Activity in a Forced Desynchrony protocol

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Abstract

In chronobiology, a forced desynchrony (FD) protocol is often used to disentangle the homeostatic and circadian effects on behavioural or psychological parameters that show a 24-hour rhythmic pattern. In this research we looked at the ability of a 3-day FD protocol to disentangle homeostatic and circadian effect, and if the question whether the effect of bright light in rhythmic output parameters is under circadian control. Specifically, we looked at the activity of participants over the course of the protocol and whether circadian time influences activity. Using percentage related cumulative frequency analysis we assessed the activity of participants over the course of the protocol and found there was no change in small, medium and large sized activity, in either light condition. An effect of circadian time on activity registered by a wrist-worn ActiGraph Actiwatch was found under dim light conditions, but not under bright light conditions. We conclude that the 3-day FD protocol can be used to disentangle homeostatic and circadian effects on rhythmic output parameters and that circadian time seems to have an effect on activity under dim light conditions.

Introduction

Our biological clock is the system that, under influence of zeitgebers (i.e. outside stimuli responsible for attenuating the biological clock) like light exposure, sends our body signals that have a near 24-hour rhythmicity (Wright KP et al., 2002, Cajochen C et al., 2000). This rhythmicity enables the body to actively anticipate, instead of passively react to, predictable changes that occur across the 24-hour day-night cycle. There are several rhythmic output parameters that are under circadian control such as metabolic rate, core body temperature, heart rate and alertness (Czeisler CA et al., 1999). One of the most prominent circadian rhythms is the sleep-wake cycle, which influences neurobehavioral, mental and physical performance, and shows a circadian rhythm with a peak and nadir within the 24-hour day (Zhou X et al., 2011, Teo W et al., 2011, Schmidt C et al., 2007). The sleep-wake cycle is regulated by two systems. The first is the endogenous circadian system, which keeps track of the nearly 24-hour oscillatory internal biological time in order to promote wakefulness during the biological day and promote sleep during the biological night. The second is the homeostatic sleep pressure, which balances time spent awake and time spent asleep through an increase in sleep pressure with increasing time awake and a dissipation of this pressure while sleeping (Aston-Jones G, 2005, Daan S et al., 1984). In most current chronobiological research these processes are studied under natural conditions, meaning that one process cannot be studied without studying the other, since these processes are synchronized with regard to each other. Due to this synchronization it is impossible to know if and how

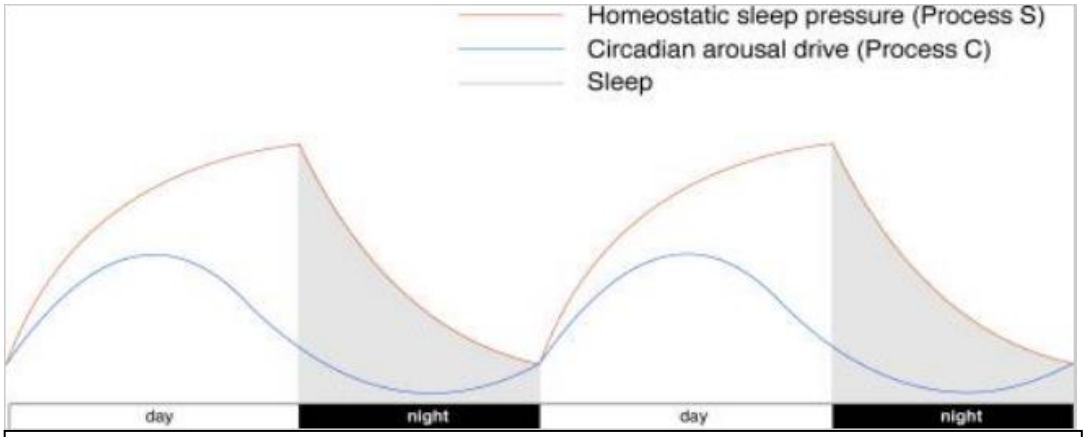

Figure 1. A schematic representation of the synchronized situation of the homeostatic and circadian processes during the day-night cycle. Sleep is initiated when circadian drive for wakefulness declines and sleep pressure is high. Sleep pressure dissipates overnight and is sufficiently low when circadian drive for wakefulness increases. (Oikonomou G et al., 2017)

much of the daily fluctuations in sleepiness are caused by circadian control or homeostatic regulation. This is not only the case for sleepiness, but also for other processes, like alertness, which are regulated by these systems.

Scientists have started applying a forced desynchrony (FD) protocol, which enables separation of the two systems. This entails enforcing subjects to an alternative day-night cycle which is much shorter or longer than the endogenous 24-hour rhythm. Therefore, the circadian clock cannot entrain anymore and keeps following its own intrinsic 24hour rhythm. This means that the subject, and therefore the homeostatic sleep pressure, follows the imposed forced rhythm. In this way, the protocol separates the two systems, allowing researchers to study the circadian and the homeostatic component separately. While parameters like neurobehavioral and physical performance have been shown to be influenced by circadian time (Zhou X et al., 2011, Teo W et al., 2011), this has not yet been studied for activity in general. When studying certain neurological and mood disorders, circadian rhythmicity in activity is often disturbed (Rock P et al., Townhill J et al., Roveda E et al.). Using actigraphy monitors, the disruption of the circadian rhythmicity of activity in subjects suffering from these disorders is monitored and compared to regular daily activity rhythms. In these studies however, circadian rhythms

in activity are mainly the consequence of the subject following their normal sleep-wake cycle. There is no separation of influence of homeostatic sleep pressure and circadian time on activity. That is why in this 3-day FD protocol we also looked at activity of participants as registered by ActiGraph actiwatches.

While activity is under obvious circadian control due to the sleep-wake pattern, we wanted to disentangle homeostatic and circadian-clock related effects on the activity of the participants, making it possible to see what the circadian and homeostatic effects on activity are. Because of relatively small shifts in the daily cycle that subjects are exposed to in current FD protocols (20-hr, 28-hr days) (Wu L et al., 2015, Postnova S et al., 2016) and to get optimal desynchronization of homeostatic and circadian systems, these protocols usually encompass several weeks. One of the aims of this study was to ascertain whether a FD protocol of 3 days (four 18-hour cycles) is enough to disentangle circadian and homeostatic effects on rhythmic output parameters, specifically activity, thereby diminishing the burden for the participants. The second goal of this experiment was to see if this FD protocol can be used to test whether the effect of light on output parameters is under circadian control. Lastly, we wanted to look at the effects of a 3-day 18-hour FD protocol on different levels of activity.

Methods

Participants

A total of 8 healthy males aged between 20 to 29 (average 23.8 ±1.21) years old participated in the current study. Participants health status was assessed using a general health questionnaire. Participants did not suffer from medical conditions, psychological disorders or sleep disorders, did not take any prescribed medication, and alcohol and caffeine consumption was low (≤3 cups a day) at the time of the study. The participants did not perform shift work, and did not travel over multiple time zones one month prior to or during the study. Sleep quality was assessed through the Pittsburgh Sleep Quality Index (PSQI) and chronotype was assessed with the Münicher Chronotype Questionnaire (MCTQ).

Protocol

Participants arrived at the human isolation facility of the University of Groningen 12 hours before habitual sleep onset (determined by the MCTQ), after which the FD intervention started. Subjects remained under <5 lux light intensity until the first sleep period, which is the start of the FD protocol (Figure 2.). After habituation and explanation of the protocol, subjects gave hourly saliva samples from 7 h before habitual sleep onset, to determine Dim Light Melatonin Onset (DLMO), which is considered to be a marker of circadian time (i.e. time as indicated by our circadian clocks to the rest of the body). The 18-hour FD schedule consists of 5 hours of sleep and 13 hours of awake time, resulting in four 18-hour FD cycles (72 hours = 3 days). During this time, subjects will

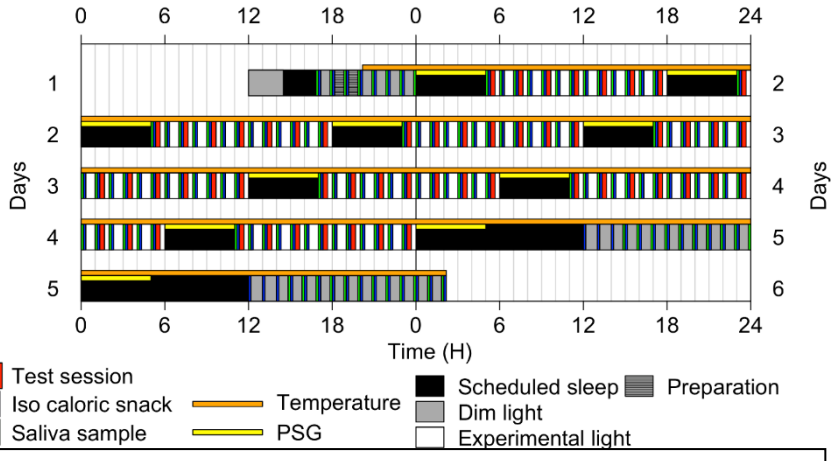

Figure 2. Schematic representation of the 3-day FD protocol.

perform a neurobehavioral performance tasks every two hours. Saliva samples to determine melatonin and cortisol levels were given every hour. Also, subjects will get isocaloric snacks every 2 hours (determined by calculating BMR according to the Harris-Benedict equation and giving the subjects the 18-hour equivalent of 1.4x BMR over 24 hours). ActiGraph wGT3X activity monitors were used, which capture and record high resolution raw acceleration data. These were placed at the wrist, waist and ankle. Weight of participants was measured immediately after arrival at the human isolation facility and on the evening of the recovery day. To determine metabolic rate, participants were asked to drink doubly labelled water. Based on their body weight, participants were given a calculated amount of doubly labelled water orally, followed by tap water. Saliva samples were taken every morning and evening of their awake time. Subjects participated two times in the FD protocol, separated by at least three weeks. One protocol was spent under bright light (>1500 lux) conditions, the other in dim light (<5 lux). Subjects were randomized and divided into two groups, both visiting with a different order of light condition.

Data analyses

For comparing activity between subjects the percentage related cumulative frequency (PRCF) method was used (Riachi M et al., 2004), which is similar to dose-response analysis used in the pharmacology field. Activity data is registered by actiwatches as counts per minute. For all individuals this activity was separated in voluntary and forced activity for the entire protocol, based on moments where participants were instructed to do something (forced activity), or were free to do as they pleased (voluntary activity), resulting in a voluntary and a forced activity dataset for every participant in every light condition. For a PRCF analysis of activity during an FD protocol, data was transcribed to logarithmic values. The range of these values is then divided into intervals. For every day of every person in every light condition the PRCF analysis then counts how many minutes with a certain amount of activity are present in any given interval. This is done for the entire range of intervals, after which the amount of minutes present in every interval are cumulatively added up. The result is a graph that shows how many minutes with a certain amount of activity were present during that day. The total amount of minutes with registered activity on that day is set as 100% and when this is plotted with the intervals on the x-axis, the result is a sigmoidal curve. From this curve EC25, EC50 and EC75 values can be calculated, which correspond to when the curve is at 25, 50 and 75%. When the curve is at 25% the corresponding interval on the x-axis stands for small amounts of activity per minute. Correspondingly, when an EC25 value is higher it is positioned further to the right on the x-axis, meaning the range of the interval where 25% of the total activity of the day is achieved is higher. This in turn means that when an EC25 value is higher there are fewer minutes with small amounts of activity present, meaning there were more minutes with more activity present. To simplify, EC values can be translated to minutes with little (EC25), medium (EC50) and large (EC75) amounts of activity registered. In this way, instead of analysing activity as a whole, the different aspects of activity can be taken into account as well. The benefit of using EC values is that they can be used for statistical analysis. Using GraphPad Prism, EC25, EC50 and EC75 were calculated for every 18-hour cycle the participants were present. Analysis was performed separately for all locations where actiwatches were worn (wrist, hip and ankle).

An additional z-transformation was performed on the activity data to complement the results from the PRCF analysis. Because of the differences in the means of activity between the dim and bright light conditions, a z-transformation is useful because the resulting z-scores of the two different conditions become comparable it is measuring in multiples of the standard deviation of that sample. Since the mean of a z-transformed sample is always zero, and the z-transformation was done over all data, the different light conditions can revolve around the x-axis when plotted and demonstrate an increase or decrease in activity compared to the total activity of both conditions. Analysis was performed for all

FD days separately, and raw activity data was LOG transformed before doing the z-transformation. Only wrist and waist actiwatches were used. Statistics were done by a two-tailed, paired t-test comparing dim and bright light conditions.

To assess effects of the circadian clock and homeostatic sleep pressure hourly averages of activity were taken. To calculate the homeostatic component hourly averages of the FD days were taken, resulting in 18 averaged hours of the sleep-wake cycle (four time points). To calculate the circadian component, hourly averages were taken from the three regular 24-hour days (three time points). The homeostatic averaged component was then subtracted from this 24-hour day average for all hours of the entire protocol. This mathematical disentanglement allows for separating circadian and homeostatic effects on activity. The resulting 24-hour activity pattern is the effect of circadian time on activity. For every subject this was linked to their DLMO to make sure every pattern of activity is on the same circadian time. Activity is displayed in a 24-hour graph with, when a circadian effect on activity is present, a sinusoidal trend. A code was written in R (R Studio 1.1.453) to make a sinus fit of the corrected 24-hourly activity, which results in a significant amplitude if a circadian effect on activity is present.

Results

Due to technical issues several ankle actiwatches did not yield usable data, making the sample group for that actiwatch too small to be used in this research. In the following results only the wrist and waist actiwatches are used.

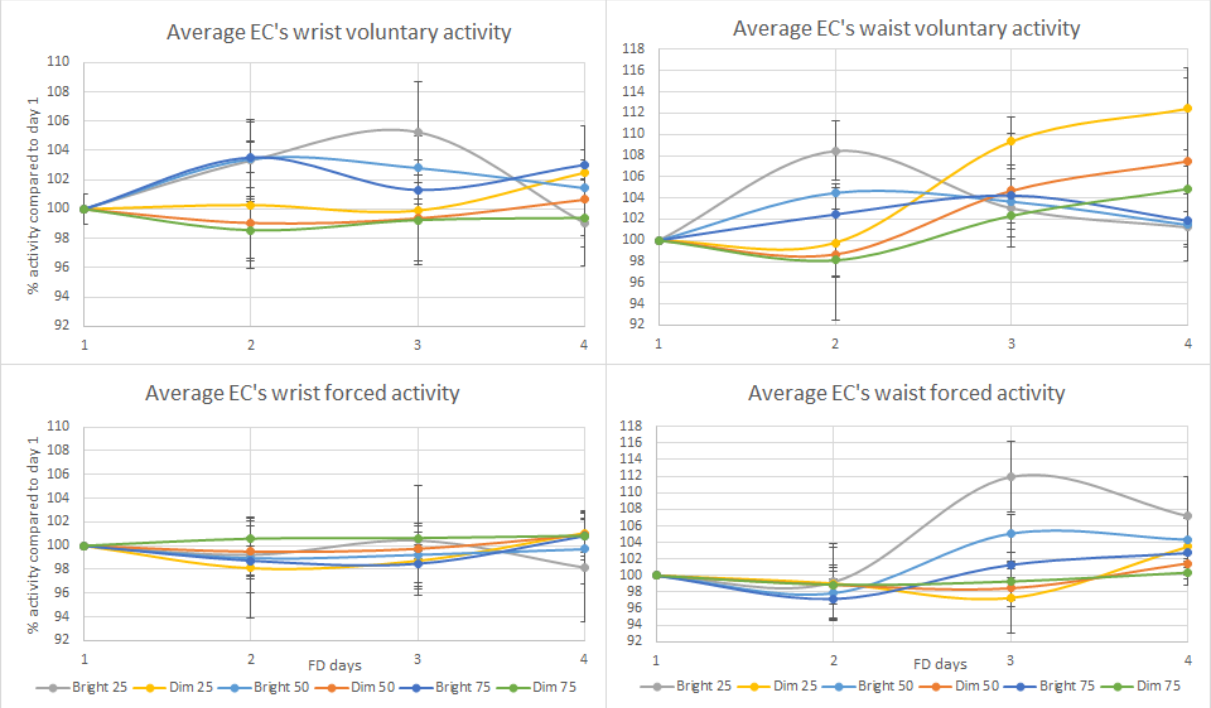

Figure 3. Results of the PRCF analysis of both forced and voluntary activity. On the x-axis the 18-hour FD days, showing the changes in activity over the course of protocol in wrist and waist actiwatches. Both bright and dim light conditions are shown, with EC25, 50 and 75 corresponding to small, medium and large sized movements, e.g. bright 25 is the small movement activity over the course of the protocol in the bright light condition. PRCF analysis resulted in EC values in both conditions. One specific value in one light condition was taken from every day, after which they were compared to the first day. This day was set as 100%, resulting in a single curve for the change in that specific EC value (or movement size) in that light condition over the course of the protocol.

PRCF analysis

For every actiwatch of every participant the activity was divided according to the four 18-hour sleep-wake cycles of the protocol. For every FD day a PRCF analysis was done, resulting in four sets of EC values for every participant for both of the two visits. EC's 25, 50 and 75 were averaged for every FD day and day 1 was set at 100%. Every series of EC values was compared in percentage to the first FD day, resulting in the graph in figure 3. In this way the change in activity over the course of the FD protocol can be registered. All EC values calculated were taken and a repeated measures ANOVA statistical test was performed. Within subject factors were light condition and time (FD-day) and between subject factors were order of the light conditions the participants were exposed to and the different actiwatches. This analysis was performed for all three EC values individually, with the values in percentage compared to day one being the dependent variable in the analysis. No significant differences were observed over the course of the protocol in any activity size or light condition in either forced or voluntary activity. The only interaction that showed a trend was the light*time*order interaction of the wrist actiwatches, which was present in all EC's and resulted in a maximum significance of p = 0.101 for EC75.

Z-transformation

For the wrist actiwatches, the results shown in figure 4 shows increased activity in dim-light conditions and decreased activity in bright light conditions. Activity in the dim light condition over the course of the entire protocol was significantly higher ($p < 0.01$) than activity in the bright light condition. A variation in wrist activity between the different FD days in the dim-light condition was also observed. The waist actiwatches showed no trends in activity.

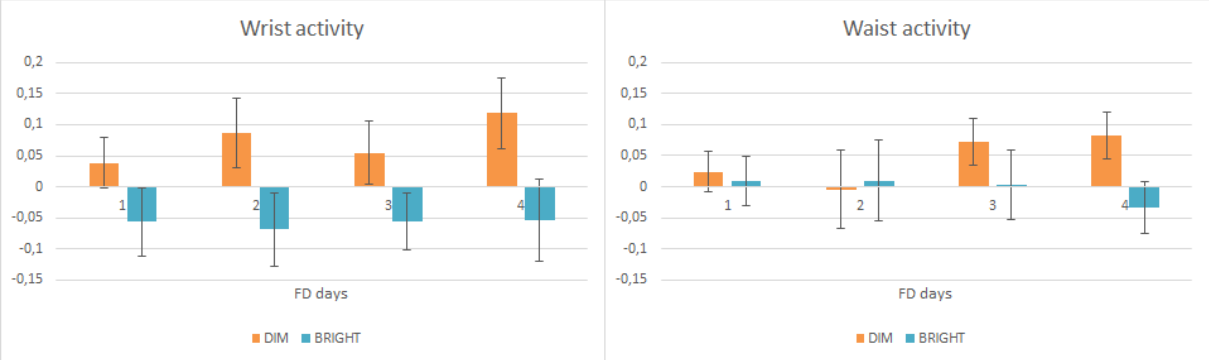

Figure 4. Results from the z-transformation of the activity data. Orange bars represent dim light conditions and blue bars bright light. Overall wrist activity differed significantly between dim and bright light conditions ($p < 0.01$). Waist activity showed no trends. X-axis is displayed in FD days and y-axis is displayed in z-scores resulting from z-transformation.

Circadian effects

To establish the effects of circadian and homeostatic time on activity, the two systems were mathematically disentangled. Activity was separated into voluntary and forced activity. In figure 6 homeostatic activity is shown, with no significant differences between dim and bright light conditions.

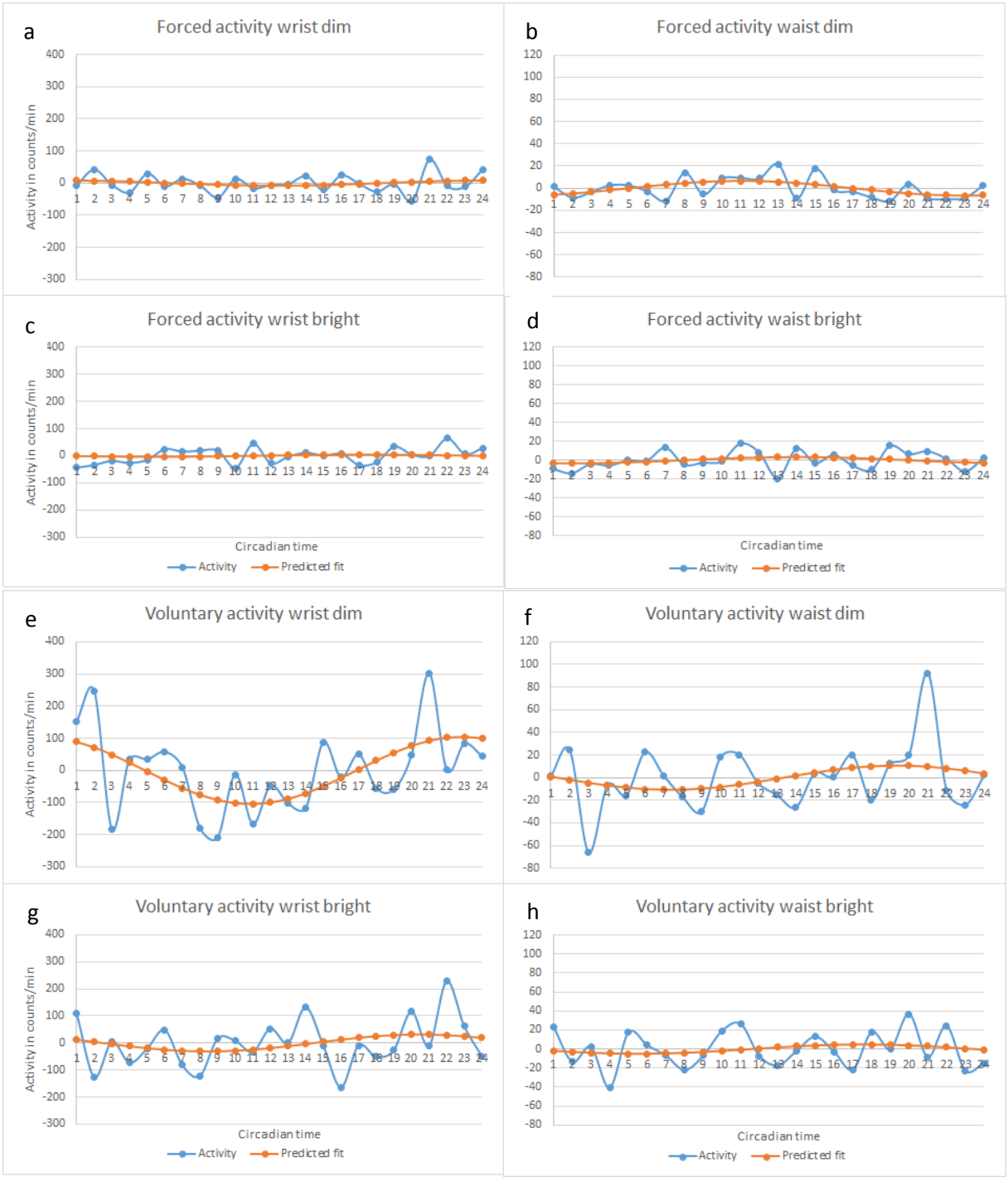

Figure 5. Schematic display of circadian effects on activity. The blue line represents the effect of circadian time on activity, as explained in the methods. The orange line is the sinusfit made by R, where a significant amplitude of the fit indicates the presence of influence of circadian time on activity. Significant amplitudes were detected in graphs b (p = 0.01084, with an amplitude of 6.655 and SE of 2.391) and e (p = 0.00234, with an amplitude of 104.32 and SE of 30.337). X-axis is displayed as circadian time beginning from the first hour after DLMO.

With regard to the effects of circadian time on activity, when looking at the voluntary activity there was no significant pattern in the waist actiwatch graph. The wrist actiwatch graph under bright light was also non-significant, but under dim light conditions, a significant 24-hour pattern in activity with a significant amplitude (p = 0.00234, with an amplitude of 104.32 and SE of 30.337) was observed. At the peak and nadir of the pattern the effect of circadian time on activity resulted in an increase or decrease of 104 counts per minute respectively. In forced activity the waist actiwatch graph showed a 24-hour pattern in the dim light condition (p = 0.01084, with an amplitude of 6.655 and SE of 2.391),

but not in the bright light condition. The wrist actiwatch graphs showed no 24-hour patterns and significant amplitude under either light condition .

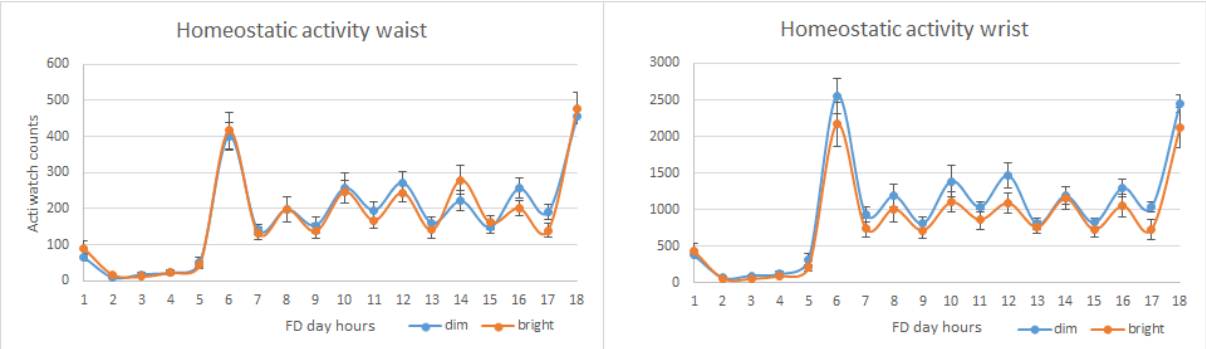

Figure 6. Schematic display of homeostatic activity in both dim and bright light conditions. On the x-axis the hours of the FD days are shown and on the y-axis the hourly averages of registered counts are shown. The first 5 hours of the 18-hour cycle are spent asleep and the last 13 awake. Dim and bright light conditions did not show significant differences.

Discussion

Disruption of circadian rhythmicity in sleep-wake patterns and daily activity is associated with several mood disorders and a compromised health status in general (Roveda E et al., 2012, Rock P et al., 2014). While human studies suggest a possible link between deficits in circadian rhythmicity and less successful healthy ageing, studies in rodents have shown direct relations between ageing and a degenerating functioning of the SCN. In one study, voluntary exercise was shown to help entrain the biological clock and reduce the desynchronization between SCN and peripheral tissues during ageing (Leise TL et al., 2013). In another study voluntary exercise was shown to enhance activity rhythms and alleviate anxiety and depression-like behaviours in a sand rat model (Tal-Krivisky K et al., 2015). While physical exercise has been shown to influence and be influenced by circadian patterns, voluntary activity has not yet been studied in this context and could be of some interest.

The results of the PRCF analysis showed that there was no significant change in activity over the course of the protocol. Even though the participants are well-rested on FD day 1 compared to FD days 2, 3, and 4, they show no decline in activity, even though they are also awake in what would normally be their night time. This can possibly be explained by the A possible explanation could be that the participants were not allowed to exercise, causing them to be inactive over the course of the entire protocol, but since PRCF analysis can analyse all aspects of activity, a decline in “small movement” activity might be expected. There is also no difference in voluntary or forced activity between the bright and dim light condition. Where you would expect bright light conditions to activate participants (Campbell SS et al., 1995), this is not the case in either wrist or waist actiwatches and any EC.

The results from the z-transformation showed a difference in activity between dim and bright light conditions. Bright light seems to have an inhibitory effect on activity in the FD protocol, whereas activity in dim light conditions seems to be increased. This could be due to the fact that in dim light, the biological clock is more true to its natural rhythm and stimulates the participants to be more active. The variation observed in the dim light condition of the wrist actiwatches could possibly be an explanation for the effect of circadian time on activity found in figure 5e. Also the seemingly constant level of decreased activity in the bright-light condition could in turn explain the lack of circadian influence on activity in bright light conditions in the wrist.

When present, significant amplitudes indicate a circadian pattern in activity. The absence of an amplitude can be explained by a homeostatic influence on activity caused by time spent awake, where individuals show the same activity pattern in every wake period of the protocol. This causes the activity pattern to be only visible in an 18-hour cycle, because over a 24-hour cycle these sleep and wake periods even each other out. In figure 6 the 18-hour cycle of the homeostatic component is clearly shown, with the activity pattern of the participants clearly showing a peak in activity at the beginning and end of the wake period, caused by general activity when getting out or getting ready for bed. The two-hourly peaks in activity are caused by participants preparing for test sessions. In the voluntary activity of the wrist actiwatch in the dim light condition, a significant amplitude was found, suggesting an effect of circadian time on activity. This effect was not seen in the bright light condition. This could be caused by the fact that bright light is a stimulating factor on the activity of individuals (Campbell SS et al., 1995), which could suppress influence of circadian time on activity, causing them to display a more homeostatic activity pattern. When the activity periods of the homeostatic pattern balance each other out, this in turn would result in the flat line we observe in some of the circadian pattern graphs. Although an influence of circadian time on forced activity was not expected, there was a significant amplitude observed in the forced activity waist actiwatch in dim light. That being said, the amplitude is relatively small, indicating that this is a very small effect, especially compared to voluntary dim light wrist actiwatch. The fact that an effect of circadian time on activity was found also suggests a link between the biological clock and the activity of an individual. Although circadian time has been linked to physical performance before, this was found to be mainly based on time spent awake after entrained awakening (Facer-Childs E et al., 2015). A direct influence of circadian time on activity, as found in our research, could be of some interest when looking into performance of, for example, athletes.

Since research into circadian patterns of activity in general is based on the sleep-wake pattern and disruption of the circadian pattern usually is caused by restless nights and napping during day-time (Castro J et al., 2015), a direct effect of circadian time on activity is a new approach of studying circadian patterns in activity. The fact that we only see a circadian pattern in activity in the wrist watch might be due to the fact that the participants were not allowed to exercise or move intensively in general. It is uncertain if our results can be directly applied to daily life, considering only the wrist showed a circadian pattern and there was no baseline of participants in the isolation facility to compare our results to. However, an effect of circadian time on voluntary activity is something that can be taken into account in future research into voluntary exercising and its effect on the amplitude of circadian activity patterns, and its subsequent effect on the general healthy ageing of individuals and the well-being of patients susceptible to mood disorders.

To conclude, the 3-day 18-hour FD protocol seems to be sufficient to distinguish an effect of circadian time on activity. From the z-transformation of the activity data we can conclude that bright light seems to constantly decrease activity compared to dim light over the course of the protocol. The circadian pattern found in the wrist activity could be due to the variation in activity between FD days. This in turn could be due to the fact that the effect of the biological clock is not suppressed in dim light conditions. The fact that the ankle actiwatches cannot be taken into account is regrettable, as they would have added a valuable third dataset to this research. The circadian pattern in activity is an intriguing new find and could stimulate research into circadian activity to not only look at amplitude of day- and night-time activity, but also direct effects of circadian time on activity levels during actual periods of activity.

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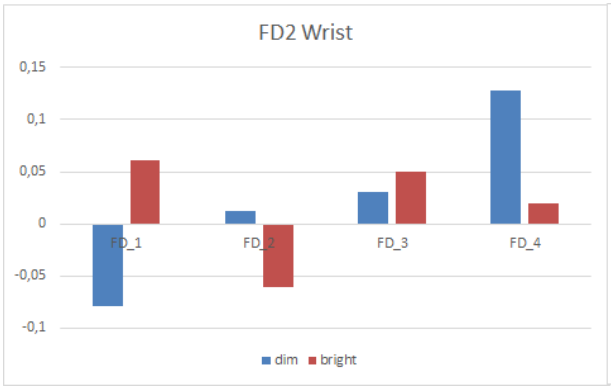
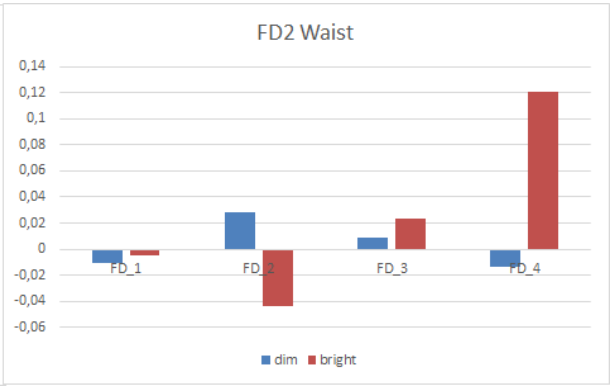
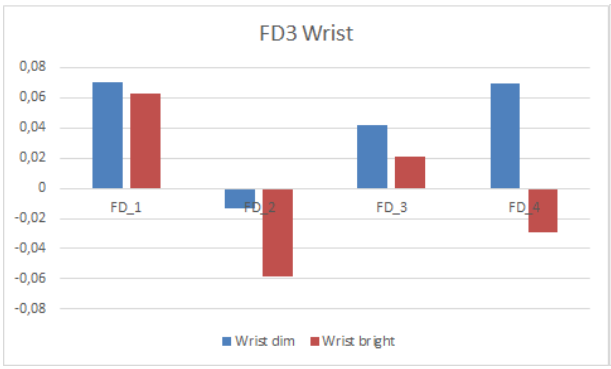
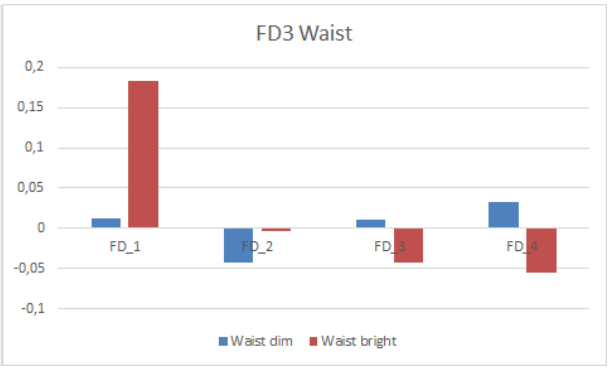
Supplementary graphs

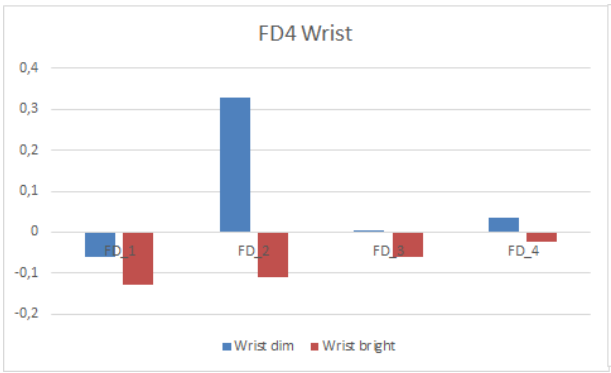
Individual z-transformation

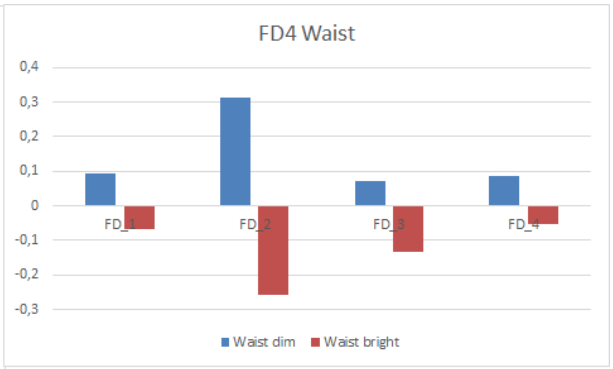


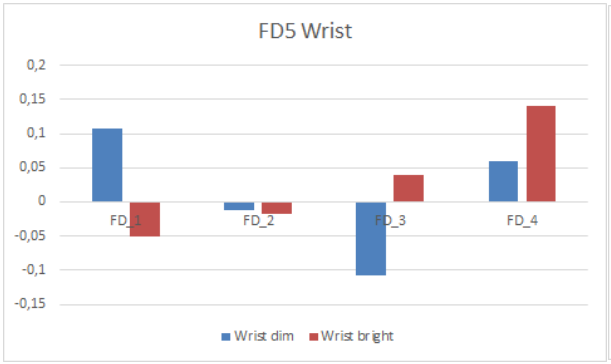


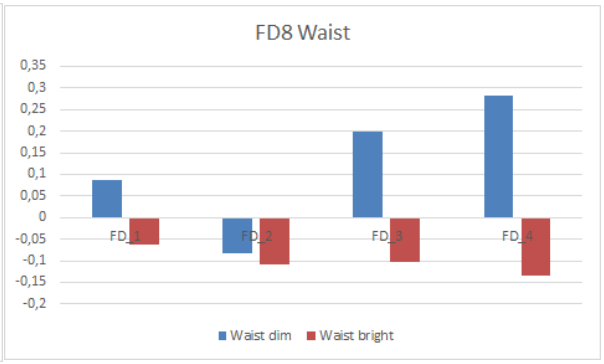


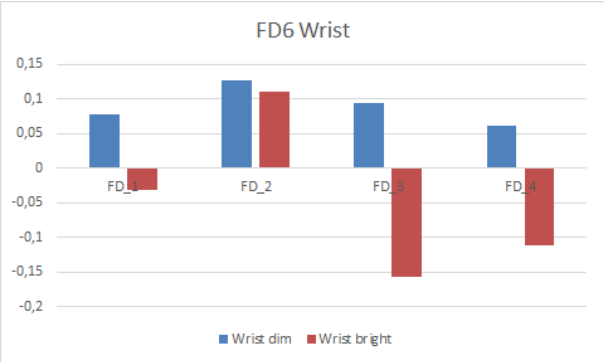








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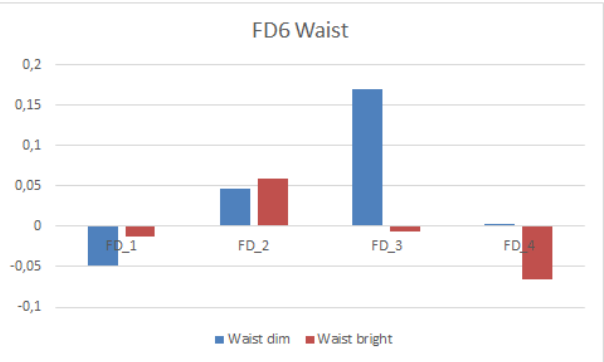
0,15
0,1
0,05
0
-0,05
-0,1
-0,15
-0,2
FD_1
FD_2
FD_3
FD_4
Wrist dim
Wrist bright


FD6 Waist
0,2
0,15
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0
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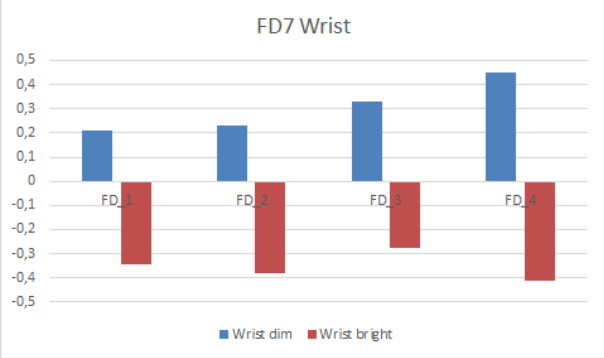
Waist dim
Waist bright


FD7 Wrist
0,5
0,4
0,3
0,2
0,1
0
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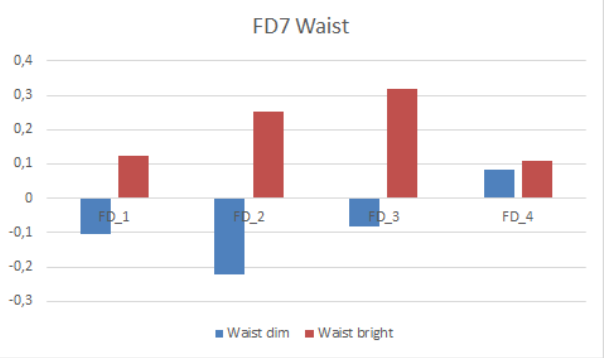
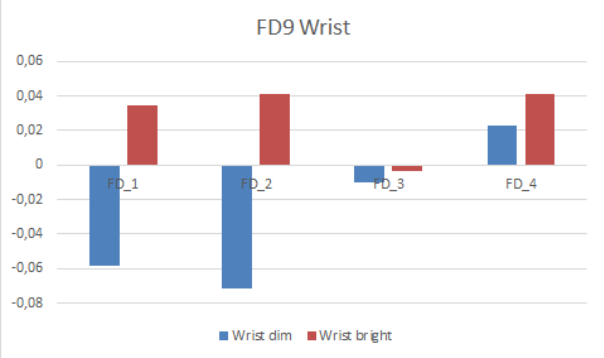
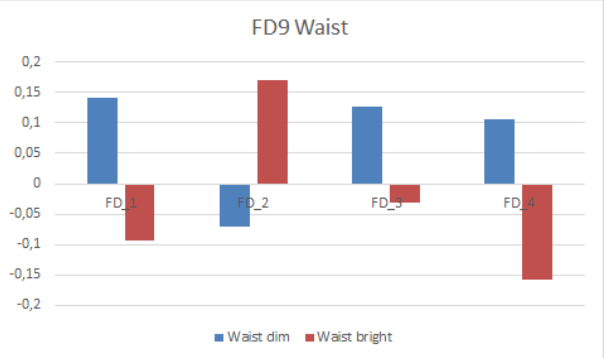
-0,5
FD_1
FD_2
FD_3
FD_4
Wrist dim
Wrist bright


FD7 Waist
0,4
0,3
0,2
0,1
0
-0,1
-0,2
-0,3
FD_1
FD_2
FD_3
FD_4
Waist dim
Waist bright


FD9 Wrist
0,06
0,04
0,02
0
-0,02
-0,04
-0,06
-0,08
FD_1
FD_2
FD_3
FD_4
Wrist dim
Wrist bright


FD9 Waist
0,2
0,15
0,1
0,05
0
-0,05
-0,1
-0,15
-0,2
FD_1
FD_2
FD_3
FD_4
Waist dim
Waist bright