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Circadian Acclimatisation in Pilots Study (CAPS)

Kennis als Vermogen

Royal NLR – Netherlands Aerospace Centre

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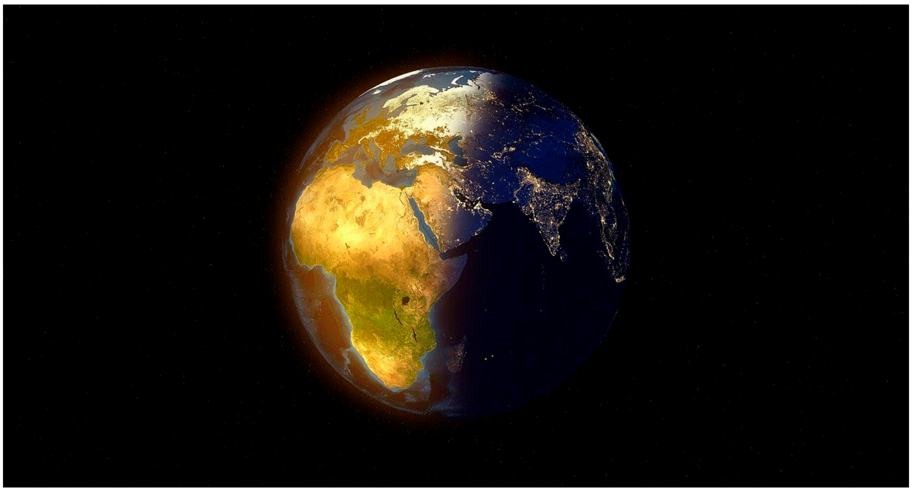

Problem area

The European Union Aviation Safety Agency defines three so called 'states of (circadian) acclimatization': 'acclimatised to the time zone of departure', 'acclimatised to the time zone of arrival' and 'an unknown state of acclimatization'. This underlines the challenge regarding the prediction of the individual level of circadian disruption. It has been shown that accurate determination of an individual's actual level of circadian disruption is difficult. Therefore, a reliable way of determining circadian disturbance during field studies is desirable. Collecting saliva to determine evening melatonin levels could be a solution for this. The aim of the current study was therefore to determine the circadian rhythm of civil airline pilots when not flying for an extended period due to the Covid-19 pandemic, and to compare this with their rhythm when flying normal schedules again, using saliva measurements.

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AUTHOR(S)
A. van Drongelen
A. Maij

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Description of work

The study population consisted of 41 intercontinental flying pilots of a large airline company. After finishing a baseline questionnaire, the participants followed an online session in which the saliva collection was explained. Subsequently, self-measurement kits were sent to the participant's home address for the baseline saliva collection. Next, the pilots started the follow-up measurements when they were flying a schedule that resembled their pre-Covid-19 situation, during a period of at least three rotations. During this period, the participants filled out a daily sleep, wake and activity log by means of a data collection app during the first two of these rotations.

Results and conclusions

The data collection started in the summer of 2020. The baseline measurements showed that the participating pilots were in good health, slept well, and were well rested during the extended period of inactivity. At the end of the study, in November 2021, 36 (88%) out of 41 pilots had performed their baseline saliva measurements and for 33 of them the Dim Light Melatonin Onset (DLMO; an accurate marker for assessing the circadian rhythm) was successfully determined. Furthermore, 15 (37%) pilots had completed all their follow-up measurements. The results showed that when these pilots started flying again, in 31% of the cases pilots experienced circadian disruption (> 45 minutes DLMO difference) the night before starting their next rotation. However, the overall level of disruption was not significantly larger than 45 minutes, indicating that on average the rest period in between rotations was sufficient for the participants to get back to their baseline circadian rhythm. Schedule related factors that proved to be positively associated with larger DLMO deviations were a higher number of time zones crossed, and a westward direction of the rotation. No other consistent pattern was found for the association of circadian disruption (y/n) with personal or schedule related factors, nor was a significant cumulative effect found.

Applicability

This report describes an innovative field study which uses saliva collection to determine the circadian disruption of airline pilots. Unfortunately, loss to follow-up was high due to the ongoing Covid-19 pandemic and the resulting disrupted schedules of the participants. It was shown, however, that flight crew members were able and willing to perform the measurements at home correctly, and that by means of the procedure, circadian disruption could be objectively measured in a valid and reliable way. With the knowledge gained, the saliva collection procedure can be applied in future field studies, to better understand the effects of certain flight schedules and flight time limitations, and to draw more specific conclusions regarding the causal factors of circadian disruption.

Royal NLR
Anthony Fokkerweg 2
1059 CM Amsterdam, The Netherlands
p) +31 88 511 3113
e) info@nlr.nl i) www.nlr.nl

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AUTHOR(S):

A. van Drongelen	NLR
A. Maij	NLR

Royal NLR – Netherlands Aerospace Centre

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Summary

Background

The start of the Covid-19 pandemic offered a unique possibility to study the level of recovery of aircrew while they are absent from flight schedules (including night flights, and time zone crossings) which they are normally exposed to. In both practice and science it is still unclear what 'sufficient recovery' is, and which factors (e.g. individual characteristics, workload, home situation) are of influence. This certainly applies to the degree of recovery after circadian disruption, and the jet lag symptoms that result from it. In addition, accurate determination of an individual's circadian level of disruption has been proved to be difficult, especially in the field. The aim of the current study was therefore to determine the recovery level and circadian rhythm of civil airline pilots while they are absent of flying, using saliva measurements. By means of follow-up measurements, it was determined what the effects were of being exposed to 'normal' flight schedules after a long period of inactivity, and which factors are of influence on circadian adjustment.

Methods

The study population consisted of 41 intercontinental flying pilots of a large airline company. After finishing a baseline questionnaire, the participants followed an online session in which the saliva collection procedure was explained. Subsequently, self-measurement kits were sent to the participant's home address for baseline saliva collection. Next, the pilots started the follow-up measurement when they were flying a schedule that resembled their pre-Covid-19 situation, during a period of at least three rotations. During this period, the participants filled out a daily sleep-, wake- and activity log by means of a data collection app during the first two rotations.

Results

The data collection started in the summer of 2020 and the baseline measurements showed that the participating pilots were in good health, slept well, and were well rested. At the end of the study, in November 2021, 36 (88%) out of 41 pilots had performed their baseline saliva measurements and for 33 of them, the Dim Light Melatonin Onset (DLMO; an accurate marker for assessing the circadian rhythm) was successfully determined. Furthermore, 15 (37%) pilots had completed all their follow-up measurements. The results showed that when these pilots started flying again, the overall level of disruption was not significantly larger than 45 minutes, indicating that on average the rest period in between rotations was sufficient for the participants to get back to their baseline circadian rhythm. In 31% of the cases, pilots experienced circadian disruption (> 45 minutes DLMO difference) the night before starting their next rotation. Schedule factors that is positively associated with larger DLMO deviations are a higher number of time zones crossed, and a westward direction of the rotation. No other consistent pattern was found for the association of circadian disruption (y/n) with personal or schedule related factors, nor was a significant cumulative effect found.

Conclusion

This study showed that during the first year of the Covid-19 pandemic, participating pilots were in good health and well rested. Also, pilots who started started flying again, got back to their baseline circadian rhythm levels the last day before their next rotation most of the time. Furthermore, our study shows that it is feasible to use a saliva collection procedure in (small scale) studies to determine the effects of flight schedules on the level of circadian disruption and adjustment in flight crew. Despite possible improvements in the protocol, flight crew members were able and willing to perform the measurements at home correctly. Consistent close contact of the researchers with the participants remains neccesary however. Although the ongoing Covid-19 pandemic was the main reason of the large loss-to follow up, this type of supervision would make it possible to gather more follow-up data and subsequently draw more specific conclusions regarding the causal factors of circadian disruption.

Contents

Abbreviations

ACRONYM	DESCRIPTION
CAPS	Circadian Acclimatisation of Pilots Study
Circadian disruption	Disruption of the human sleep-wake rhythm
CIS	Checklist Individual Strength
DLMO	Dim Light Melatonin Onset
EASA	European Union Aviation Safety Agency
FDP	Flight Duty Period
fte	fulltime-equivalent
FTL	Flight Time Limitations
KSS	Karolinska Sleepiness Scale
MEC-U	Medical Research Ethics Committees United
NLR	Royal NLR - Netherlands Aerospace Centre
PSQI	Pittsburg Sleep Quality Index
PVT	Psychomotor Vigilance Task
RIA	radioimmunoassay
rMEQ	reduced Morningness-Eveningness Questionnaire
SCN	Suprachiasmatic nucleus
SF-36	Short Form 36

1 Introduction

Since early 2020, the Covid-19 pandemic has had a huge and worldwide effect on healthcare, economics and international relations. One of the sectors that was hit the hardest from the start was the civil aviation industry. The number of flight movements decreased with 73.3% from 2019 to 2020 (*Air Transport Statistics*, n.d.) and airline operators were forced to make drastic economic cuts. In the meantime, flight personnel that was still in service could only fly a fraction of their ‘pre-Covid’ flights, and were spending a lot of time at home. This situation at the beginning of the pandemic offered a unique possibility for a ‘natural experiment’: because of not flying, airline pilots had the opportunity to really recover from their flight schedules (including night flights, and time zone crossings) which they are normally exposed to.

Irregular working hours and time zone crossings can lead to fatigue, sleep deprivation and disruption of the biological clock in the short-term (Caldwell, 2005). Cumulative exposure to these working hours can, amongst other severe health problems, lead to chronic sleep and fatigue issues, especially when insufficient options for recovery are provided (van Drongelen et al., 2017). Despite the fact that work schedule, flight time, and rest regulations indicate how many hours and/or days aircrew should be able to recover between duty periods, from a scientific point of view, it is still unclear what 'sufficient recovery' actually is, and which factors (e.g. individual characteristics, workload, home situation) are of influence. This certainly applies to the degree of recovery after circadian disruption (disruption of the human sleep-wake rhythm, which is controlled by the biological clock) and the jet lag symptoms that result from it. The severity and duration of jet lag symptoms are thought to be largely determined by the direction and duration of time zones crossed, the possibility and ability to sleep while traveling, and exposure to environmental time-giving cues at the destination (Drake & Wright, 2011). In both military and civil aviation, it is assumed that after crossing at least three time zones, circadian disruption takes place (Waterhouse et al., 1997). This frequently used rule of thumb indicates that in the case a layover exceeds 48 hours or more, it is best to try and adapt to the local time zone (Flower, 2001; Sack, 2010). During a layover that is shorter than 48 hours, it should be best to remain in the ‘home rhythm’ (e.g. the Dutch time zone) as much as possible. However, this rule is controversial amongst both scientists and flight crew members. It is, for instance, hard to remain in your home rhythm while you have to spend up to 48 hours on the other side of the world. In addition, it is known that a large number of contextual (previous flight schedules, current flight direction, current flight times) and individual factors (exposure to light, napping, nutrition, exercise) can influence the degree of circadian disruption and the subsequent adjustment (Waterhouse et al., 2007).

Despite what is stated above, the European Union Aviation Safety Agency (EASA) defines three so called ‘states of acclimatization’ as a function of the number of time zones crossed and time elapsed since reporting at reference time. A crewmember can be considered to be (i) acclimatized to the reference time (the departure location), (ii) acclimatized to the new time zone (the destination); and (iii) in an unknown state of acclimatization. The third type of acclimatization underlines the challenge regarding the prediction of the individual level of circadian disruption, as a consequence of the multiple influential factors involved. This is part of the reason why EASA designated ‘*duties of more than 11 hours for crew members in an unknown state of acclimatization*’ as a Flight Duty Period (FDP) for which additional research should identify the effectiveness of the current corresponding European Flight Time Limitations (FTL).

The question remains however, if the state of acclimatization of the crew members is indeed 'unknown'. Accurate determination of an individual's circadian rhythm (level of disruption) is has been proven to be difficult though, especially in the operational field. That is why the degree of circadian disturbance is often estimated based on the outcomes of a biomathematical model (which uses the work schedule and sleep-wake rhythm as input) (Skeldon et al., 2017), or is approximated by the local time where the trip began (Gander et al., 2015). Moreover, in general evidence regarding the extent of circadian disruption due to jet lag and its effect on fatigue and performance of flight crewmembers is scarce. Previous studies that monitored pilots on trans-meridian flights often (i) focused on recovery/adaptation and not on performance/fatigue (Gander et al., 2013), (ii) had no adequate reference condition (van den Berg et al., 2016), and/or (iii) used estimations of circadian phase that are likely inaccurate (e.g., based on changes in sleep patterns, which do not necessarily reflect changes in circadian phase (Gander et al., 2016).

Given these limitations, a more direct and reliable way of determining circadian disturbance during real life aviation is desirable. A possibility to do this is, is by means of collecting saliva to determine evening melatonin levels. The circadian rhythm of melatonin in saliva (or plasma), is a defining feature of the suprachiasmatic nucleus (SCN) function, the endogenous circadian pacemaker (the biological clock). It has been shown that the onset of melatonin secretion under dim light conditions in the circadian evening (the Dim Light Melatonin Onset - DLMO) is the most accurate marker for assessing the circadian rhythm (Bonmati-Carrion et al., 2014). Additionally, DLMO has also been shown to be useful for assessing circadian phase delays or advances (disruption) and for identifying optimal application times for therapies such as bright light or exogenous melatonin treatment (Pandi-Perumal et al., 2007). Melatonin levels and/or DLMO have been used in a variety of industrial settings to measure circadian disruption as a result of shift schedules and occupational exposures (Ferguson et al., 2012; Harris et al., 2010). In the offshore sector for instance, it has been shown that circadian phase determination by means of collection of saliva is a valid possibility in field studies (Merkus et al., 2015; Riethmeister et al., 2018). Studies that have applied this method with flight crew members in real life are still rare however. More than 20 years ago, Härmä et al. (1994) performed one of the few studies that determined circadian variation and resynchronization rates by means of in the field saliva measurements with flight attendants.

Based on the above, the aim of the current study was to determine the circadian rhythm of civil airline pilots by means of saliva measurements and DLMO determination during the Covid-19 pandemic. In addition, subjective levels of recovery, sleep characteristics, and fatigue were measured. Furthermore, by means of additional follow-up measurements when the pilots started flying 'normal' schedules again, the object was to determine:

(i) if saliva collection and DLMO determination is a valid, reliable and practical option for circadian disruption measurement in field studies with airline pilots;
(ii) what the effect on circadian acclimatization is of being exposed to 'normal' flight schedules after a long period of (Covid-19) inactivity;
(iii) if the recovery time between flight schedules is sufficient to get back to DLMO baseline levels;
(iii) which contextual factors (personal, environment, destinations) have a significant influence on circadian acclimatization.

2 Methods

The CAPS study started in July 2020. The study design and procedure was assessed by the Medical Research Ethics Committees United (MEC-U), Utrecht, the Netherlands (registration number W20.162). According to Dutch law, this study was proven to be exempt from further medical ethical review.

2.1 Participants

The study population consisted of intercontinental flying pilots of a large airline company. The pilots could participate if they were not, or only limited flying as a result of Covid-19 at the start of the measurement campaign. In addition, they should not have been flying commercially for at least three weeks at the time of the baseline saliva collection. Other exclusion criteria were sleep medication usage, having a chronic sleep disorder, living abroad, and leaving the company (or retire) within one year from the start of the study. It was determined that in order to be able to detect an effect size of 0.45 (45 minutes DLMO difference), with a significance level (α) of 0.05, and a power of 0.8, at least 40 pilots had to participate in the study (Giménez et al. 2016).

2.2 Procedures and outcome measures

All potential participants were made aware of the study by means of announcements on the airline company's news app. The announcement consisted of a link to a website with additional information. Here, it was explained that the participants could withdraw from the study at any time without giving any reason. In addition, the pilots were asked to sign an informed consent, indicating they were informed about the study procedures, after which they were directed to the online baseline questionnaire.

2.2.1 Baseline questionnaire

By means of the online questionnaire the following outcomes were collected at baseline:

- The demographic variables age (years), experience as a pilot (years), type rating, function, contract rate, secondary position (y/n), household composition (single / cohabiting / married), and children living at home (no / yes, ≥4 years old / yes <4 years old).
- Chronotype, measured with one item from the rMEQ questionnaire (Loureiro & Garcia-Marques, 2015).
- Sleep Quality, using the 4-item Jenkins Sleep Scale (Jenkins et al., 1988).
- Sleep characteristics (sleep length, sleep latency, sleep efficiency, and sleep medication usage) were measured using subscales of the Pittsburg Sleep Quality Index (PSQI) (Buysse et al., 1989).
- Long-term fatigue, as measured with the 20-item Checklist Individual Strength (CIS) (Beurskens et al., 2000).
- Current general health, using one item from Short Form 36 (SF-36) (Ware & Gandek, 1994).

After finishing the baseline questionnaire, the researchers contacted the individual participants to schedule an online instruction session, in which both the saliva collection method and usage of the data collection app was explained.

2.2.2 Baseline saliva collection

After the online instruction session, a self-measurement kit was sent to the participant's home address. The kit contained an additional explanation sheet, six Salivette® saliva collection tubes, plastic seals, and a return envelope. The pilots were instructed to pick an average evening at home to collect the saliva hourly, starting five hours before normally going to bed, until bedtime (six in total). From one hour before the start of the collection period onwards, exposure to light had to be limited as much as possible. Consumption of food and beverages other than water were forbidden 30 minutes before sampling (Figure 1). The tubes with the collected saliva had to be stored in a normal fridge at home, and sent to the laboratory within 3 days after the measurement. Once received by the laboratory, the samples were kept in a deep freezer (-80°C) before being analyzed. The samples were centrifuged, and a double-antibody radioimmunoassay (RIA) was performed during the analysis. DLMO was marked as the first time when linear interpolated melatonin concentrations exceeded the 3 pg/mL threshold (Woelders et al., 2017).

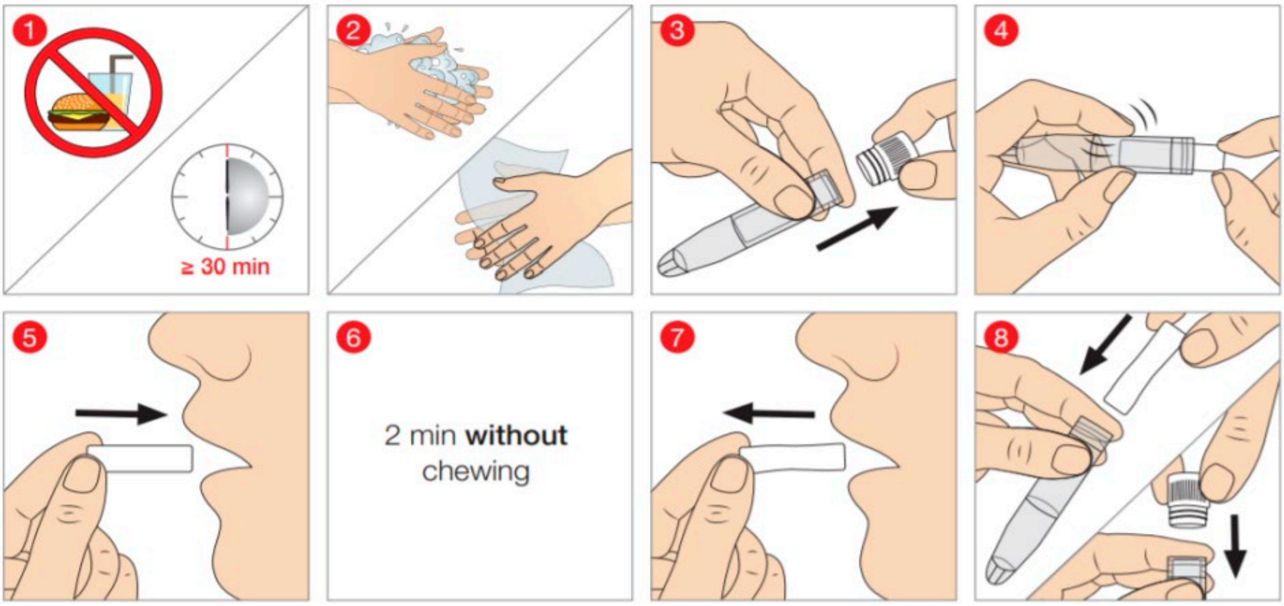

Figure 1: Instruction animation shown to the participants about the usage of the self-measurement saliva collection kit

2.2.3 Follow-up measurements

After the baseline saliva measurement was received by the laboratory, two additional saliva measurement kits were sent to the participants. The participating pilots were instructed to start with the follow-up measurement when they were going to fly a schedule again that resembled their pre-Covid-19 situation, for a period of at least three rotations (three outbound, and three inbound flights). They were asked to perform the saliva measurements during the last night at home before the next (i.e. the second and third) rotation in their schedule to determine the level of recovery between the rotations. The saliva collection procedure itself was the same as during baseline.

During the first two rotations, the participants were also asked to fill in a daily sleep-, wake- and activity log by means of the NLR data collection app (NLR Study, Figure 2). The questions in the app concerned sleep timing, sleep length, sleep quality, napping behavior, fatigue (Karolinska Sleepiness Scale - KSS), and commuting and flight schedule details. In Table 1 an overview of the follow-up measurements is shown by means of a fictional flight schedule sequence.

Table 1: Fictional flight schedule sequence with corresponding follow-up measurements

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Schedule		outbound	layover	inbound				outbound	layover	layover	inbound				outbound
Saliva collection							x							x	
Sleep-wake log	x	x	x	x	x		x	x	x	x	x	x		x	

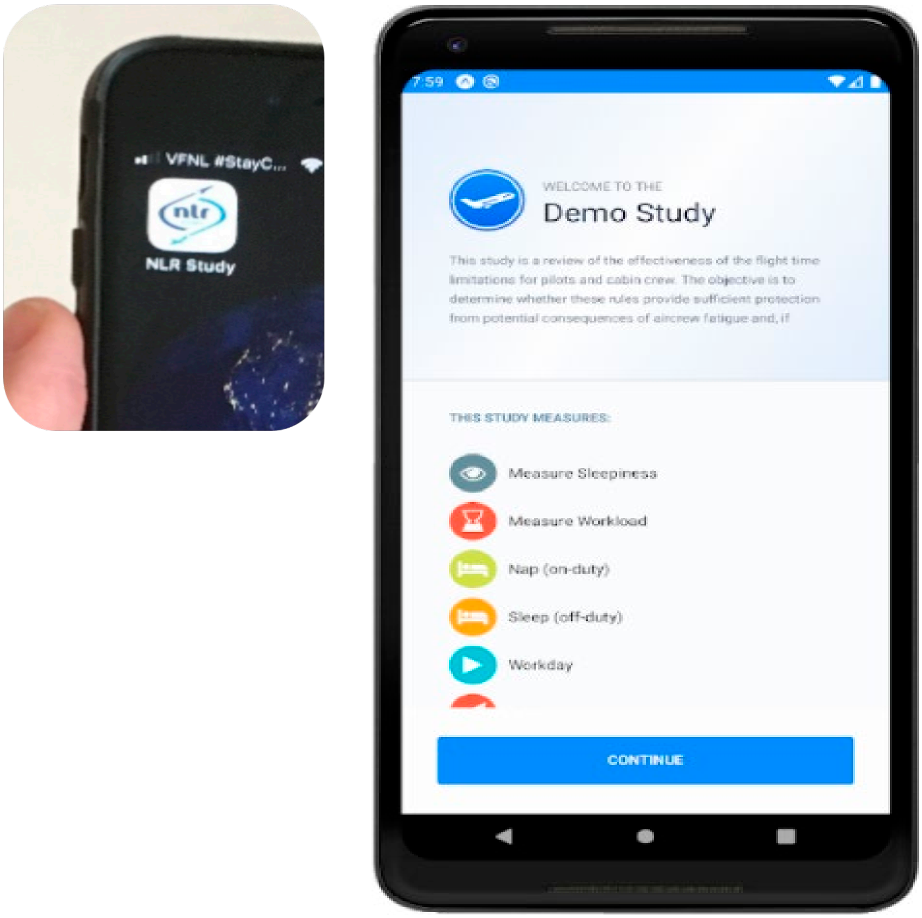

Figure 2: Icon and screenshot of the NLR Study data collection app.

2.3 Statistical Analysis

Descriptive analyses techniques were used to describe the baseline characteristics of the participants. Furthermore, the number of correctly performed baseline and follow-up measurements of saliva collection and qualitative information on reasons for dropping out of the study were collected to determine the feasibility of DLMO determination through saliva in flight crew.

A one-sample t-test was used to determine if the DLMO, after being exposed to a normal rotation, significantly differed from the DLMO at baseline (following a long period of inactivity) and if this difference led to significant circadian disruption (y/n), defined as more than 45 minutes difference between the follow-up measurement and the

DLMO measured at baseline (Giménez et al. 2016). Furthermore, non-parametric correlation techniques were used to determine which schedule related and personal factors were significantly related to circadian changes. In addition, univariate logistic regression analyses were used to determine if these factors led to circadian disruption (y/n). Moreover, univariate ANOVA analysis were performed to determine whether schedule related factors had a significant effect on the level of DLMO deviation compared to baseline. A significance level of $P<0.05$ was considered to be statistically significant in all analyses. All analyses were conducted with the Statistical Package for Social Sciences (SPSS) version 26 (IBM Corp, Armonk, NY, USA).

3 Results

The study announcement was placed on the news app of the airline company in the beginning of July 2020. After one month, a reminder announcement was sent through the app. As shown in Figure 3, 87 of the approximately 2.700 pilots (3%) were interested to participate and filled in the baseline questionnaire. Based on the information that was filled out, 36 of those pilots had to be excluded due to one of the exclusion criteria (being not flying for at least two weeks, sleep medication usage, a chronic sleep disorder, living abroad, leaving the company or retire within one year). The remaining 51 pilots were invited to join the online instruction session in which the measurement procedures were explained more thoroughly. As a result of these sessions, 10 more pilots proved not able to participate or decided they were not willing to. Consequently, baseline saliva measurement kits were sent to 41 pilots. Of those 41 pilots, 36 pilots (88%) performed the baseline saliva measurements and sent their saliva kits to the laboratory. Of those, 33 baseline DLMO times could successfully be determined.

At the end of the study period (November 2021), 19 (46%) pilots had performed one follow-up measurement, while 15 (37%) completed both their follow-up measurements, being both the saliva measurements and the daily sleep-, wake- and activity log, after commencing their (near) normal flight schedules again. Most pilots who did not succeed in performing their (complete) follow-up measurements indicated that they were not flying frequently yet or were grounded for a longer period of time due to the low traffic demand as a result of the ongoing Covid-19 pandemic. One participant indicated to withdraw from the study.

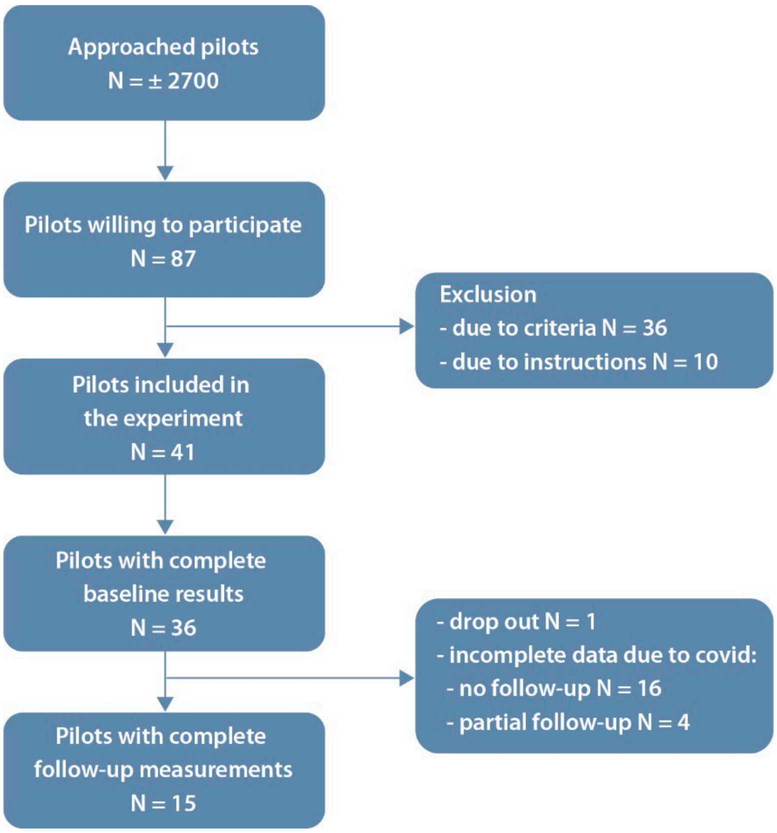

Figure 3: Flow chart with the number of participating pilots

3.1 Descriptive statistics

The demographic characteristics of the participating pilots at baseline and at follow-up completion are shown in Table 2. At baseline, the mean age of the participants was 43 (sd 9.6) years old, they were predominately male (85%), cohabiting (88%), and having children (71%). Most of the participating pilots were captain (44%) or first officer (46%) and had either a 0.8 fte (n=15) or a fulltime contract (n=20). The majority of the pilots (n=26) had an Airbus type rating (several participants had multiple type ratings).

The 15 pilots who completed their follow-up measurements had a mean age of 44.1 (sd 10.2). Comparison analyses of the baseline dataset with that of the 15 participants with follow-up measurements showed that this group was representative for the whole group regarding age (mean difference = -1.403, t = -.444, p = .660); gender (χ^2=.032, p = .858); household composition (χ^2=2.208, p = .332); children (χ^2=1.849, p = .604); contract rate (χ^2=3.716, p = .446); ancillary position (χ^2=1.213, p = .271); current position (χ^2=.557, p = .757). Although aircraft type was also statistically representative (χ^2=.108, p = .743), it can be seen that there were no B747 pilots who completed their follow-up measurements, this was due to the fact that this aircraft type was phased out during the course of the study.

Table 2: Demographic characteristics of all participating pilots at baseline (n=41) and of the participants who completed their follow-up (n=15)

Demographic Characteristic		n = 41	%	n = 15	%
Age	mean (sd)	43.2 (9.6)		44.1 (10.2)	
Gender	male	35	85.4	13	86.7
	female	6	14.6	2	13.3
Household composition	alone	4	9.8	2	13.3
	together	36	87.8	12	80
	other	1	2.4	1	6.7
Children	no	12	29.3	6	40
	yes, one or more < 4 years old	4	9.8	1	6.7
	yes, all > 3 years old	24	58.5	8	53.3
	other	1	2.4	0	0.0
Contract rate	<80	3	7.3	2	13.4
	80	15	36.6	4	26.7
	90	3	7.3	2	13.3
	100	20	48.8	7	46.7
Ancillary position	no	39	95.1	15	100
	yes	2	4.9	0	0.0
Current position	captain	18	43.9	7	46.7
	first officer	19	46.3	6	40.0
	second officer	4	9.8	2	13.3
Aircraft type	A330	26	63.4	10	66.7
	B737	11	26.8	3	20.0
	B747	8	19.5	0	0.0
	B777	11	26.8	5	33.3
	B787	7	17.1	3	20
	Other	4	9.8	3	20

The baseline health, sleep and fatigue related outcomes are shown in Table 3. All chronotypes are present in the included group of pilots, with the majority (n=31, 75%) somewhat in the middle of the range (being either more a morning type than an evening type; neither; or more an evening type than a morning type). Most participants indicated they had an excellent or good health, and none of them indicated to have a (very) bad health. The total mean score on the Checklist of Individual Strength (CIS) was 50.5 points (range 20-140), and that of the fatigue severity subscale 19.8 (range 8-56). Higher scores mean that more fatigue is experienced. The Jenkins sleep quality score of the participants was 6.3 (range 0-20; a higher score indicates a lower sleep quality). The mean sleep length of the participants at baseline was 7h21m, and the mean sleep latency 19 minutes. The mean overall sleep efficiency was higher than 85%. In total, 36 pilots completed their baseline DLMO measurement. As a result of a multiple regression analysis, it could be seen that age, chronotype, general health, CIS fatigue, Jenkins sleep quality, sleep length and sleep latency were not significantly associated with the timing of the DLMO.

The characteristics of the pilots who completed their follow-up measurements (n=15) showed that there were no statistically significant differences with the whole group of participants regarding chronotype (χ^2=.0552, p = .968); general health (χ^2=3.919, p = .141), sleep quality (mean difference = -.86923, t = -.721, p = .475); sleep length (mean difference = -0:10, t = -.844, p = .404), sleep latency (mean difference = -3.418, t = -.571, p = .571), and sleep efficiency (mean difference = -.372, t = -.135, p = .893). The group with follow-up measurements was however significantly different with regard to the scores on the Checklist Individual Strength (CIS total mean difference = 12.747, t = 2.188, p < .05 and CIS fatigue mean difference = 7.016, t = 2.535, p < .05).

Table 3: Baseline outcomes of the sleep and health related outcomes of all participating pilots at baseline (n=41) and of the participants who completed their follow-up (n=15)

Variable		n = 41	%	n = 15	%
Chronotype	Definitely a morning type	3	7.3%	1	6.7%
	More morning type than an evening type	9	22.0%	4	26.7%
	Neither	10	24.4%	4	26.7%
	More evening type than a morning type	12	29.3%	4	26.7%
	Definitely an evening type	7	17.1%	2	13.3%
General health	Excellent	15	36.6%	8	53.3%
	Good	23	56.1%	7	46.7%
	Reasonable	3	7.3%		
CIS total (range 20-140)	Mean (sd)	50.5 (18.4)		42.2 (13.5)	
CIS fatigue (range 8-56)	Mean (sd)	19.8 (8.9)		15.2 (5.9)	
Jenkins Sleep Quality (range 0-20)	Mean (sd)	6.0 (3.7)		6.6 (4.3)	
PSQI sleep length (h:mm)	Mean (sd)	7:21 (0:38)		7:28 (0:26)	
PSQI sleep latency	Mean (sd)	19:38 (18:19)		17:28 (12:37)	
PSQI sleep efficiency	Percentage (sd)	85.6 (8.4)		85.8 (7.0)	

The results of the 15 pilots who completed their follow-up measurements are shown in Table 4. It can be seen that after the first rotation (before rotation 2), the saliva measurement pointed out that 29% (4 out 14) of the pilots experienced circadian disruption (> 45 minutes difference in comparison with baseline DLMO, for all pilots in which this could be determined). After the second rotation (before the third), at least 39% (5 out of 13) of the pilots for whom the DLMO could be determined experienced circadian disruption.

In four occasions, the DLMO could not be determined because the threshold value of 3 pg/ml of the melatonin concentration was not reached during the measurement procedure with six saliva samples. For those cases it was clear that the DLMO occurred after the final saliva sample only. As a result, the corresponding data could not be included in the comprehensive analysis. In Figure 4 an example can be seen of a measurement in which the DLMO could be determined (left, melatonin concentration in saliva crossing the threshold of 3 pg/ml) and one where this was not possible (right, the melatonin concentration does not reach 3 pg/ml).

In Table 4 it can be seen that two participants did not succeed in communicating the right information regarding their schedule (NA): these data points could not be included in the analysis regarding the association with schedule related factors. This could partly be the result of the data collection app which was malfunctioning at certain moments in time (being down and/or disconnected with the server). Participants who reported problems with the app received an alternative diary in the form of a digital word file, but not everyone was able or willing to fill out and return this file.

Table 4: DLMO results of the pilots who completed their follow-up measurements

participant	DLMO at baseline (T0)	rotation 1				DLMO before rotation 2 (T1)	rotation 2				DLMO before rotation 3 (T2)
		direction	time zones crossed	layover hours (n)	rest days (n)		direction	time zones crossed	layover hours (n)	rest days (n)	
1	21:22	W	6	27	7	21:07	W	9	47	6	23:00*
2	after 1:20	N-S	0	36	9	0:56	W	7	50	NA	after 0:25
3	21:48	E	3.5	26	4	21:54	E	7	43	5	20:44*
4	21:29	E	7	34	5	21:46	E	3.5	26	5	22:07
5	21:22	W	6	27	6	19:39*	W	8	50	4	21:11
6	20:08	E	3	26	7	20:09	W	6	50	10	20:22
7	21:16	W	8	50	9	21:28	E	3	27	7	21:16
8	21:30	N-S	2	25	4	21:31	W	6	27	4	22:11
9	21:18	W	6	27	5	20:32*	W	6	76	5	21:21
10	20:52	N-S	1	27	5	21:02	W	6	44	5	22:08*
11	22:38	W	5	26	5	23:41*	W	6	43	7	23:54*
12	23:19	N-S	1	27	8	after 1:00*	W	8	26	NA	after 0:00
13	21:15	E	7	57	6	20:53	N-S	0	50	6	21:15
14	18:55	N-S	0	50	8	19:35	E	7	58	5	18:32
15	20:48	N-S	1	46	5	20:33	W	6	75	3	22:34*

E=East, W=West, N-S=North-South (crossing maximal two time zones). NA = Not Applicable: variable could not be determined through the data collection app. *DLMO >45 different compared to the DLMO time measured at baseline.

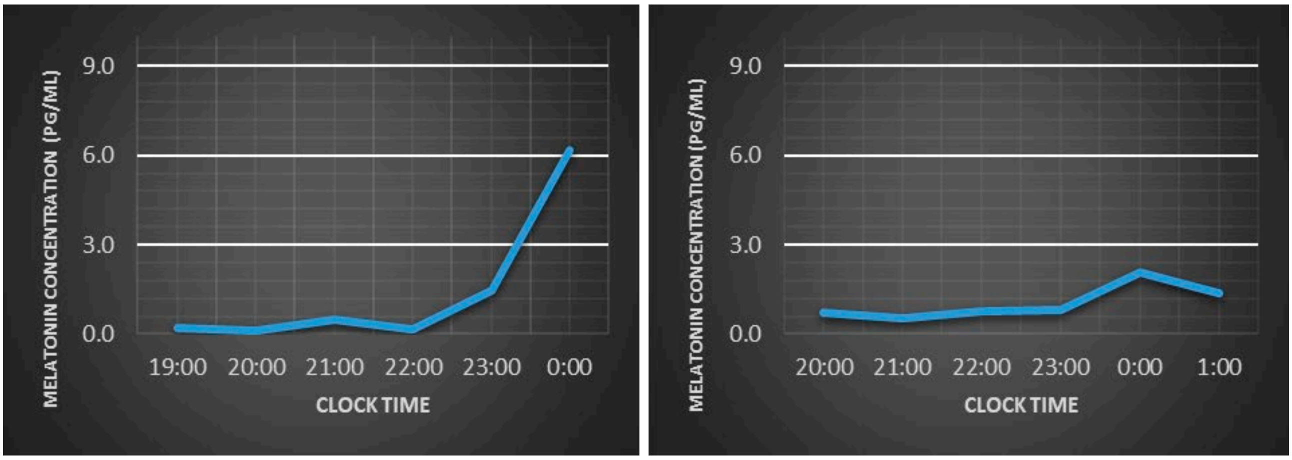

Figure 4: Example of the outcomes of two saliva measurements. In the graph on the left the 3pg/ml DLMO threshold was reached. In the graph on the right, the threshold was not reached for which the timing of the DLMO could not be determined

3.2 Comprehensive analyses

Comprehensive statistical analyses were carried out with the data of the 15 participants that were complete. A one-sample t-test was performed first to determine if the DLMO after being exposed to a normal rotation, significantly differed from the DLMO measured at baseline (following a long period of inactivity). The mean absolute difference between the baseline and follow-up measurement of the DLMO at T1 turned out to be 28.8 minutes ($p<0.05$), [95%CI 11.1 – 46.4]. The absolute difference between baseline and DLMO at T2 was also significant, and increased to 41.1 minutes ($p<0.05$), [95%CI 18.0 – 64.2]. The mean absolute difference when T1 and T2 outcomes were taken proved to be 34.9 minutes ($p<0.001$), [95%CI 21.2 – 48.6].

No significant associations between personal factors and DLMO deviation could be found. Because of the relative low number of full datasets and the large variation in schedule characteristics, few associations between schedule related factors and the level of DLMO deviation could be determined. However, using the Spearman Rho non-parametric correlation technique, a significant positive correlation between the number of time zones and DLMO deviation was found (Spearman Rho = 0.393, $p<0.05$). Thus, the more time zones were crossed, the larger the DLMO deviation.

To determine whether the direction of the rotation had an effect on the level of DLMO deviation compared to baseline, an univariate ANOVA analysis was performed. When comparing three types of directions (westward, eastward, and north-south flights crossing maximal two time zones) a significant effect on the level of DLMO deviation was found ($F(2) = 3.735$, $p < 0.05$). By means of the multiple comparisons test, a trend could be seen between westward and eastward flight ($p = 0.071$), indicating that westward flights could lead to more circadian disruption in comparison with eastward flights. This result was confirmed when the north-south flights were extracted from the analysis, and the direction of the DLMO deviation was taken into account ($F(1) = 4.4$, $p < 0.05$) (Figure 5).

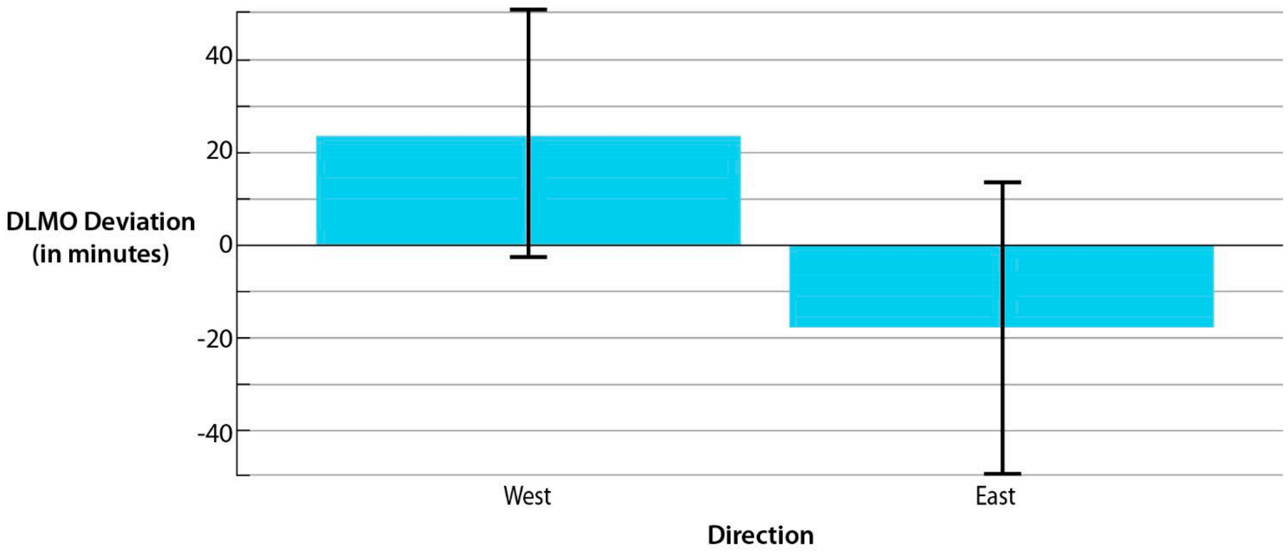

Figure 5: DLMO deviation as a result of flight direction

Additional analyses were performed to find out if any cumulative effects of rotation direction could be seen. This was not the case for participants flying in the same direction ($F(1)=0.249$, $p=0.627$), although only based on data of seven participants. Moreover, when looking at subsequent rotations in the opposite direction, a notable (non-significant) increase could be seen for the three participants who flew eastward first, and had a westward rotation next (Figure 6).

Finally, a stepwise logistic regression model was used to determine if the schedule related factors led to circadian disruption (y/n), defined as an absolute difference of more than 45 minutes between the DLMO during follow-up and DLMO measured at baseline. Although both flight duration and flight direction remained in the eventual model, the outcomes did not reach statistical significance ($\chi2 = 11.668$, $p = 0.112$).

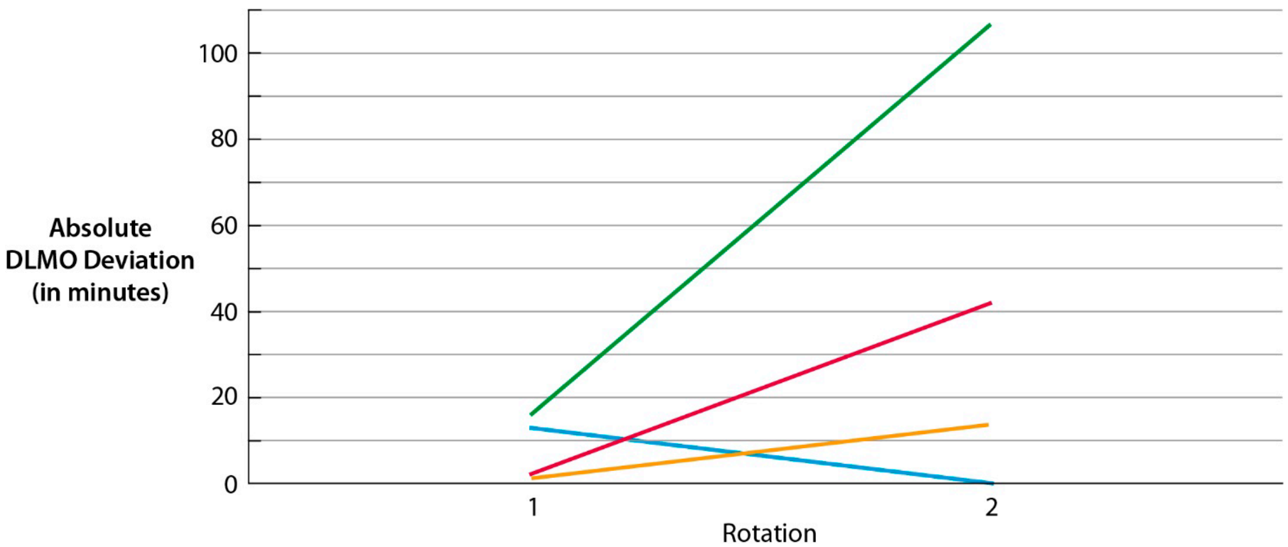

Figure 6: Absolute DLMO deviation for participants having subsequent rotations in opposite directions. Participant green, red and orange had an eastward rotation first

4 Discussion

This report describes an innovative study that uses a saliva collection procedure to determine the circadian disruption of airline pilots by calculating the Dim Light Melatonin Onset (DLMO). The Covid-19 pandemic made it possible to perform a realistic baseline measurement with pilots who had not been flying for a longer period of time for which unexposed circadian rhythms, sleep and recovery characteristics could be determined. Before the pandemic, this would have been quite difficult, due to the cumulative and continuous exposure of most pilots to a variety of irregular working hours and time zone crossings. After an announcement on the airline company’s news app during the summer of 2020, 87 pilots indicated to be interested participating. This reflects a low response rate (about 3% of the total population), but many pilots could have been excluded beforehand, for example because they only flew within Europe and were therefore not (sufficiently) exposed to time zone crossings. Furthermore, other factors such as the discomfort of the required (saliva) measurements, the fact that most pilots were still flying occasionally, and job insecurity may have contributed to the low response. Eventually, 41 pilots remained eligible to participate after the selection procedure.

From the results of the baseline questionnaires we saw that, at the time the pilots were at home due to the Covid-19 pandemic, the participants were in good health, slept well, and were well rested. In an intervention study amongst more than 500 active airline pilots from the same company, a mean total CIS score of 62.5 was found, and a fatigue severity score of 26.3 (van Drongelen et al., 2014). In comparison, the mean scores of the participants of the current study at baseline were 50.5 (range 20-140, higher score indicates more overall fatigue) and 19.8 (range 8-56, higher scores indicate more fatigue severity) respectively. In addition, the mean Jenkins Sleep Quality score in the mentioned intervention study was 7.4, while the baseline sleep quality score of the participants in the current study was 6.1 which indicated that the sleep quality of the CAPS participants was better. The duration of sleep at home was adequate as well: the mean sleep length of 7h21m of the CAPS participants at baseline is within the recommendations of the US National Sleep Foundation to sleep 7 to 9 hours per night (Hirshkowitz et al., 2015). Moreover, although varying between individuals, an average sleep duration of close to 8 hours per night is thought to be needed to maintain psychological and physiological health (Bendak & Rashid, 2020; Van Dongen et al., 2003).

The first aim of this study was to find out if one of the most accurate markers for circadian rhythm -DLMO- could be assessed by means of a saliva self-collection procedure in airline pilots. The results show that it is indeed possible to measure circadian disruption of airline pilots this way. Despite the large variation in flight schedule exposure, and the relatively demanding collection procedure which lasts five hours, includes six measurements, and contains restrictions with respect to light exposure and nutrition, the DLMO time could be determined for the vast majority of pilots who managed to send in samples for analysis. In addition, we did not receive many questions or complaints regarding the procedure, which could indicate that the pilots were able and willing to perform the actions involved once the instructions were clear to them. Moreover, only a few remarks were made on the explanation forms we added to the measurement kits.

It has to be mentioned, however, that there was quite a large percentage of pilots who were lost to follow-up. Eventually, only 15 of the 41 pilots (37%) could provide a complete dataset. It is possible that some participants were not motivated anymore as a result of their experiences during the first baseline saliva measurement. The participants with whom we have communicated during the follow-up period, however, indicated that it was mostly due to the ongoing Covid-19 situation that they were not able to perform their follow-up measurements. The majority of the participating pilots had an Airbus type rating and especially the routes flown with this aircraft by the involved airline company were affected most by the Covid-19 pandemic. In addition, since the B747 aircraft was phased out during the course of the study, these pilots had to train to fly another aircraft type and could therefore not conduct their follow-up measurements. Nevertheless, our study does show that it is possible to use the saliva measurement procedure in future (small scale) studies to determine effects of flight schedules on circadian disruption. Active supervision by the researchers seems appropriate, however, since in the current study several participants who were flying frequently again provided only one follow-up measurement (n=4), performed a measurement on the wrong day (i.e. not in accordance with the measurement schedule provided (Table 1: Fictional flight schedule sequence with corresponding follow-up measurements, or performed a saliva measurement from which the DLMO threshold could not be determined (n=2).

In addition, for a good interpretation of the DLMO measurements during future studies, a better functioning sleep-, wake- and activity log is needed. In the current study the NLR data collection app proved to be malfunctioning at certain moments in time. Because the app was still in the development phase during the study, this resulted in several bugs, the app being down and/or occasionally disconnected with the server. Although participants who reported problems with the app received an alternative diary in the form of a digital word file, relevant contextual information might have been lost due to the problems with the app. Furthermore, although subjectively measured information about the sleep/wake schedule has been proven to be of added value (van den Berg et al., 2016), for future studies it will be worthwhile to add objective sleep/wake measures to a well-functioning data collection app, for instance by means of a short Psychomotor Vigilance Task (PVT) (Basner et al., 2011).

The second aim of this study was to determine the effect of being exposed to 'normal' flight schedules again after the period of inactivity, to find out if the recovery time between flight schedules was sufficient to get back to DLMO baseline levels, and to establish which contextual factors had a significant influence on the level of recovery. The results showed a mean difference of 35 minutes between the DLMO at baseline and the DLMO measured one day before the next rotation during a (near) normal schedule, indicating that on average the rest period in between rotations was sufficient for the participants to get back to their baseline circadian rhythm. In addition, during the majority of the measurements, the data of the participants showed that they did get back to their baseline circadian rhythm levels. In 31% (8 out of 26) of the measurements the provided recovery time in between schedules proved to be insufficient: the DLMO deviation during these measurements was larger than 45 minutes the night before the outbound flight, indicating circadian disruption (Giménez et al. 2016). Although this level of disruption might seem small, it is definitely relevant since it can have both performance (fatigue, vigilance) and health (sleep, cardiometabolic) effects (Waterhouse et al., 1997; Sack, 2010).

No cumulative effect of exposure on circadian disruption could be found, nor were any schedule related factors significantly associated with the cut-off for circadian disruption. Although these results might partly be due to the low number of participants with follow-up measurements, they could also be influenced by the composition of this specific group of pilots. The baseline questionnaire outcomes showed that compared to the whole group, these participants scored 23% better on the Checklist Individual Strength and 32% better on the subscale fatigue, indicating that their level chronic fatigue was lower, and this might be due to a better ability to recover from irregular working hours and circadian disruption.

Despite the large loss to follow-up, it was possible to show that the more time zones were crossed, the larger the DLMO deviation was. In addition, westward rotations proved to lead to higher levels of DLMO deviation compared to eastward rotations. These outcomes partly coincide with previous research which showed that the severity and duration of circadian disruption can be determined by flight direction, flight duration, layover length and the number of time zones crossed (e.g. Drake & Wright, 2011; Lamond et al., 2006). Which factors are most influential depends on the specific airline schedules the subjects are exposed to, the possibility and ability to sleep during layover, exposure to environmental zeitgebers, and the characteristics of the flight crewmembers themselves (e.g. Caldwell, 2005; Gander et al., 2013). Future studies with more participants who are able to complete their follow-up measurements would be able to develop a more targeted approach, for instance focusing on westward rotations only to have less data variance and a higher change to discover the disruptive characteristics in these schedules. A larger dataset might also make it possible to compose a multivariate predictive model to detect the most influential contextual factors of circadian disruption within a certain operation.

5 Conclusion

Previous literature made clear that it is difficult to predict and measure the individual level of circadian disruption as a result of flight schedule exposure. This report shows that a validated saliva collection procedure to determine the melatonin onset, can be performed at home in between flight schedules. By means of this procedure, objective measurement of individual circadian disruption and/or adjustment is therefore possible in future field studies with flight crew members.

The current study also showed that participating pilots were in good health, slept well, and were well rested in the midst of the Covid-19 pandemic during summer 2020. The follow-up measurement of the 15 pilots who started flying frequently again and were willing to perform the saliva measurements got back to their baseline circadian rhythm for most of the time. In 31% of the cases, the pilots experienced circadian disruption (> 45 minutes difference in melatonin onset compared to baseline) the day before their next rotation. No cumulative effect could be found, nor was an association found with personal characteristics of the participants. The number of timezones crossed and the direction of the rotation (westward compared to eastward) were found to be associated with more circadian deviation compared to baseline. The limited number of associations found are thought to be the result of the considerable loss to follow-up due to the ongoing pandemic, and the wide variation in schedules flown. Future field studies should therefore try to gather more follow-up data to subsequently draw more specific conclusions regarding the causal factors of flight schedule dependent circadian disruption.

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Postal address
PO Box 90502
1006 BM Amsterdam, The Netherlands
e) info@nlr.nl i) www.nlr.org

Royal NLR
Anthony Fokkerweg 2
1059 CM Amsterdam, The Netherlands
p) +31 88 511 3113

Voorsterweg 31
8316 PR Marknesse, The Netherlands
p) +31 88 511 4444