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Diurnal variation of circulating interleukin-6 in humans: a meta-analysis

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Abstract

The pleiotropic cytokine interleukin-6 (IL-6) has been proposed to contribute to circadian regulation of sleepiness by increasing in the blood at night to signal for sleepiness. Earlier studies have reported diurnal variations of IL-6, but phase estimates are conflicting. We have therefore performed a meta-analysis on the diurnal variation of circulating IL-6. Studies were included if they reported circulating levels of IL-6 recorded at least twice within 24 hours in the same individual. A systematic search resulted in the inclusion of 43 studies with 56 datasets, for a total of 1100 participants. Individual participant data were available from 4 datasets with a total of 56 participants. Mixed-effects meta-regression modelling confirmed that IL-6 varied across the day. Contrary to many previous claims, we found that the most consipuous effect was a trough in the morning, rather than a peak in the evening or night. We propose that the diurnal variation of IL-6 may be explained to some degree by suppression of IL-6 secretion by the morning cortisol peak.

Introduction

Sleepiness is regulated by two main processes: the circadian process, which makes us sleepier in the night, and the homeostatic process, which causes sleepiness to increase with time awake [1]. It has been proposed that interleukin-6, a pleiotropic cytokine, participates in circadian sleepiness regulation by increasing at night and inducing increased tiredness through signalling in the brain [2–6]. Early studies of diurnal variation of IL-6 in humans found a peak in the night-time [7, 8], and it is this observational relationship that forms the main line of evidence for a regulatory effect of circulating IL-6 on sleep. However, further studies have found peaks at different times or no peaks at all. Table 1 shows locations of peaks and troughs that have been estimated in the literature so far, demonstrating wide ranges. One previous meta-analysis of IL-6 and time of day has been reported [9] (repeated in [10] and [11]), but it included only a subset ($k = 11$) of all published studies. Thus, the observational relationship between IL-6 and time of day remains contentious. Therefore, we have performed a meta-analysis, aiming to investigate the diurnal variation of IL-6 in the blood stream, while accounting for possible effects of sleep.

Materials and Methods

Literature search and data acquisition

The PubMed database was searched on 2016-01-03 using the search terms "interleukin-6 AND (sleep OR diurnal OR circadian)", and the limit "human". Records were reviewed by one investigator (GN). Studies were included if they reported IL-6 in plasma or serum from healthy participants with a time-course including two or more time-points within 24 hours. Figure 1 shows a flowchart of data inclusion. Table 2 shows characteristics of included studies. Table 3 lists studies that fulfulled inclusion criteria but which could nonetheless not be included, etiher because data could not be estimated, because of duplicate publication, or because the reported levels of IL-6 were much higher than expected in healthy humans and were therefore judged to be incredible.

Data were estimated from published tables or from graphs using GetData Graph Digitizer, version 2.25.0.25 (`getdata-graph-digitizer.com`). Error bars were assumed to represent standard errors unless otherwise indicated. Time of day was coded, as well as sleep or wake, time asleep, and time awake. Data obtained at the time when the lights-out period began were coded as awake and data from when the lights-out period ended were coded as asleep. When times for falling asleep and waking up were not recorded or not reported, we assumed that they were 23:00 and 07:00. When applicable, time from catheter insertion was also coded. Unless otherwise specified, serial sampling with more than two samples within the same 24-hour period was assumed to have been performed using an indwelling catheter inserted at the first sampling time point. Data recorded during sleep deprivation were not included. IL-6 data were

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ln-transformed to better approximate a normal distribution. For datasets where individual participant data were not available, transformation was performed as described in [12].

Individual participant data were available from 4 datasets, which were coded separately (see table 2). In these datasets, data points below assay detection limits (meaning lowest known point of assay linear range) were conservatively re-coded to the value of the detection limit. In Sothern 1995 [13], 7 values out of 88 (8%), ranging from 0.5 to 0.96 pg/ml, were re-coded to 1 pg/ml. In Karshikoff 2015 [14], 23 values out of 83 (28 %), ranging from 0.01 to 0.88 pg/ml, were re-coded to 0.9 pg/ml.

Ethical approval was not required. The study protocol was not registered. All data and the full analysis code are freely available at `https://github.com/GNilsonne/IL6_diurnal`.

Meta-analysis

To investigate the diurnal time course of IL-6 in plasma and possible moderator variables, we used hierarchical mixed-effects models. This approach allows for more complex model fitting and is expected to have higher statistical power compared to fitting models separately in each dataset and then analysing summary measures such as acrophase and amplitude. Diurnal variation was investigated by fitting cosinor functions with periods of 24, 12, and 6 hours. Time from catheterisation was included with a random slope for each data set in order to account for the effect of local inflammation, which has been proposed [15,16]. Effects of sleep were investigated exploratively with a binary factor for sleep/wake, as well as time asleep and time awake. Datasets were weighted by the number of participants multiplied by the square root of the number of time-points in each study. Models were compared using likelihood ratio tests. Analyses were performed using R version 3.2.0 [17] with the nlme package [18].

Results

Diurnal variation of IL-6

First, we fitted a null model including only time from catheterization and random intercepts for each dataset. Figure 2 shows residuals after these effects have been accounted for, suggesting that there remains variation to explain. Next, we compared a model with a 24 h cosinor function to the null model. The 24 h cosinor model fit better (log likelihood -483.5 vs -528.8, $p < 0.0001$, figure 3). We then added another cosinor function with 12 h period. We did this for two reasons. The first reason was that addition of shorter periods allows a better estimation of non-sinusoidal effects, albeit at a cost of higher risk of overfitting. The second reason was that 12 h periods have been proposed by earlier investigators [19], and we considered that these claims should be tested. The model with both 24 h and 12 h cosinor functions fit better than the model with only the 24 h period (log likelihood -460.1 vs -483.5, $p < 0.0001$, figure 3). Next, we exploratively investigated the addition of yet another cosinor with a 6 h peroid, but that did not improve model fit (log likelihood -457.7 vs -460.1, $p = 0.09$, prediction not shown).

Attempting to disentangle diurnal variation from effects of sleep, we investigated the addition of model effects for sleep (asleep/awake), time asleep (hours since sleep onset), and time awake (hours since wake onset). Starting with the best-fitting model including 24 and 12 h periods, we found that the addition of sleep, time asleep and time awake, or all three variables, did not improve model fit (log likelihoods -459.9, -459.2, and -459.0, respectively, vs -460.1, with p values 0.80, 0.36, and 0.36). Finally, we investigated the addition of sleep, time asleep and time awake, and all three variables, to the model with 24 h period. When comparing these models to the best-fitting model with 24 and 12 h periods, we saw worse fit (log likelihoods -475.5, -471.5, and -471.5, vs -460.1, with p values ≤ 0.0001). When comparing to the model with 24 h period only, we found that the addition of sleep, time asleep and time awake, or all three variables, yielded better-fitting models (log likelihoods -475.5, -471.5, and -471.5, vs -483.5, p values 0.0003, < 0.0001, and < 0.0001).

The best-fitting model had a conspicuous trough between 09:00 and 10:00 in the morning and a second less pronounced trough close to 22:00, and two peaks located close to 17:00 and 02:00 (figure 3). Since diurnal rhythms are commonly investigated using cosinor functions with 24 h periods, we show predictions from this simpler model too (figure 3). This model estimated bathyphase at 08:05 and acrophase 20:05, with an amplitude of 0.166. All the included datasets, with predictions from the best-fitting model, are shown in figures 4 and 5. Individual participant data are shown in figure 6, from those 4 studies where individual participant data were available.

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Assessment of risk of bias

Our literature review identified 37 eligible studies which could not be included. Not counting participants with duplicate data, these studies reported 488 participants, compared to the 1100 included in our meta-analysis. Additionally, an unknown number of studies with eligible data have never been published. It is likely that unreported studies were more likely not to have found significant effects. However, with regard to estimating diurnal phase, we suspect that earlier investigators have been happy to report effects regardless of the location of peaks and troughs, reflected in the wide variety of published estimates (table 1). Therefore, even though the first reports of diurnal effects reported peaks in the night, we suspect that the studies included here were not strongly biased towards reporting effects at any particular time of day.

Included datasets comprise both data from studies that were designed to measure diurnal or circadian variation, and data from studies that incidentally happened to fulfil our inclusion criteria. The latter group generally had fewer time points for measurements. Since this meta-analysis uses mixed-effects meta-regression by time of day, and is hence not based on single summary measures of included studies, is is not possible to investigate bias by usual means such as a funnel plot. The meta-analysis is not strongly dependent on any single study. The most influential study was Mehra 2006, with a total regression weight of 11.19% over five different datasets. Based on the above considerations, we judge that the risk of bias due to selective publishing and data inclusion is probably moderate to low.

Discussion

Our meta-analysis confirmed that circulating IL-6 shows diurnal variation. The most marked effect was a morning trough. The best-fitting model included a 24 h and a 12 h cosinor component. The LOESS curve shown in figure 2 suggests a relatively flat curve from the afternoon to the late night, and the better fit obtained by adding the 12 h component may reflect the rather steep change occurring between the morning and the afternoon. For this reason, we are reluctant to consider the best-fitting model as proof that there are two distinct peaks or troughs during the 24 h day.

Our analytical approach treats sleep as a confounder to be eliminated, in order to best estimate diurnal variation. The effect of sleep on circulating IL-6 is an interesting question in its own right, but is better addressed by sleep deprivation experiments, data from which were not included here. A recent meta-analysis of the effect of sleep deprivation on IL-6 included 12 studies and found no significant effect [20].

One previous meta-analysis [9] (repeated in [10] and [11]) has investigated diurnal variation of circulating IL-6. Compared to this earlier meta-analysis, we have used a more systematic approach and we include more datasets ($k = 56$ compared to $k = 11$). The present findings contrast markedly with those of the earlier meta-analysis, as it located a morning peak at approx. 06:00 (table 1).

Since this analysis concerns an observational relationship, confounding from a variety of sources cannot be ruled out. For instance, physical activity can affect levels of IL-6, but any instructions to participants about physical activity, and measures of their behavior, were for the most part not reported in included studies.

Our finding of a diurnal variation in circulating IL-6 does not contradict the notion that IL-6 in the blood may signal to the brain to promote sleepiness. However, the shape of the estimated curve is not immediately suggestive of such a regulatory function. A simpler explanation for lower levels in the morning is regulation from the HPA axis. Because cortisol rises in the morning, and because glucocorticoids suppress secretion of IL-6, the observed variation in IL-6 could be explained to some degree by this mechanism.

The diurnal variation estimated here is large enough to pose a risk of confounding if sampling is performed without regard to time of day, and we therefore recommend that time of day should be taken in to consideration in studies recording IL-6 in plasma or serum from healthy humans. The present findings raise the question whether other cytokines also show diurnal variation. As far as we are aware, IL-6 is the the only cytokine to date to be subject to a meta-analysis of diurnal variation.

Author Contributions

Conceived of the study: GN. Designed the study: GN, MI. Collected data: GN. Analysed data: GN, MI. Interpreted results: GN, TÅ, ML, JA, MI. Drafted the manuscript: GN. All authors read and approved the final version of the manuscript.

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Competing interests

The authors have no competing interests to declare.

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Figures and Tables

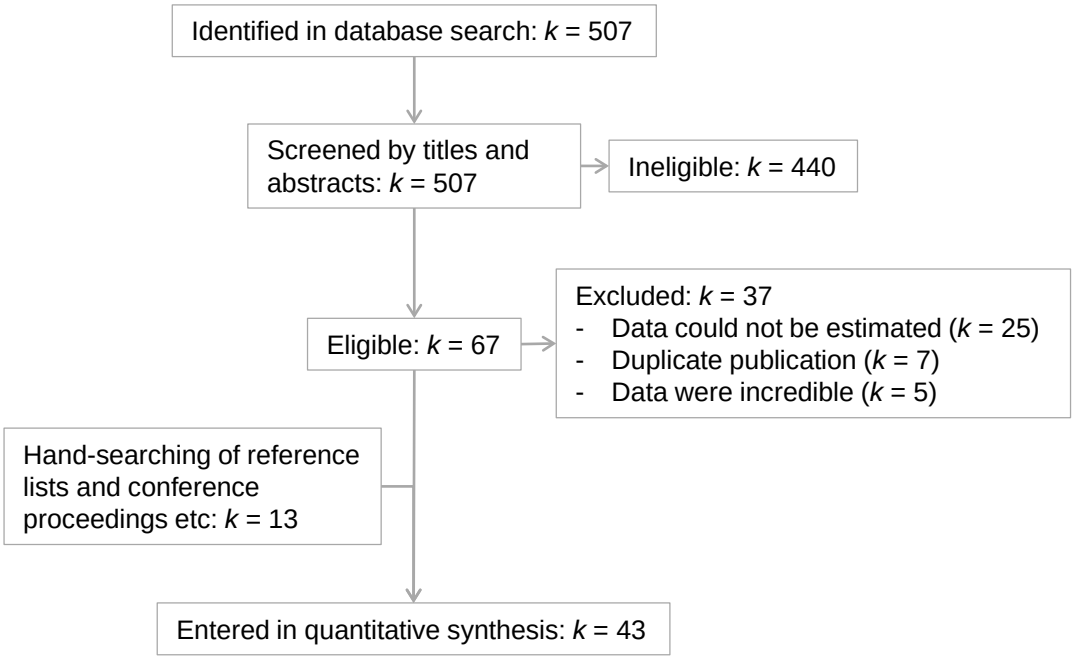

Figure 1. Data inclusion

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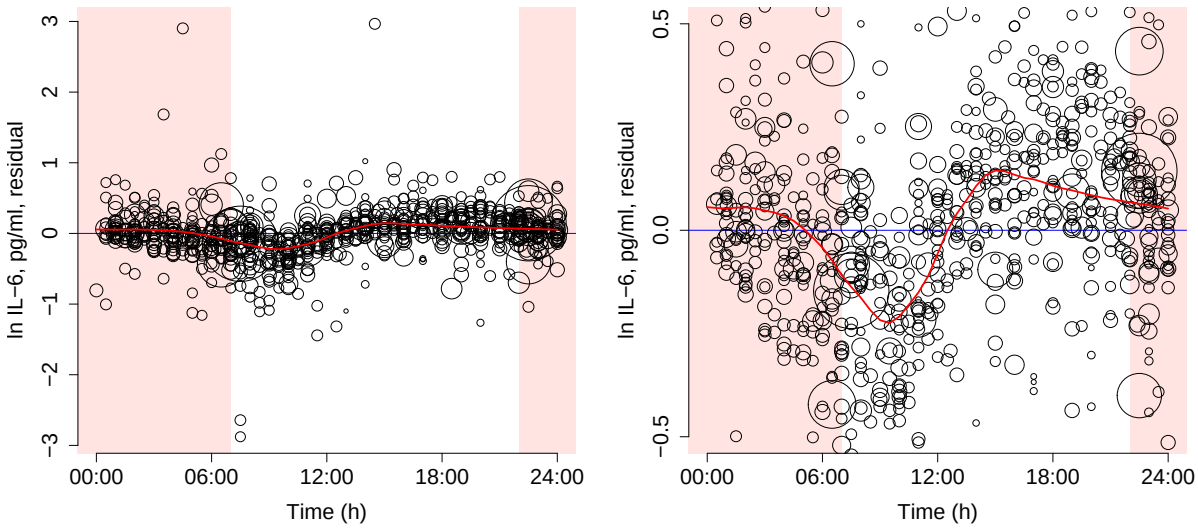

Figure 2. Residuals from null model. Data points sized by regression weight. In the null model, a random intercept for each study and a linear effect of time from catheterization have been included. Therefore, these residuals show the putative diurnal variation to be modeled. For the purpose of illustration, a weighted LOESS curve is shown in red. The LOESS curve was fitted on three repeated days of the same data, and the curve for the second day shown, to ensure that the estimates would converge at 00:00 and 24:00. Time from 22:00 to 07:00 has been shaded to indicate the night. Left: All data points shown. Right: Y axis range restricted to increase resolution.

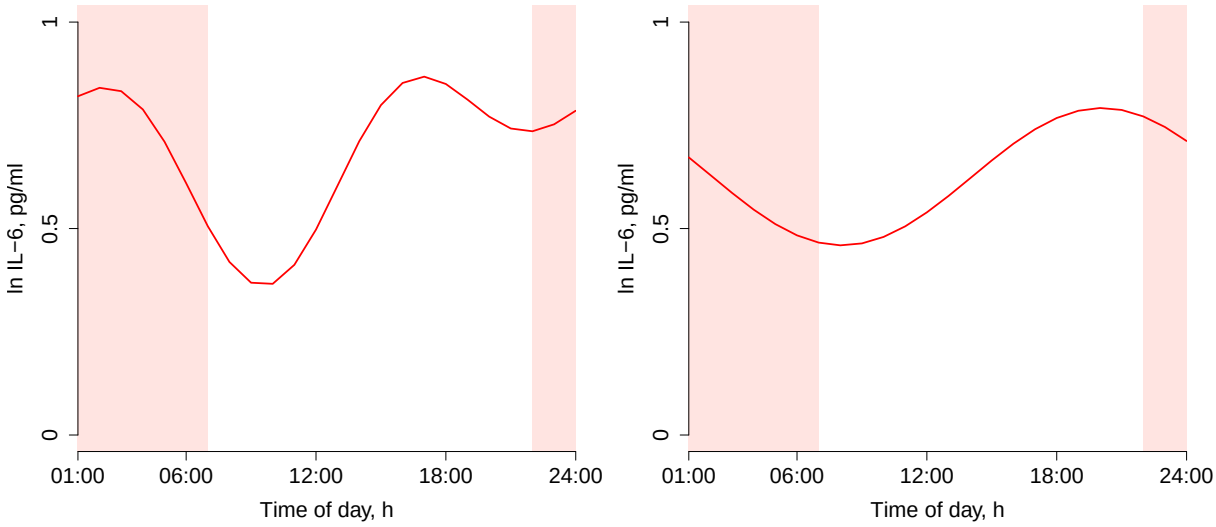

Figure 3. Predicted diurnal time courses from meta-regression models. Left: Best-fitting model including cosinor functions with 24 and 12 h periods. Right: Model including only 24 h period. Time from 22:00 to 07:00 has been shaded to indicate the night.

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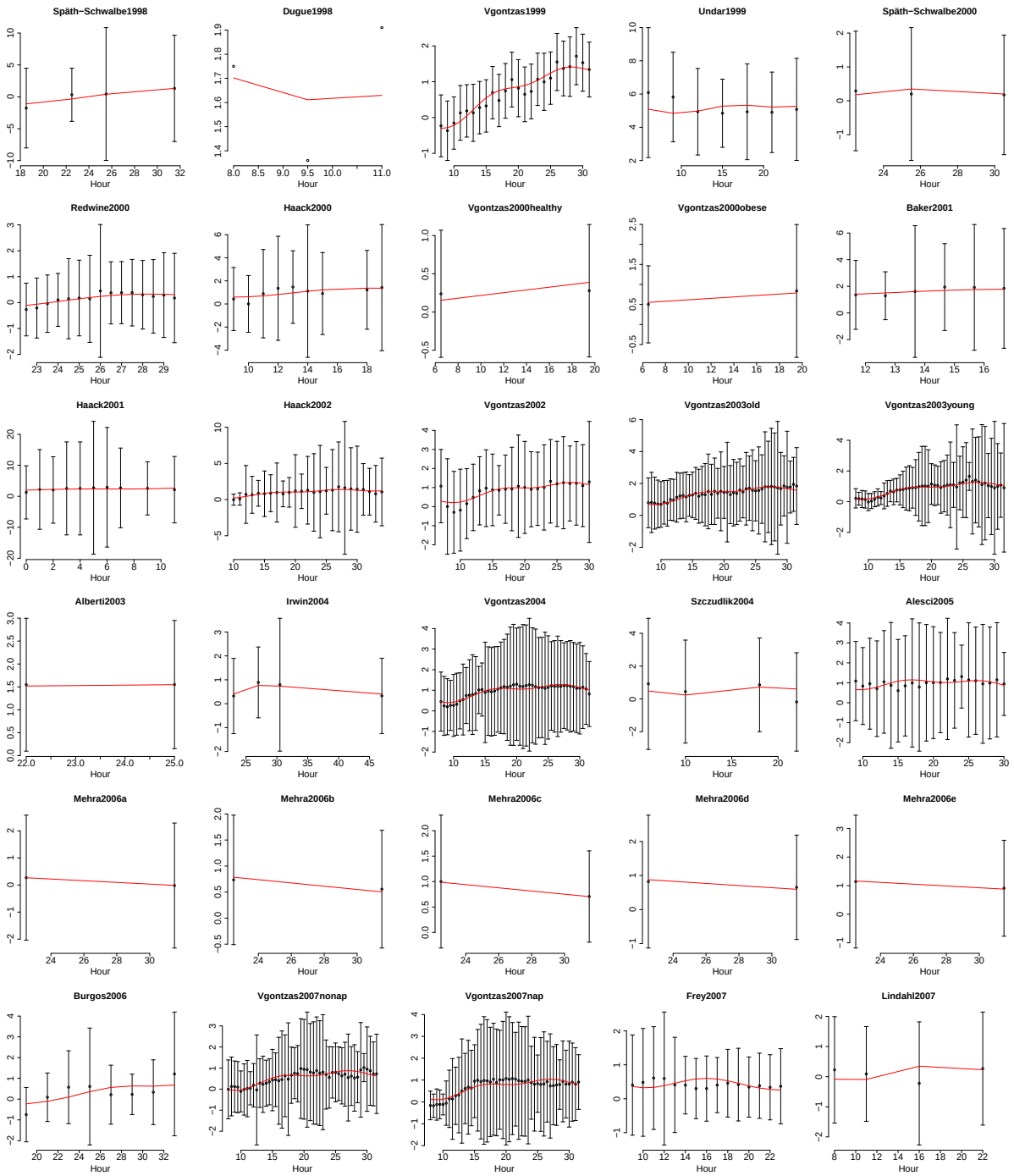

Figure 4. Data and fitted time courses, showing the first 30 out of 56 datasets. Data are shown as estimated from original publications, with error bars showing standard deviations. Y axes show ln IL-6 (pg/ml) throughout. Hours are in chronological time where 1 is 01:00 on the first day. Red lines show predictions from the best-fitting model.

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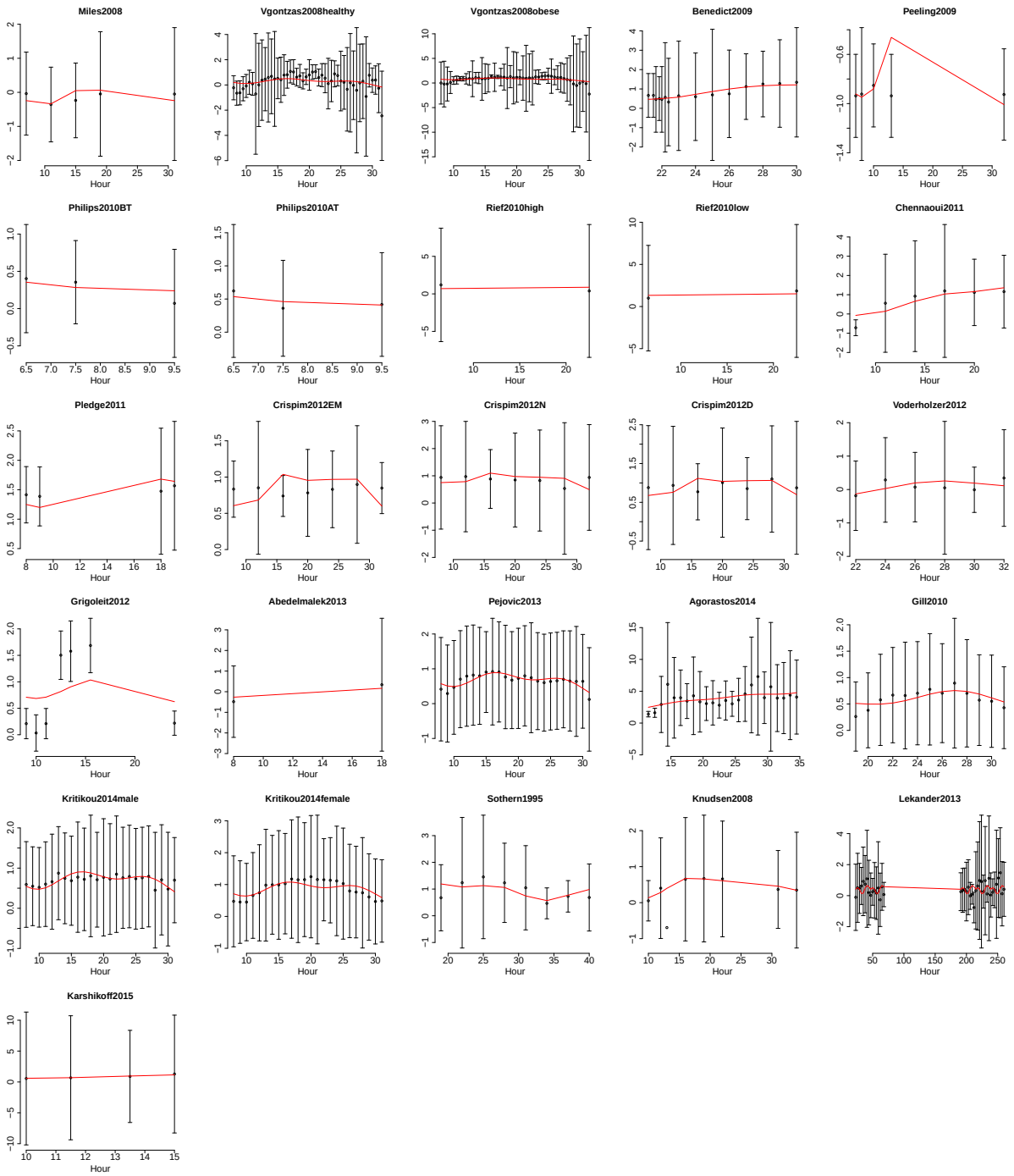

Figure 5. Data and fitted time courses, showing the last 26 out of 56 datasets. Data are shown as estimated from original publications, with error bars showing standard deviations. Y axes show ln IL-6 (pg/ml) throughout. Hours are in chronological time where 1 is 01:00 on the first day. Red lines show predictions from the best-fitting model.

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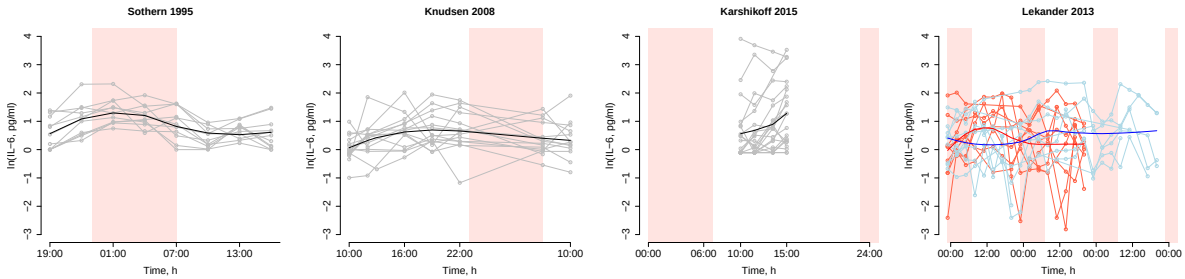

Figure 6. Individual participant data. Individual participant data were available from 4 datasets and are shown here for the purpose of illustrating the high degree of variability within individuals. To illustrate summary effects within each dataset, thick lines show loess functions fitted to each dataset. Time from 22:00 to 07:00 has been shaded to indicate the night. For Lekander 2013, two sets of measurements, made with a few days' interval, have been plotted over the same time course, in red and blue respectively.

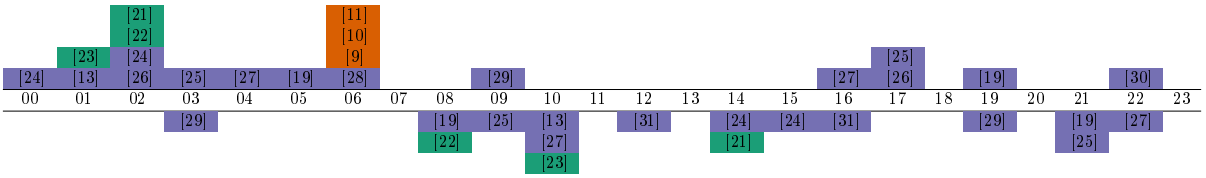

Table 1. Estimates of phase reported in earlier literature. Every count represents one claim of having located a peak (box above time-line) or a trough (box below time-line) in a dataset. Blue: Studies included in quantitative review. Green: Studies excluded from quantitative review. Orange: Meta-analysis. Review papers are not included.

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1st Author	Year	Ref.	$n_{subjects}$	$n_{timepoints}$	Weight, %	Notes
Sothern	1995	[13]	11	8	0.99	a
Späth-Schwalbe	1998	[32]	16	4	1.01	
Dugué	1998	[33]	22	3	1.21	
Vgontzas	1999	[19]	8	24	1.24	
Ündar	1999	[28]	10	7	0.84	
Späth-Schwalbe	2000	[34]	18	3	0.99	
Redwine	2000	[35]	31	15	3.80	b
Haack	2000	[36]	20	9	1.90	
Vgontzas	2000	[37]	12; 11	2; 2	0.54; 0.49	c
Baker	2001	[38]	8	6	0.62	
Haack	2001	[39]	10	10	1.00	
Haack	2002	[16]	12	25	1.99	
Vgontzas	2002	[26]	11	24	1.71	
Vgontzas	2003	[25]	15; 13	48; 48	3.29; 2.85	
Alberti	2003	[40]	20	2	0.49	
Irwin	2004	[41]	15	4	0.95	
Vgontzas	2004	[42]	25	48	5.49	
Szczudlik	2004	[43]	17	4	1.08	
Alesci	2005	[24]	9	22	1.34	
Mehra	2006	[44]	150; 23; 12; 35; 30	2; 2; 2; 2; 2	6.72; 1.03; 0.54; 1.56; 1.34	d
Burgos	2006	[29]	11	8	0.99	
Vgontzas	2007	[45]	20; 20	48; 48	4.39; 4.39	e
Frey	2007	[46]	19	15	2.33	b
Lindahl	2007	[47]	14	4	0.89	
Miles	2008	[31]	51	5	3.61	
Vgontzas	2008	[48]	15; 13	48; 48	3.29; 2.85	
Knudsen	2008	[30]	15	8	1.14	a, f
Benedict	2009	[49]	17	15	2.09	
Peeling	2009	[50]	8	5	0.57	
Phillips	2010	[51]	7	3; 3	0.38; 0.38	g
Rief	2010	[52]	60; 52	2; 2	2.69; 2.33	
Gill	2010	[53]	14	13	1.60	
Chennaoui	2011	[54]	12	6	0.93	
Pledge	2011	[55]	6	4	0.76	
Crispim	2012	[56]	6; 7; 9	7; 7; 7	0.50; 0.59; 0.75	
Voderholzer	2012	[57]	16	6	1.24	
Grigoleit	2012	[58]	10	7	0.84	
Abedelmalek	2013	[59]	12	2	0.54	
Pejovic	2013	[60]	30	24	4.65	
Lekander	2013	[61]	9	40	1.30	a
Agorastos	2014	[27]	11	24	1.71	
Kritikou	2014	[62]	18; 21	24; 24	2.79, 3.26	
Karshikoff	2015	[14]	21	4	1.31	a, h
Total			1100	789	100	

Table 2. Characteristics of included studies. Where studies reported several datasets, these are specified separately. a: Individual participant data were available. b: Some data were given in time relative to sleep onset or wake-up, and were re-coded using mean chronological time as a best approximation. c: Averaged over 3 consecutive days. d: 358 of 385 participants were included in analyses of IL-6. Final n for each sub-group was not given, and was therefore conservatively coded as the lowest possible n in each sub-group. Error bars were denoted as standard deviation, but were coded as standard errors because they were incredibly small for standard deviations. e: Each dataset was said to have 50% of the total participants ($n = 41$), and both were conservatively coded as $n = 20$. f: 15 of 16 participants could be identified in the graph. g: The same 7 participants were included twice with a 10-week interval, yielding two different data sets. h: We have previously published IL-6 data from this study openly at [63].

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1st author	Year	Ref.	$n_{subjects}$	Reason for exclusion
Lemmer	1992	[64]	12	Data could not be estimated (below detection limit)
Gudewill	1992	[7]	12	Data could not be estimated (given as counts)
Pollmächer	1993	[65]	15	Data could not be estimated (not shown; were "close to assay detection limit")
Bauer	1994	[8]	5	Data could not be estimated (given as arbitrary units)
Dinges	1994	[66]	20	Data could not be estimated (not shown)
Arvidson	1994	[67]	10	Data could not be estimated (below detection limit)
Seiler	1994	[15]	6	Data could not be estimated (clock time not given, and too low resolution)
Seiler	1995	[68]	6	Data same as in [15]
Sothern	1995	[23]	10	Data same as in [13]
Pollmächer	1996	[69]	20	Data could not be estimated (given as difference between treatments)
Entzian	1996	[22]	10	Data were incredible (extremely high levels)
Korth	1996	[70]	20	Data could not be estimated (too low resolution)
Crofford	1997	[71]	5	Data could not be estimated (largely below detection limit)
Gudmundsson	1997	[72]	1	Data were incredible (extremely high levels)
Born	1997	[73]	10	Data were incredible (extremely high levels)
Lissoni	1998	[74]	10	Data could not be estimated (largely below detection limit)
Bornstein	1998	[75]	9	Data could not be estimated (not shown)
Hermann	1998	[76]	10	Data could not be estimated (too low resolution)
Kanabrocki	1999	[21]	11	Data same as in [13]
Genesca	2000	[77]	8	Data could not be estimated (largely below detection limit)
Mastorakos	2000	[78]	5	Data same as in [71]
Mullington	2000	[79]	19	Data could not be estimated (given as change between conditions), also possibly same as in [70]
Johansson	2000	[80]	18	Data could not be estimated (largely below detection limit)
Shaw	2001	[81]	10	Data could not be estimated (largely below detection limit)
Lange	2002	[82]	18	Data could not be estimated (given as z-transformed change between time points)
Domínguez-Rodríguez	2003	[83]	40	Data were incredible (extremely high levels)
Domínguez-Rodríguez	2004	[84]	60	Data were incredible (extremely high levels)
Haack	2007	[85]	18	Data could not be estimated (given as change between conditions)
Eisenberger	2009	[86]	16	Data could not be estimated (too low resolution)
Eisenberger	2010	[87]	16	Data same as in [86]
Haimovich	2010	[88]	2	Data could not be estimated (given as change between conditions)
Sauvet	2010	[89]	12	Data same as in [54]
Grigoleit	2011	[90]	34	Data could not be estimated (control condition not shown)
Miles	2012	[91]	30	Data same as in [31]
Schrepf	2014	[92]	28	Data could not be estimated (time of sampling not shown)
Wegner	2014	[93]	18	Data could not be estimated (too low resolution)
Scott	2015	[94]	14	Data could not be estimated (clock time not given)
Total			578	

Table 3. Characteristics of excluded studies