marginal, a clearer  
 557 case can be made for chronovaccination in adults 60 years and older, where morning  
 558 vaccination was more beneficial<sup>104</sup>. It has been speculated that immunosenescence  
 559 - the gradual age-related decline in both innate and adaptive immune function -  
 560 may play a role<sup>106,117</sup>. Similarly, immunosuppressed individuals might benefit from  
 561 TODV-mediated boosted vaccine effectiveness<sup>113,114</sup>, suggesting that TODV may  
 562 find a role in targeted age group and co-morbidity cohorts. The potential of infant  
 563 chronovaccination is largely an unexplored area, with most studies focussed on adult  
 564 COVID-19 and influenza programmes. A recent study, however, examined diurnal  
 565 patterns of varicella vaccine effectiveness in children - finding that immunisation  
 566 during the late morning to early afternoon led to fewer breakthrough infections  
 567 compared with those vaccinated in the evening<sup>103</sup>. And what of urban-rurality?  
 568 How might 'Place' - the extent of coupling to 'sun time' - influence sensitivity to  
 569 TODV? This and the influence of seasonality, for example, on TODV are largely  
 570 unexplored and might contribute to understanding the complex relationship between  
 571 the environment, clocks, and immune function. Although a discussion of the role  
 572 of sleep in immune function, and therefore its contribution chronovaccination, is  
 573 not developed in this review, Livieratos *et al.*<sup>118</sup> have reviewed the evidence of the  
 574 impact of circadian and sleep factors on influenza vaccine-induced immune responses,  
 575 emphasising that yet another variable – sleep duration – should be considered for  
 576 understanding vaccine-induced antibody responses.

577 It was hoped that the award of the clock biology Nobel Prize in 2017 would emphasise  
 578 the time-of-day reporting in biology and medicine. However, Nelson *et al.*<sup>37</sup> report  
 579 that the time-of-day variable remains largely ignored. Much commentary on the  
 580 future direction of chronovaccination emphasise the importance of systematically  
 581 recording the time-of-day at which vaccines are administered, yet to many chronobi-  
 582 ologists it remains a puzzle why such a fundamental biological variable is regularly  
 583 overlooked in the first place. One factor may be the increased resources and costs that  
 584 accompany properly designed multi time-point circadian assays; however circadian  
 585 biologists would no doubt argue that biological studies cannot be reliably replicated –  
 586 a fundamental tenet of science – unless time-of-day is recorded. Another important  
 587 consideration is that internal biological time (circadian phase) can vary considerably,  
 588 the consequence of modern lifestyles and the high chance of desynchrony due to  
 589 many of the Person, Place and Time factors described here (Table 1). For example,  
 590 nurses' circadian rhythms in body temperature have been shown to be significantly  
 591 misaligned due to shift working patterns, causing work and sleep schedules to be  
 592 out of phase with each other<sup>119</sup>. Both external time of vaccination and internal  
 593 (biological) time recording are therefore important factors, with internal time record-  
 594 ing representing a particular technical challenge<sup>53</sup>. However, research on circadian  
 595 wearable devices that continuously track body movement, heart rate, temperature,  
 596 and light exposure has increased significantly over the past decade, are continually  
 597 improving and offer a non-invasive method to capturing real-life circadian rhythms<sup>26</sup>.

598 'Time will tell' if chronovaccination finds a role in delivering an edge to vaccine effec-  
 599 tiveness. There remain many challenges, not only in establishing clear clinical benefits,  
 600 but other hurdles akin to practical and societal issues. The general public's growing  
 601 interest of the role of circadian rhythms in relation to health and well-being, or  
 602 'circadian hygiene', might ultimately drive individual awareness of optimal times for  
 603 vaccination. Therefore, as Vink *et al.*<sup>104</sup> allude to in their review, the future vaccine  
 604 customer may well regard optimal vaccination as an hourly continuous variable in  
 605 relation to their personal circadian phase rather than the more blunt binary (morning  
 606 vs afternoon) variable we currently consider.

## 607 8 Conclusions

608 Chronovaccination studies have been demonstrably hampered by the panoply of  
 609 confounding factors inherent to population structures and our modern lifestyles.  
 610 Understanding endogenous human biological time-keeping in the context of their  
 611 exogenous settings is likely key to disentangling the relative influences of covariates.  
 612 Nonetheless, TODV as a chronotherapy shows promise for older age and immuno-  
 613 suppressed groups, and while the effect size may prove to be modest, their practical  
 614 significance could be viewed as a marginal gain in vaccine effectiveness, in the same  
 615 way that knowledge of clockwork is harnessed as a competitive advantage in numerous  
 616 other areas of society. Key hurdles in the adoption of chronovaccination are whether  
 617 society buys into the idea and whether health agencies conclude that the fragility of  
 618 vaccine uptake might ultimately be further jeopardised by changes to well established  
 619 vaccination programmes.

## 620 9 Acknowledgements

621 I recognise that the scope of this review, due to space restrictions, prevents the  
 622 citation of every relevant publication. I apologise for any omissions of important work  
 623 in this topic area.

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