

**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 immunology¹, unequivocal evidence for clinical benefit outweighing other
9 societal and practical considerations has yet to be established². 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.

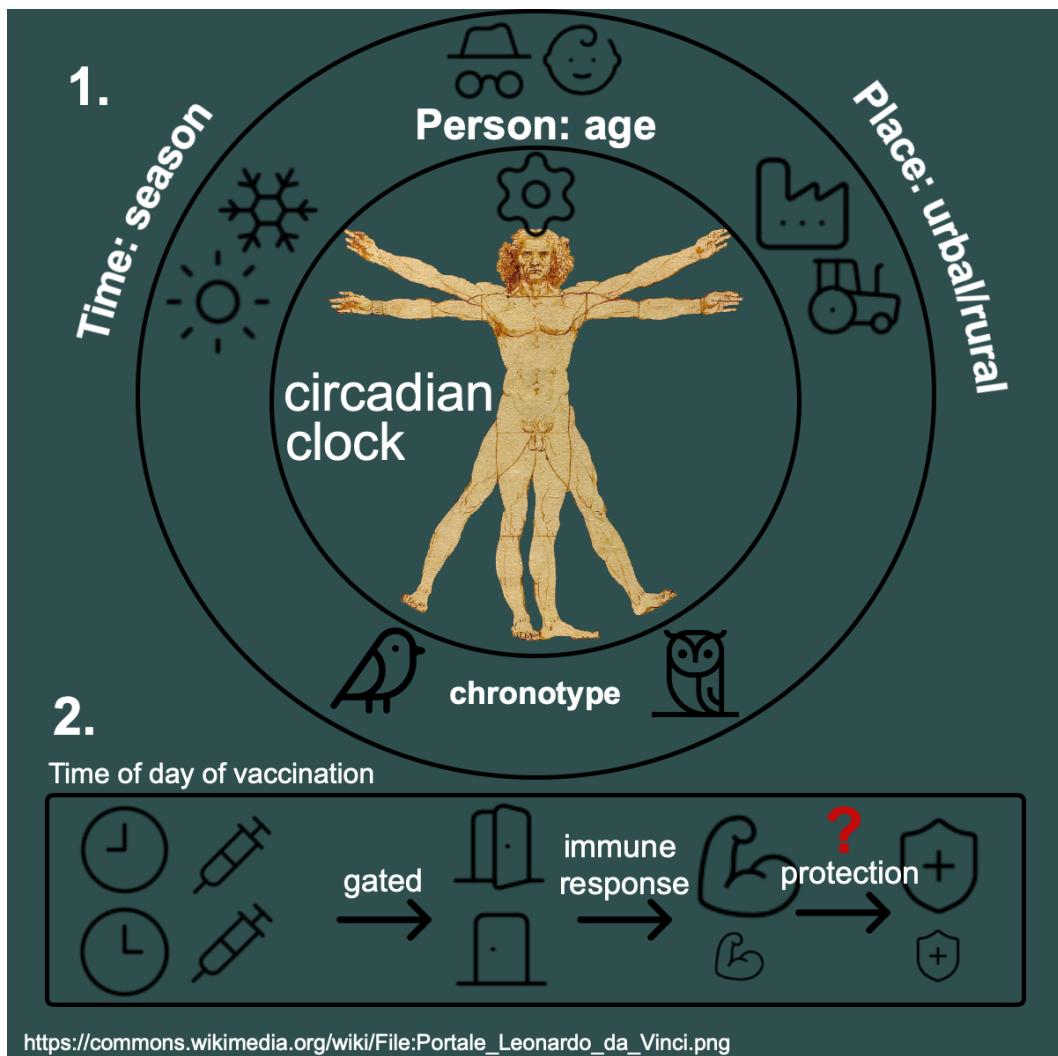


Figure 1: Graphical abstract

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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^{3–8}. A copy of the clock is found in most cell types including cells of
 49 the immune system^{8,9}, but precise and robust coordination of circadian rhythmicity
 50 is the product of a network of tissue and organ clocks, including a central 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 peripheral
 53 clocks located in virtually all tissues and organs of the body¹⁰.

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^{11,12} and even social signals¹³.
 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 jetlag¹⁴ - 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 central pacemaker itself adjusts to changes in the environment relatively quickly, the
 69 peripheral clocks can take longer to adjust^{15,16}. 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^{17,18}.

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^{19,20}. 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 genes⁹. 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 cycle²¹. 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 optimised²². In mammalian

systems the liver and intestine clocks anticipate, rather than react to, mealtimes to coordinate food availability with nutrient processing and energy demand^{4,23}.

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 regimes²⁴. ‘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^{25,26}.

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 day²⁷. 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 agrochemicals²⁸, to the administration of anti-malaria drugs at specific times of the day²⁹, to the rhythmic responses to drugs in mammals^{30,31}. 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^{31,32}. A seminal study in 2014 by Zhang *et al.*³⁰ 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 significant³⁰. For example, statins are a class of drug that lower cholesterol levels by inhibiting HMGCR (HMG-CoA reductase)³³. 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)³⁰. There are now numerous examples of health conditions where timed drug treatments align with the circadian gated biological target, so called ‘clocking the drugs’³¹, examples include hypertension, diabetes and anti-inflammation^{31,34,35}. Despite this, many in the scientific community still consider ‘time-of-day’ a neglected variable in fundamental biological and clinical research^{35–37}. 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 time³⁸. 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^{35,38,39}. Indeed, chronotherapies that consider time of day may significantly improve clinical trial success and therefore ultimately patient care³⁹.

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 memory⁴⁰. 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 exposure⁴¹. 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^{40,42,43}, where the clock essentially modulates leukocyte migration across the body, effectively gating the number of leukocytes at specific sites throughout the body across the day⁴⁴.

It is therefore generally regarded that the circadian clock is a potent regulator of immune function^{6–8,40,45}, 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 partitioned⁴⁶. 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 phenotypes⁴⁷. 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 needed⁹, 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 fitness⁴⁵.

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^{48,49}. 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 morning⁵⁰, or increased joint stiffness and pain in the early morning with rheumatoid arthritis sufferers⁵¹. 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 biomarkers⁵² 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 efficiency⁵².

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 lag⁵³.

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 types⁵⁴. 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^{55,56}. Chronotype is a risk factor for cardiometabolic and mental health, with evening types being more susceptible to conditions such as obesity and bipolar disease^{57,58}. A systematic review of evidence has also shown a trend for evening chronotypes to experience more serious symptoms for immune-mediated inflammatory diseases⁵⁹. 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^{60,61}. 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 work⁶².

222 3 Place - Circadian clocks and our modern lifestyles

223 Modern life and our environment also shape chronotype. Roenneberg & Merrow¹⁸
 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^{63,64}. 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 clock¹⁸. 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 morning⁶⁵. 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)⁶⁶.
 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 chronotype⁶⁷.

256 In 2022, around 27% of the UK workforce were employed in the evening or during the
 257 night¹, compared to a reported 16% of adults who usually work a non-daytime schedule
 258 in 2018 in the US¹⁷. 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 quality⁶⁸. A 2002 publication estimated that around 70% of US workers are
 265 employed in indoor work environments⁶⁹.

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 Environment² 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 disconnect⁷⁰. 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 areas³. 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 investigation⁴.

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.*¹³ 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

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

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

³ 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/>

⁴ 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 size¹³. 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 entrainment⁷¹. 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^{72,73}. 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 west⁷⁴. 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)⁷⁵. 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 rhythmicity⁷³.

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 days⁷⁶ – studies have
 325 suggested that the timing of the initial immune stimulus can have lasting effects on
 326 immune (patho)physiology^{76–79}. 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 responses⁸⁰. Experimental
 329 evidence for variation in antibody production after vaccination has been obtained^{81–85},
 330 for example a large, randomised trial of different times of vaccination showed that
 331 morning vaccination enhanced the antibody response to the influenza vaccine⁸⁴. There
 332 has been some scepticism about the role of the clock in the development of adaptive
 333 immunity⁸⁶ – 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 processes⁷⁶. 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 contrast⁶⁴. Daylength modulates with season and both the intensity and
 346 spectral composition of light to which people are exposed vary with season⁸⁷, and
 347 while direct evidence for seasonality in humans is limited⁸⁸, 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 subjects⁸⁷. 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 rhythms⁸⁷. 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 performance⁸⁹, 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^{90,91}, 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 night⁹², and has been shown to be a
 362 risk factor for psychological disorders⁹³, and obesity⁹⁴.

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 day⁹⁵. Both
 366 circalunar rhythms and circatidal rhythms are widespread in marine environments,
 367 but recent evidence suggests their potential influence on human health⁹⁶. 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 moon⁹⁷. 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 temperature⁹⁵ and
 373 a recent seminal study in humans intriguingly demonstrated 12-h rhythms in gene
 374 transcript levels⁹⁸. 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 dusk⁹⁹. 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 light⁹⁵. 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 secondary oscillators, in peripheral organs (brain, heart, liver, gut, pan-
 397 creas, adipose tissue, adrenal glands, lungs, and skeletal muscle¹²) entrain with the
 398 SCN central pacemaker and how potential misalignment mechanistically contributes
 399 to human health. Roenneberg & Merrow¹⁸ point out, however, that there is likely
 400 to be a constellation of possible phase entrainments, or ‘phase maps’, describing
 401 the entrainment between the central and peripheral clocks; reflecting the idea that
 402 we entrain differently at different times of our lives, at different times of the year
 403 and amongst each other, and whether we live in cities or in the country¹⁸. Table 1
 404 summarises Person, Place & Time variables relevant to chronovaccination studies.

Table 1: Person, Place & 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 vaccine⁸⁴. Using a prospective
 413 cohort study design Zhang *et al.*¹⁰⁰ 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.*¹ 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 vaccine⁸⁴, an assessment
 420 of the effect of TODV on specific and non-specific immunity induced by the TB
 421 vaccine, Bacillus Calmette-Guérin (BCG)⁸¹, 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^{100,101}.

424 The Otasowie *et al.*¹ review, however, sets out conflicting evidence for the optimum
 425 TODV. The work by Zhang *et al.*¹⁰⁰ 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.*¹⁰¹ 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.*¹⁰¹ speculated that the use of the inac-
 433 tivated whole virus in the Zhang *et al.*¹⁰⁰ 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.*¹⁰² 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)¹⁰². 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 clock¹⁰².
 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 vaccination¹⁰³. 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. Exploratory post-
 458 hoc analysis of a clinical trial (DANFLU-1¹⁰⁴) of the relative effectiveness of high
 459 dose versus standard dose quadrivalent influenza vaccines established that earlier
 460 TODV was associated with fewer respiratory hospitalisations¹⁰⁵, although the authors
 461 noted that this association was likely limited by relatively few outcomes in subjects
 462 vaccinated later in the day.

463 Two independent systematic reviews^{2,106} have surveyed published studies on the
 464 human immune response to vaccination at different times of day in order to gauge the
 465 evidence supporting diurnal variation in the effectiveness of vaccination. Wyse *et al.*²
 466 noted the challenge in generalising the findings based on the heterogeneity of the stud-
 467 ies surveyed, however the authors concluded that overall there was insufficient overall
 468 evidence that administration of vaccines at different times of day affected immune
 469 outcomes. On the other hand, Vink *et al.*¹⁰⁶ concluded that that morning vaccination
 470 enhanced antibody responses in adults aged 60 years and older, noting that this age
 471 group is a key demographic for influenza and COVID-19 vaccination. Studies qualified
 472 for inclusion in the Vink *et al.* review if they measured antigen-specific antibody or
 473 T-cell responses following vaccination, and if these immune responses were compared
 474 between participants vaccinated at different time points during the day, whereas for
 475 Wyse *et al.* studies were included if they reported any immune or clinical outcome
 476 following vaccination at a defined time of day.

477 The Wyse *et al.*² review assessed over 3,100 studies, 23 of which met their inclusion
 478 criteria. These studies were published between 1967 and 2024 and almost half of them
 479 included reported data collected for SARS-CoV-2 vaccination programs during the
 480 COVID-19 pandemic. The review authors found that most studies were biased by
 481 failing to account for immune status prior to vaccination, self-selection of vaccination
 482 time, or due to the presence of confounding factors such as sleep, chronotype, and
 483 shift work. Of these 23 studies, the optimum TODV was concluded to be afternoon
 484 (5 studies), morning (5 studies), morning and afternoon (1 study), midday (1 study),
 485 and morning or late afternoon (1 study), with the remaining 10 studies reporting
 486 no effect. Of the studies that reported an association between TODV and outcome
 487 of vaccination, 3 presented data that could be used to estimate the size of this
 488 effect^{100,102,107}. For example, Hazan *et al.*¹⁰² estimated that optimising the time of
 489 vaccination might improve vaccine effectiveness by 8.6–25%.

490 Vink *et al.*¹⁰⁶ identified 860 records through their literature search, 17 of which
 491 met their eligibility criteria; five investigated the effect of vaccination timing on
 492 immune responses to influenza vaccines^{84,85,108–110} and nine explored how time of
 493 day influences immune responses to SARS-CoV-2 vaccines^{100,101,107,111–116}. Eleven
 494 out of the 17 studies reported statistically significant effects of vaccination timing,
 495 with ten reporting stronger antibody responses following morning vaccination, while
 496 one study favoured vaccination later in the day. The strongest evidence for diurnal
 497 variation was found for influenza vaccines in older adults; sub-group analysis for age
 498 stratification in the included studies consistently showed stronger antibody responses
 499 for 60-year-olds or older for morning vs afternoon vaccination. Pooled results from
 500 two randomised controlled trials^{84,108} for influenza vaccination showed a statistically
 501 significant small-to-medium standardised mean difference (SMD) in antibody titers
 502 for adults aged 65 and older (effect size; SMD = 0.32, 95% CI = 0.21–0.43), with
 503 morning vaccination consistently yielding higher titers 1 month post-vaccination.
 504 However, Vink *et al.*¹⁰⁶ caution over-interpretation of this reported effect size due to
 505 the limited number of randomised controlled trials used in the meta-analysis.

506 **7 Discussion**

507 The role of circadian rhythms in many diverse strands of modern society are increas-
 508 ingly apparent, in areas such as health and well-being - from increasing understanding
 509 of chronic clock disruption (e.g., shift work, social jet lag) through to optimal living
 510 – the integration of personal circadian rhythms into daily schedules for eating, exer-
 511 cising, and sleeping to maximise health and performance, increasingly regarded as a
 512 ‘game changer’ in elite sports regimes²⁴. Another example, in agriculture – harnessing
 513 knowledge of crop clocks to increase yields and resilience, more and more topical with
 514 the gathering storm of climate change and food security challenges²⁸. Both examples
 515 under the cloak of cultural disconnect from natural cycles of light and dark and the in-
 516 sidious creep of artificial light, including blue light at inappropriate phases of our day.
 517 Modern lifestyles embolden living against our natural clock settings, yet decades of
 518 fundamental scientific research in biological clockwork, rewarded with the 2017 Nobel
 519 prize in Physiology or Medicine⁵, are offering opportunities to harness aspects of clock
 520 function as chronotherapies. One such therapy – chronovaccination - might offer an
 521 extra edge in vaccine effectiveness. However, is the current evidence strong enough
 522 to support the idea that it can be a low-cost, low-risk strategy for boosting vaccine
 523 effectiveness? The case for chronovaccination – surveyed in systematic reviews^{2,106} –
 524 is somewhat patchy. While the influence of TODV is compelling in animal studies
 525 where environmental conditions are strictly controlled^{76,80}, human populations feature
 526 a panoply of individual clock types set in a multitude of environments. Young clocks,
 527 teenage clocks, old clocks, with their spectrum of lark and owl clock ‘settings’. Clocks
 528 in rural and modern industrial environments, seasonal clocks, socially jetlagged clocks
 529 - all muddling interpretation of human circadian studies assessing the effect of TODV.
 530 Large population cohort epidemiological studies have been successful in teasing
 531 apart relationships between our endogenous clock function and the environment – for
 532 example revealing the association between where we live in a Time Zone and chrono-
 533 disruption¹³. Population-level approaches have also been used in chronovaccination
 534 studies^{102,103}, but report modest effect sizes and suffer from potential biases due to
 535 non-randomisation of cohorts. Vink *et al.*¹⁰⁶ demonstrated that the strongest evidence
 536 for diurnal variation in vaccination effectiveness was found for influenza vaccines using
 537 randomised control trials and Wyse *et al.*² argue that large population randomised
 538 trials are the ideal, with the power of thousands of participants enabling adjustment
 539 for multiple demographic and lifestyle factors that might otherwise confound detection
 540 of an effect of TODV. These would be costly enterprises, but Wyse *et al.*² reason that
 541 the onus of determining whether effectiveness is a function of the TODV should fall to
 542 the vaccine producers during clinical trials.

543 Even if there was a compelling advantage of TODV, would it be readily integrated
 544 into vaccination programmes? One only has to look at the debate around Daylight
 545 Saving Time (DST)¹¹⁷ to understand the sensitive nature of policies that have
 546 disruptive effects on social routines. Another relevant example is the idea of later
 547 school start times for adolescents – designed to align better with their clock driven
 548 ‘owl’ behaviours – that illustrates how interventions, however well intended, can
 549 shift the problem onto other groups of individuals, in this case later scheduling
 550 working against teachers and parents own chronotypes and social schedules. Would
 551 the potential benefit of TODV justify its disruption to well-established vaccination
 552 programming? On the one hand boosted vaccine effectiveness can help improve
 553 vaccine hesitancy, with hesitancy identified by the WHO as one of the 10 threats to
 554 global health⁶. On the other hand, the convenience of accessing vaccinations is an

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

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

important factor for vaccine uptake², 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 vaccinate¹¹⁸, and may therefore be a useful strategy for fostering confidence in time-of-day vaccinations.

While the benefits of TODV across whole populations remains marginal, a clearer case can be made for chronovaccination in adults 60 years and older, where morning vaccination was more beneficial¹⁰⁶. It has been speculated that immunosenescence - the gradual age-related decline in both innate and adaptive immune function - may play a role^{108,119}. Similarly, immunosuppressed individuals might benefit from TODV-mediated boosted vaccine effectiveness^{115,116}, suggesting that TODV may find a role in targeted age group and co-morbidity cohorts. The potential of infant chronovaccination is largely an unexplored area, with most studies focussed on adult COVID-19 and influenza programmes. A recent study, however, examined diurnal patterns of varicella vaccine effectiveness in children - finding that immunisation during the late morning to early afternoon led to fewer breakthrough infections compared with those vaccinated in the evening¹⁰³. And what of urban-rurality? How might 'Place' - the extent of coupling to 'sun time' - influence sensitivity to TODV? This and the influence of seasonality, for example, on TODV are largely unexplored and might contribute to understanding the complex relationship between the environment, clocks, and immune function. Although a discussion of the role of sleep in immune function, and therefore its contribution chronovaccination, is not developed in this review, Livieratos *et al.*¹²⁰ have reviewed the evidence of the impact of circadian and sleep factors on influenza vaccine-induced immune responses, emphasising that yet another variable – sleep duration – should be considered for understanding vaccine-induced antibody responses.

It was hoped that the award of the clock biology Nobel Prize in 2017 would emphasise the time-of-day reporting in biology and medicine. However, Nelson *et al.*³⁷ report that the time-of-day variable remains largely ignored. Much commentary on the future direction of chronovaccination emphasise the importance of systematically recording the time-of-day at which vaccines are administered, yet to many chronobiologists it remains a puzzle why such a fundamental biological variable is regularly overlooked in the first place. One factor may be the increased resources and costs that accompany properly designed multi time-point circadian assays; however circadian biologists would no doubt argue that biological studies cannot be reliably replicated – a fundamental tenet of science – unless time-of-day is recorded. Another important consideration is that internal biological time (circadian phase) can vary considerably, the consequence of modern lifestyles and the high chance of desynchrony due to many of the Person, Place and Time factors described here (Table 1). For example, nurses' circadian rhythms in body temperature have been shown to be significantly misaligned due to shift working patterns, causing work and sleep schedules to be out of phase with each other¹²¹. Both external time of vaccination and internal (biological) time recording are therefore important factors, with internal time recording representing a particular technical challenge⁵³. However, research on circadian wearable devices that continuously track body movement, heart rate, temperature, and light exposure has increased significantly over the past decade, are continually improving and offer a non-invasive method to capturing real-life circadian rhythms²⁶.

'Time will tell' if chronovaccination finds a role in delivering an edge to vaccine effectiveness. There remain many challenges, not only in establishing clear clinical benefits, but other hurdles akin to practical and societal issues. The general public's growing interest of the role of circadian rhythms in relation to health and well-being, or 'circadian hygiene', might ultimately drive individual awareness of optimal times for vaccination. Therefore, as Vink *et al.*¹⁰⁶ allude to in their review, the future

vaccine customer may well regard optimal vaccination as an hourly continuous variable in relation to their personal circadian phase rather than the more blunt binary (morning *vs* afternoon) variable we currently consider. In this regard it will be particularly interesting to learn the outcome of the ‘Chrono-Vax’ clinical trial that aims to determine the effect of influenza vaccination timing on antibody and T-cell responses across a continuous time window from early morning to late afternoon for participants aged between 60 and 85 years old¹²². Importantly, participants will be asked to provide data on their history of shift work, comorbidities, (sleep) medication use, lifestyle characteristics, as well as insights into their chronotype¹²².

618 8 Conclusions

619 Chronovaccination studies have been demonstrably hampered by the panoply of
 620 confounding factors inherent to population structures and our modern lifestyles.
 621 Understanding endogenous human biological time-keeping in the context of their
 622 exogenous settings is likely key to disentangling the relative influences of covariates.
 623 Nonetheless, TODV as a chronotherapy shows promise for older age and immuno-
 624 suppressed groups, and while the effect size may prove to be modest, their practical
 625 significance could be viewed as a marginal gain in vaccine effectiveness, in the same
 626 way that knowledge of clockwork is harnessed as a competitive advantage in numerous
 627 other areas of society. Key hurdles in the adoption of chronovaccination are whether
 628 society buys into the idea and whether health agencies conclude that the fragility of
 629 vaccine uptake might ultimately be further jeopardised by changes to well established
 630 vaccination programmes. While the timing of vaccinations may not be at the forefront
 631 of our minds during interpandemic peace times, continued research on the potential
 632 benefits of chronovaccination should be an important strand of our approach to
 633 preparing for future pandemics.

634 9 Acknowledgements

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

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