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Thoracic surface temperature rhythms as circadian biomarkers for cancer chronotherapy

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Abstract

The disruption of the temperature circadian rhythm has been associated with cancer progression, while its amplification resulted in cancer inhibition in experimental tumor models. The current study investigated the relevance of skin surface temperature rhythms as biomarkers of the Circadian Timing System (CTS) in order to optimize chronotherapy timing in individual cancer patients. Baseline skin surface temperature at four sites and wrist accelerations were measured every minute for 4 days in 16 patients with metastatic gastro-intestinal cancer before chronotherapy administration. Temperature and rest-activity were recorded, respectively, with wireless skin surface temperature patches (Respironics, Phillips) and an actigraph (Ambulatory Monitoring). Both variables were further monitored in 10 of these patients during and after a 4-day course of a fixed chronotherapy protocol. Collected at baseline, during and after therapy longitudinal data sets were processed using Fast Fourier Transform Cosinor and Linear Discriminant Analyses methods. A circadian rhythm was statistically validated with a period of 24 h ($p<0.05$) for 49/61 temperature time series (80.3%), and 15/16 rest-activity patterns (93.7%) at baseline. However, individual circadian amplitudes varied from 0.04 °C to 2.86 °C for skin surface temperature (median, 0.72 °C), and from 16.6 to 146.1 acc/min for rest-activity (median, 88.9 acc/min). Thirty-nine pairs of baseline temperature and rest-activity time series (75%) were correlated ($r>|0.7|$; $p<0.05$). Individual circadian acrophases at baseline were scattered from 15:18 to 6:05 for skin surface temperature, and from 12:19 to 15:18 for rest-activity, with respective median values of 01:10 (25–75% quartiles, 22:35–3:07) and 14:12 (13:14–14:31). The circadian patterns in skin surface temperature and rest-activity persisted or were amplified during and after fixed chronotherapy delivery for 5/10 patients. In contrast, transient or sustained disruption of

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DECLARATION OF INTEREST
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these biomarkers was found for the five other patients, as indicated by the lack of any statistically significant dominant period in the circadian range. No consistent correlation ($r<|0.7|$, $p \geq 0.05$) was found between paired rest-activity and temperature time series during fixed chronotherapy delivery. In conclusion, large inter-patient differences in circadian amplitudes and acrophases of skin surface temperature were demonstrated for the first time in cancer patients, despite rather similar rest-activity acrophases. The patient-dependent coupling between both CTS biomarkers, and its possible alteration on a fixed chronotherapy protocol, support the concept of personalized cancer chronotherapy.

Keywords

Chronotherapy; circadian biomarkers; colorectal cancer; rest-activity; skin temperature

INTRODUCTION

The adequate circadian timing of chemotherapy significantly improves tolerability up to 5-fold and nearly doubled antitumor efficacy as compared to constant rate or wrongly timed administrations in rodent models and in cancer patients (reviewed in Lévi et al., 2001, 2007, 2010). However optimal circadian timing differs by up to 8 hours, according to mouse sex and genotype, despite exposure to the same light–dark schedule as recently shown for irinotecan, a topoisomerase I inhibitor that is effective against gastro-intestinal malignancies (Li et al., 2013). The clinical relevance of this finding is shown in a recent meta-analysis of three international randomized trials, where a fixed circadian delivery schedule of 5-fluorouracil-leucovorin-oxaliplatin significantly improves progression-free and overall survival in male but not in female patients with metastatic colorectal cancer (Giacchetti et al., 2012). Both survival endpoints are also twice as long in cancer patients whose rest-activity rhythm was robust, as compared to those with an altered pattern, in a pooled study involving 436 patients with metastatic colorectal cancer (Lévi et al., submitted). Nonetheless the square wave pattern of the rest-activity rhythm provides poor precision regarding the circadian phase, as compared to body temperature. Despite both rhythms reflect the central circadian pacemaker, they are generated in distinct areas of the supra-chiasmatic nuclei (SCN) (Moore & Danchenko, 2002; Silver & LeSauter, 1993; Silver & Schwartz, 2005). The concurrent measurement of both circadian biomarkers could thus refine Circadian Timing system CTS assessment in cancer patients, and help personalize chronotherapy timing and dosing. Such non-invasive circadian biomarkers could further provide early warning signals of circadian disruption, a finding also associated with poor survival in patients with metastatic colorectal, breast, lung or kidney cancer (Cohen et al., 2012; Innominato et al., 2009, 2012, 2013; Lévi et al., submitted; Mormont et al., 2000; Sephton et al., 2000, 2013).

The circadian rhythm in body temperature further coordinates the molecular circadian clocks in peripheral organs, such as liver, lung, kidney, intestine and possibly tumors through its regulatory effects on Heat Shock Factor (HSF), Heat Shock Proteins (HSPs) and Cold Inductible RNA Binding Protein (CIRBP) (Li et al., 2010; Reinke et al., 2008).

Supra-chiasmatic nuclei ablation or chronic jet lag suppresses the circadian patterns in rest-activity and core body temperature in mice, resulting in a 2-to-3-fold acceleration of experimental cancer progression (Filipski et al., 2002, 2004, 2006). In contrast, CTS re-enforcement with meal timing, a potent synchronizer of peripheral clocks, nearly halves experimental tumor growth (Filipski et al., 2005; Li et al., 2010; Wu et al., 2004). Anticancer drugs can also disrupt both circadian activity and temperature patterns, as well as molecular clocks, as a function of dose and circadian timing in mice (Ahowesso et al., 2011; Lévi et al., 2010; Li et al., 2002; Ohdo et al., 2001). Thus, the temperature circadian rhythm appears both as a biomarker of CTS function and as an endogenous synchronizer of peripheral clocks.

Although earlier studies suggested the relevance of human temperature monitoring for CTS assessment, the lack of non-invasive and convenient technology precluded further testing in cancer patients (Boudreau et al., 2008; Gunga et al., 2009). Recently developed thermal patches enabled a pilot study to be conducted in healthy subjects and in cancer patients, with challenging data suggesting the greater sensitivity of the circadian rhythms in skin surface temperature as compared to rest-activity for the detection of inter-individual differences in CTS function (Scully et al., 2011).

Here, we provide the first simultaneous longitudinal assessment of both multisite skin temperature and rest-activity measurements every minute for 4–12 days in 16 cancer patients. The temperature is higher in the daytime and lower at night. Such rhythms result from the behavioral changes that are associated with the sleep-activity cycle (Waterhouse et al., 2012). Core body temperature and rest-activity rhythms are in phase. Skin temperature should be the mirror of core body temperature, high at night and low during the day (Ortiz-Tudela et al., 2010). The study aimed at the identification of sensitive methods of signal analyses that would provide quantitative estimates of CTS function in individual patients. Inter-patient differences in internal timing were further sought, as possibly supporting the concept of personalized cancer chronotherapy.

SUBJECTS AND METHODS

Patients

Eligible patients had unresectable locally advanced or metastatic colorectal or pancreatic cancer with measurable disease. They received a fixed circadian-based chemotherapy protocol (chronotherapy) and had an adequate level of physical activity as indicated by a Performance status (PS) of <3 according to the World Health Organization. Brain metastasis, acute infection and expected poor patient cooperation were the only non-inclusion criteria. Thus patients of any age or with any prior surgery, radiotherapy and/or chemotherapy were eligible. The study was performed according to Helsinki declaration on clinical trials and followed the recommendations for chronobiological studies in human subjects (Portaluppi et al., 2010). The study objectives and methods were explained to each eligible patient. Sixteen volunteering patients signed an informed consent. The study took place between October 2010 and October 2011 at the Oncology Department of Paul Brousse University Hospital in Villejuif, France.

Circadian-based chemotherapy protocols

All the registered patients received a fixed chronotherapy protocol according to their medical condition, type and stage of cancer and previous treatment history, using an external ambulatory programmable multichannel pump (Mélodie, Aguettant, Lyon, France). Each course consisted of daily chronomodulated delivery of 5-fluorouracil (median dose per course: 2100 mg/m^2 [range: 1200–3200]) and leucovorin (1200 mg/m^2 [500–1200]) from 22:15 to 9:45, with peak delivery at 4:00, combined with irinotecan (160 mg/m^2 [130–180] from 2:00 to 8:00, with peak delivery at 5:00 on day 1) and/or oxaliplatin (77 mg/m^2 [66–140], from 10:15 to 21:45, with peak delivery at 16:00) (Bouchahda et al., 2009; Garufi et al., 2001; Gholam et al., 2006; Giacchetti et al., 2006; Lévi et al., 2011). Chronotherapy was delivered as 4-day courses intravenously or into the hepatic artery at home and/or at the hospital. Ten patients received a 1-to-2-h intravenous infusion of monoclonal antibodies Cetuximab, Bavacizumab or Panitumumab on the first day of the chonotherapy delivery, after the consultation and before the beginning of the 2-, 3- or 4-drug treatment infusion, if it was indicated for the treatment of their cancer (Bouchahda et al., 2008, 2011; Lévi et al., 2011). Chronotherapy courses were repeated every 14 days.

Study design

Thoracic skin surface temperature and wrist rest-activity were continuously monitored for 4 days at baseline, i.e. before receiving a course of chronotherapy, in 16 patients. Monitoring was continued for 4 days during chronotherapy and for 4 days after treatment administration in 10 of these patients. All patients kept a precise diary of their daily activities including times of awakening and retiring, time of shower, meal times and times and doses of medication intake on a specific form.

Temperature rhythm monitoring

Skin surface temperature (°C) was monitored at four locations on the front thorax, including two “warm” and two “cool” areas, based on infrared (IR) imaging exploration conducted in the nine initial patients (Scully et al., 2011). For this purpose, an IR camera with 0.015 °C temperature resolution (model SC7700 camera FLIR Systems ATS, Croissy-Beaubourg, France) operating in the 3–5 μm wavelengths with 640 × 512 pixels per image was used. This method identified both upper (U1 and U2) thoracic areas as “warm” and both lower (L1 and L2) thoracic areas as “cool” (Figure 1A). Skin surface temperature was measured and transmitted every minute using wireless skin surface temperature patches and a wearable monitor kept within a 2-m reception range (VitalSense®, Philips Respironics, Andover, MA). This device had a temperature sensitivity of 0.01 °C and battery life of 240 h. The patches were placed on each of the four selected front thoracic areas (Figure 1B). Patches were changed after the baseline or the treatment study span recording.

Environmental temperature was concurrently measured every minute using an iButton temperature logger (Maxim Integrated Products, Inc., San Jose, CA), placed in the pocket containing the VitalSense monitor, in order to evaluate the possible effect of external temperature on skin surface temperature. Both skin surface temperature and ambient temperature data were downloaded from the monitor and from the iButton to a personal

computer. The real skin temperature data were stored with day, hour and minute of data collection in an electronic spreadsheet form.

Rest-activity monitoring

The circadian rest-activity rhythm was monitored using a wristwatch accelerometer (Mini-Motionlogger, Ambulatory Monitoring Inc., Ardsley, NY) worn on the non-dominant wrist (Mormont et al., 2000) (Figure 1C). The count in wrist accelerations was recorded each minute concurrently with skin surface temperature for each 4-day span. The wrist accelerations per minute (acc/min) corresponded to the number of times when the accelerometer changed direction during 1 min, according to the Zero Crossing Mode (ZCM). Data were downloaded using an infrared interface linked to a personal computer and transferred into an electronic spreadsheet form.

Data analyses

Time series with >20% localized data loss were discarded since this prevented accurate interpolation. Adequate temperature and rest-activity time series were then interpolated in order to account for occasional data loss and chronograms were displayed using Matlab R2009b® (The Mathworks, Natick, MA). Fourier analysis periodogram was used in order to determine the dominant periods with corresponding amplitudes in each 4-day span for each temperature patch and for rest-activity in each patient. Cosinor parameters (mesor, amplitude and acrophase) were calculated with data from all patients respecting to each study span. It implies that these results combine intra- and inter-variations. Cosinor analyses with a period of 24 h were further performed on data from each 4-day span, in order to determine the relevant circadian parameters with their 95% confidence limits, whenever the probability of a sinusoidal rhythm was statistically validated with $p<0.05$. Mesor and amplitude, respectively, corresponded to the average value and to half the difference between maximum and minimum values of the best-fitting cosine function. The acrophase was the time of the maximum in the best-fitting cosine function. Intra- and inter-patient variability in circadian parameters were first evaluated using Spearman correlations, through the computation of 2 by 2 correlations between the five time series in the same study span for each patient, and between corresponding time series gathered during the three study spans for the 10 patients monitored before, during and after chronotherapy.

Linear Discriminant Analyses (LDA) were performed on all the time series in each study span for each patient in order to best discriminate the three study spans. Biological time series were also correlated with ambient temperature records. The coupling between two time series was considered as robust if $r \geq |0.7|$ and $p<0.05$. Mesors, amplitudes and acrophases from upper chest temperature time series were compared with corresponding lower chest temperature parameters with a *t*-test.

The dichotomy index (I<O) in rest-activity was also computed in each 4-day rest-activity time series using manufacturer's program (Action 4 v1.10, Ambulatory Monitoring Inc., Ardsley, NY). I<O was defined as the percentage of the activity counts measured when the patient was in bed that were inferior to the median of the activity counts measured when the patient was out of bed (Minors et al., 1996). This index could theoretically vary between 0%

and 100%, with a high I<O reflecting a marked rest-activity rhythm. This parameter was shown as a robust and clinically relevant parameter that integrated the circadian regulation of sleep and activity over the 24 h (Garaulet & Madrid, 2009; Innominato et al., 2009, 2012; Lévi et al., submitted; Ma et al., 2013; Mormont et al., 2000).

A descriptive analysis investigated the relations between selected circadian parameters in skin surface temperature or rest-activity and patient outcomes, including symptoms, toxicity on chemotherapy and survival. For such purpose, overall survival and treatment toxicities were compared between patients whose rest-activity parameter I<O was <97.5%, or equal to or above this value (Innominato et al., 2012; Levi et al., 2012). Survival and treatment toxicities were also compared according to categorized highest circadian temperature amplitude values above its median value or equal to it or below. The relations between temperature acrophases at baseline and toxic events subsequent to fixed chronotherapy delivery were further examined in a descriptive manner.

RESULTS

Patient and time series characteristics

About 11 male and 5 female patients with metastatic colorectal or pancreatic cancer, aged 51–89 years were enrolled in the study (Table 1). The majority of patients had a good Performance Status (PS of 0 or 1), and a normal body mass index. Most patients had liver and/or lung metastases (81% and 63%, respectively). Four patients withdrew from the study after baseline CTS evaluation. Adequate time series were obtained for 61 skin surface temperature and 16 actimetry records from 16 patients at baseline. Thirty-nine adequate temperature time series and 10 actimetry records from 10 of these patients were collected during and after chronotherapy delivery as well.

Treatment consisted of chronotherapy with irinotecan, 5-fluorouracil, leucovorin and/or oxaliplatin, possibly combined with antibodies against Epidermal Growth Factor Receptor such as cetuximab or panitumumab, or against Vascular Endothelial Growth Factor such as bevacizumab (Table 1). Chronotherapy was delivered during hospitalization for three patients and at home for seven patients.

Circadian rhythms at baseline

Chronograms of interpolated time series revealed large differences in the 24-h patterns of skin surface temperature according both to the individual patient and to the recording site. Maximum skin surface temperature values usually occurred at early night for 10 patients, at a time when activity was reaching its nadir (Figure 2A). In contrast, three patients displayed arrhythmic or out-of-phase temperature patterns.

A circadian rhythm with a 24-h period was identified as dominant with FFT and statistically validated with Cosinor ($p<0.05$) for 49 of 61 baseline temperature time series (80.3%) in 13 patients (81.2%), and for 15/16 baseline rest-activity records (93.7%).

The 24-h amplitude of the skin surface temperature rhythms ranged from 0.04 °C to 2.86 °C with a median of 0.72 °C among the 49 validated patches (Table 2). The 24-h amplitude in

rest-activity varied from 16.6–146.1 acc/min, with a median value of 89 (25–75% quartiles: 66.3–101.9) (Table 2). The circadian temperature amplitude was nearly twice as large for the lower thorax patches as compared to the upper ones (*t*-test, $p = 0.001$). In contrast no difference was found for mesor values according to patch location (Table 3). The median acrophase was localized at 01:10 (25–75% quartiles, 22:35–3:07) for the 49 rhythmic temperature patches; Individual values were staggered between 15:18 and 6:05, i.e. over a duration of 14 h and 47 min.

The median acrophase in rest-activity occurred at 14:12 (25–75% quartiles, 13:14–14:31), with individual values ranging from 12:19 to 15:18, i.e. over a time span of 3 h and 59 min. Inter-patient variability in circadian rest-activity was further documented with dichotomy index I<O whose median was 97.6%, yet with individual values ranging from 75.6% to 99.9%. No consistent correlation was found between ambient temperature and corresponding skin surface temperature or rest-activity time series ($r<0.17$; $p>0.01$).

Dynamics of skin temperature and rest-activity circadian patterns on chronotherapy

Temperature and activity patterns were robustly rhythmic at baseline, as well as during and after chronotherapy in five patients, as illustrated with chronograms and Fourier periodogram analysis (Figure 2A and B). A circadian rhythm with a 24-h period was identified as dominant with FFT and statistically validated with Cosinor ($p<0.05$) for 39 temperature time series obtained during or after chronotherapy in 10 patients and for rest-activity in all the patients. Nevertheless, a major circadian alteration was encountered for temperature and/or activity time series in four patients during and/or after treatment. Recovery toward baseline patterns was obvious for skin surface temperature in two patients and for rest-activity in three patients. In contrast, circadian disruption held up for temperature in one patient and for both temperature and activity in another patient (Figure 2C). Results from LDA revealed different behaviors (Figure 2E). The blue scatter plot corresponding to the before study span is farther from the red (during) and green (after) ones for the pt 1 than the pt 2, meaning the temperature values distribution is more different in the first patient. This could be interpreted by the impact of the drug delivery on the metabolism, a desynchronization compare to baseline that represent here the auto-individual control. Furthermore, scatter plots corresponding to during (red) and after (green) are closer. However, red and green scatter plots are closer for pt 2 explaining his difficulty to recover (Figure 2C and D). For the eight other patients, four had the blue scatter plot farther than the two other ones. Moreover, the during and after representing scatter plots were more or less superposed meaning that there was a disturbance of temperature rhythms occurring during treatment delivery and these patients had difficulty to recover. One patient had all the three scatter plot superposed and three other patients had the three scatter plots totally farther from each other meaning for this first patient the presence of disturbed temperature rhythms already at baseline and for three other ones, the robustness of his temperature rhythms and the non-impact of the drugs delivery on these later. Circadian amplitudes with a 24-h period were largest in the lower thorax patches (L1/L2) consistently with baseline data (Table 3 and Figure 3A). In contrast, a circadian rhythm in rest-activity was validated for 93.8% of the patients at baseline, and for 9 of 10 patients during and after chronotherapy. No obvious relation was found between temperature and activity amplitudes during chronotherapy. At

baseline, temperature acrophases ranged from 6 h and 41 min to 12 h and 2 min. During chronotherapy, it was staggered from 12 h and 26 min to 13 h and 41 min and after treatment from 11 h and 55 min to 13 h and 39 min with a median occurring at night, in good agreement with baseline data. In contrast the real range in rest-activity acrophases was <7 h, with a median value at daytime (Table 2 and Figure 4). The median dichotomy index (I<O) was 94.6% (25–75% quartiles, 92.3–96.3) during chronotherapy and 96.7% (93.7–98) after chronotherapy, respectively, ranging from 85.8% to 98.9% and from 77.7% to 99.7% according to individual patient.

Effect of a fixed chronotherapy protocol on 24-h synchronization

Circadian amplitudes in skin surface temperature and rest-activity were correlated at baseline ($0.733 \leq r \leq 0.857$; $p<0.05$), and after fixed chronotherapy ($0.740 \leq r \leq 0.857$; $p<0.05$) but not during treatment delivery in individual patients. This finding supported the concept of treatment-induced internal circadian desynchronization. No consistent relation was found between circadian amplitudes of temperature and activity at baseline and corresponding amplitudes during or after chronotherapy (Figure 3). However, this was the case for four patients throughout the 12-day recording span ($r>|0.7|$; $p<0.05$). This observation suggested inter-patient differences in circadian robustness.

Potential relevance for patient outcomes

The patients with highest baseline temperature amplitude above median value had a median overall survival of 14.4 months (from 7.2 to 30.3), as compared to 7.4 months (from 3.6 to 8) for those patients with lower amplitudes (Figure 5). In good agreement with these findings, the patients whose baseline I<O was equal to or exceeded 97.5% had a median overall survival of 17.7 months (from 7.4 to 30.3), as compared to 7.2 months (from 3.6 to 26.5) for those with a lower I<O. Similar survival trends were found according to temperature amplitude values and rest-activity I<O measured during or after chronotherapy (Figure 5). The patients with highest during treatment temperature amplitude above median value had a median overall survival of 12.2 months (from 11 to 23), as compared to 3.6 months (from 1.4 to 10.2) for those patients with lower amplitudes. After chronotherapy, the patients with highest temperature amplitude above median value had a median overall survival of 14.4 months (from 10.7 to 23.9), as compared to 8 months (from 3.1 to 12.8) for those patients with lower amplitudes. No correlations were observed between I<O and overall survival during or after treatment.

Toxicity was modest in the 10 patients receiving a fixed chronotherapy protocol. In this context, no apparent relation was found between adverse events and temperature amplitude. In contrast, an I<O <97.5% during or after chronotherapy was associated with Grade 2 fatigue and anorexia for four patients, and Grade 1 diarrhea for four patients while, a single patient with a higher I<O displayed Grade 1 fatigue and anorexia.

DISCUSSION

This longitudinal study is the first one that highlights large differences in the robustness of the CTS of cancer patients at baseline, using a combination of two physiological biomarkers.

Inter-patient differences were shown during and after the administration of a fixed combination chronotherapy protocol as well. We expected that the combined circadian monitoring of rest-activity and skin surface temperature at several sites would improve the precision of CTS assessment, especially regarding circadian phase, a critical information for the determination of optimal treatment timing in individual patients.

Accurate and sensitive non invasive measurements of wrist accelerations and skin surface temperature were obtained every minute for 4 days in 16 patients and for an additional 8 days in 10 of these patients, including 7 that were treated on an outpatient basis. Multiple state-of-the-art signal processing and analyses methods were adapted and helped interpret these unique longitudinal data sets. Thus, inter- and intra-patient changes were demonstrated for the coupling of 24-h patterns in skin surface temperature at four sites during chemotherapy delivery and thereafter. Rest-activity and temperature circadian parameters were significantly correlated before and after chronotherapy, while circadian desynchronization was documented during chronotherapy delivery in some patients. This finding confirmed experimental results in mice (Ahowesso et al., 2011; Li et al., 2002, 2007) and validated the usefulness of both biomarkers for proper dynamic CTS assessment.

Both progression-free and overall survival were significantly longer in those patients whose rest-activity rhythm was robust rather than disrupted, according to a pooled analysis of records from 436 patients with metastatic colorectal cancer (Lévi et al., submitted). Thus, median overall survival was twice as long in the patients whose dichotomy index I<O, a measure of the relative amount of activity In-bed versus Out-of-bed, exceeded the median value of 97.5% as compared to those with a lower I<O (Lévi et al., submitted). Circadian disruption was associated with increased circulating levels of Transforming Growth Factor α (TGFα), Interleukin-6 and Tumor Necrosis Factor α (TNFα) in metastatic colorectal cancer patients (Rich et al., 2005). These pro-inflammatory chemo-cytokines directly interfered with circadian rhythm generation in the SCN and/or at the molecular clock level in experimental models (Cavadini et al., 2007; Kramer et al., 2005).

The doubling of median overall survival according to the same I<O threshold was confirmed here. The study further revealed that the patients with a circadian temperature amplitude above its median baseline value of 0.72 °C had a nearly 3-fold increase in median survival. Thus, both rest-activity and skin surface temperature monitoring provided complementary information regarding CTS robustness and circadian phase. The determination of skin surface temperature acrophases could indeed help the identification of the optimal internal timing for chemotherapy delivery in individual patients. Thus robust circadian temperature cycles are able to entrain molecular clocks and clock-controlled pathways in mammalian cells (reviewed in Kornmann et al., 2007; Schey et al., 2009), and inhibit experimental cancer progression (Li et al., 2010). Indeed, temperature acrophase constitutes a reference biomarker for timing cancer chemotherapy (Lévi et al., 2010; Ortiz-Tudela et al., 2013). The clinical relevance of anterior thoracic skin surface temperature is investigated here, as a substitute to core body temperature. However, the technical requirement for proper circadian records of body temperature continuous measurement in ambulatory patients is cumbersome (Gunga et al., 2009; Schey et al., 2009). Thus, core body temperature fluctuates from ~36.5 °C to ~37.5 °C with a maximum ~16:00–18:00 and a minimum ~4 a.m.–5 a.m in

physiological conditions (Waterhouse et al., 2012). In contrast, skin surface temperature reportedly ranges from 32.1 °C to 36.1 °C, with an acrophase at 1:10 at night, i.e. near the expected nadir in core body temperature (Del Bene, 1990). Such timing is confirmed here for the average baseline circadian acrophase of skin surface temperature in 16 cancer patients. The rise in skin temperature at early night is related to the heat dissipation resulting in central temperature drop and triggering of sleep (Kräuchi, 2002). The possible contamination of the skin surface temperature data by environmental temperature changes was suspected for axillary temperature (Motohashi et al., 1987). However it was ruled out for the thoracic measurements, since these variables were not correlated in this study. Nearly half of the patients maintained robust circadian rhythms as indicated by consistent 24-h temperature amplitudes before, during and after chronotherapy. These patients could be amenable to personalized chronotherapy without any additional intervention. Conversely, treatment induced transient or sustained circadian disruption in the other half, possibly because of the wrong timing of the fixed chronotherapy protocol in relation to the internal phase of the patients.

Excessively dosed or wrongly timed chemotherapy suppresses the circadian patterns in rest-activity and body temperature, as well as those in liver clock genes expression in mice (Ahowesso et al., 2011; Lévi et al., 2010; Li et al., 2007; Ohdo et al., 2001). Thus, it is crucial to properly time and dose anticancer drug delivery in individual patients, in order to jointly improve tolerability and efficacy of anticancer treatments (Innominato et al., 2011, 2013; Ortiz-Tudela et al., 2013). Indeed, the incidence of severe adverse events varies up to 5-fold as a function of peak drug delivery clock hour in patients with colorectal or non-small cell lung cancer (Lévi et al., 2007). Yet, optimal circadian timing could differ between male and female cancer patients (Giacchetti et al., 2012). In mice, the circadian timing of irinotecan which achieves best tolerability varies by up to 8 h according to sex and genetic background (Li et al., 2013). A mathematical model based on the circadian transcription patterns of clock genes *Rev-erbα* and *Bmal1* adequately predicts for the optimal timing of irinotecan in different mouse categories (Li et al., 2013).

In conclusion, the current study shows that rest-activity and skin surface temperature rhythms constitute complementary circadian biomarkers. They convey distinct CTS information regarding synchronization, robustness and internal clock timing, before and throughout treatment administration. The broad dispersion of temperature amplitudes and acrophases found here in cancer patients further supports prospective testing of this biomarker combination for optimizing cancer chronotherapy timing in individual patients.

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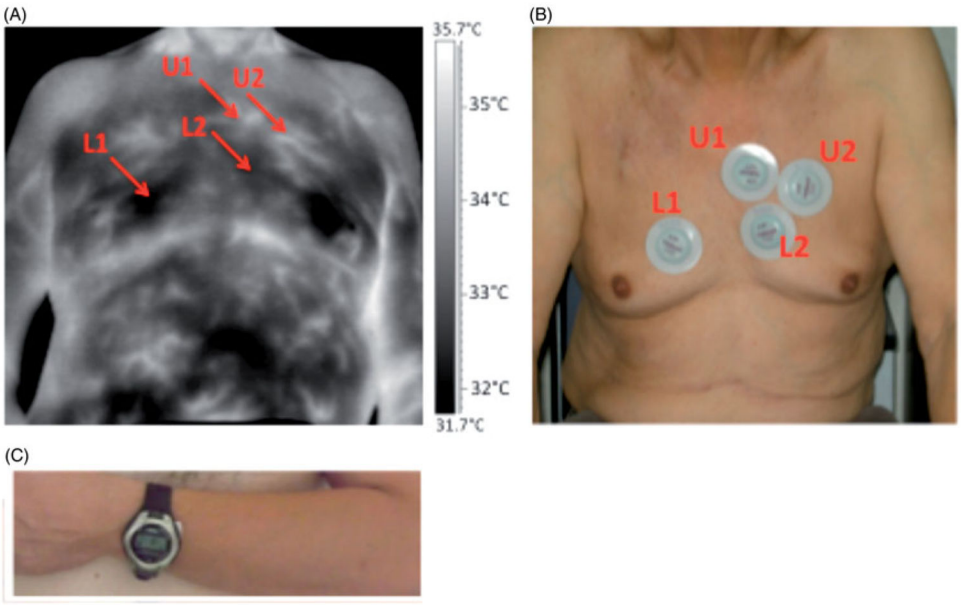

FIGURE 1.

Location of the skin temperature patches and the wristwatch accelerometer. (A) Identification of two warmer and two cooler areas in the anterior thorax of patients, through height resolution infrared (IR) imaging. (B) Patches placement on the four sites. (C) Wrist watch accelerometer worn on the non-dominant arm.

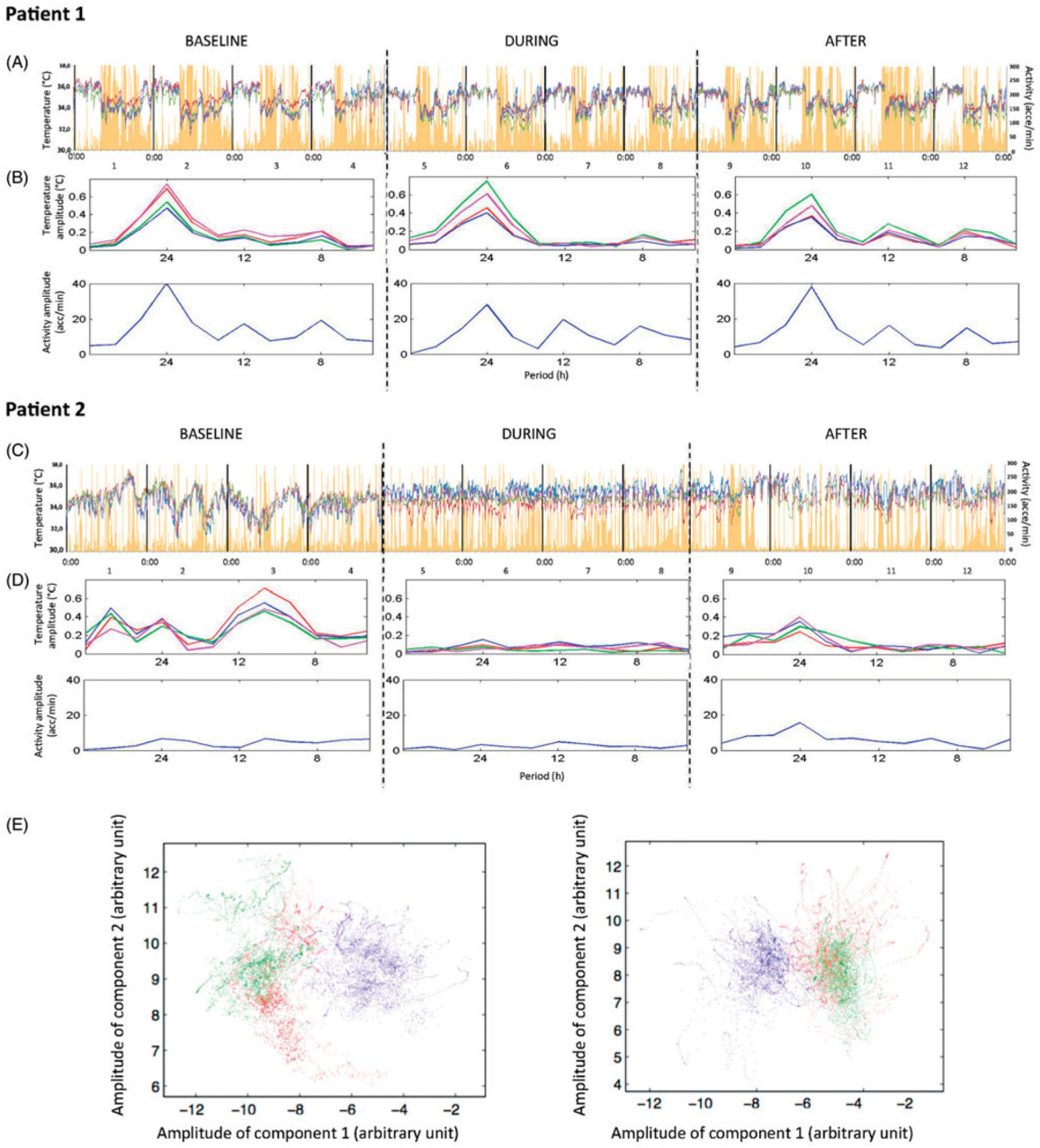

FIGURE 2.

Twenty-four-hour patterns in multisite skin surface temperature and wrist activity measured every minute before, during and after chronotherapy in two cancer patients (pt 1 and pt 2). (A and C) Chronograms of skin temperature at both upper thoracic sites (U1, blue; U2, red) and at both lower thoracic sites (L1, green; L2, purple) in patient 1 who obviously maintained robust circadian patterns throughout the 12 days record and patient 2 with obvious disrupted rhythms. (B and D) Corresponding power spectra note dominant 24-h period for all temperature patches and rest-activity with halved amplitudes during chronotherapy delivery, as compared to baseline and post-chronotherapy spans for pt 1, and the lack of dominant period with relevant amplitudes for pt 2. (E) LDA scores plot in which

each variable represents all individual temperature data collected before (blue), during (green) of after (red) chronotherapy delivery for patient 1 (left) and patient 2 (right). Chronotherapy delivery clearly modified the baseline time series. Indeed, the blue scatter plot corresponding to the baseline study span is farther from the red (during) and green (after) ones for the pt 1 than the pt 2, meaning the robustness of the pt 1 rhythms and the desynchronization observed in pt 2. Furthermore, scatter plots corresponding to during (red) and after (green) are closer justifying the previous sentence. However, red and green scatter plots are closer for pt 2 explaining his difficulty to recover.

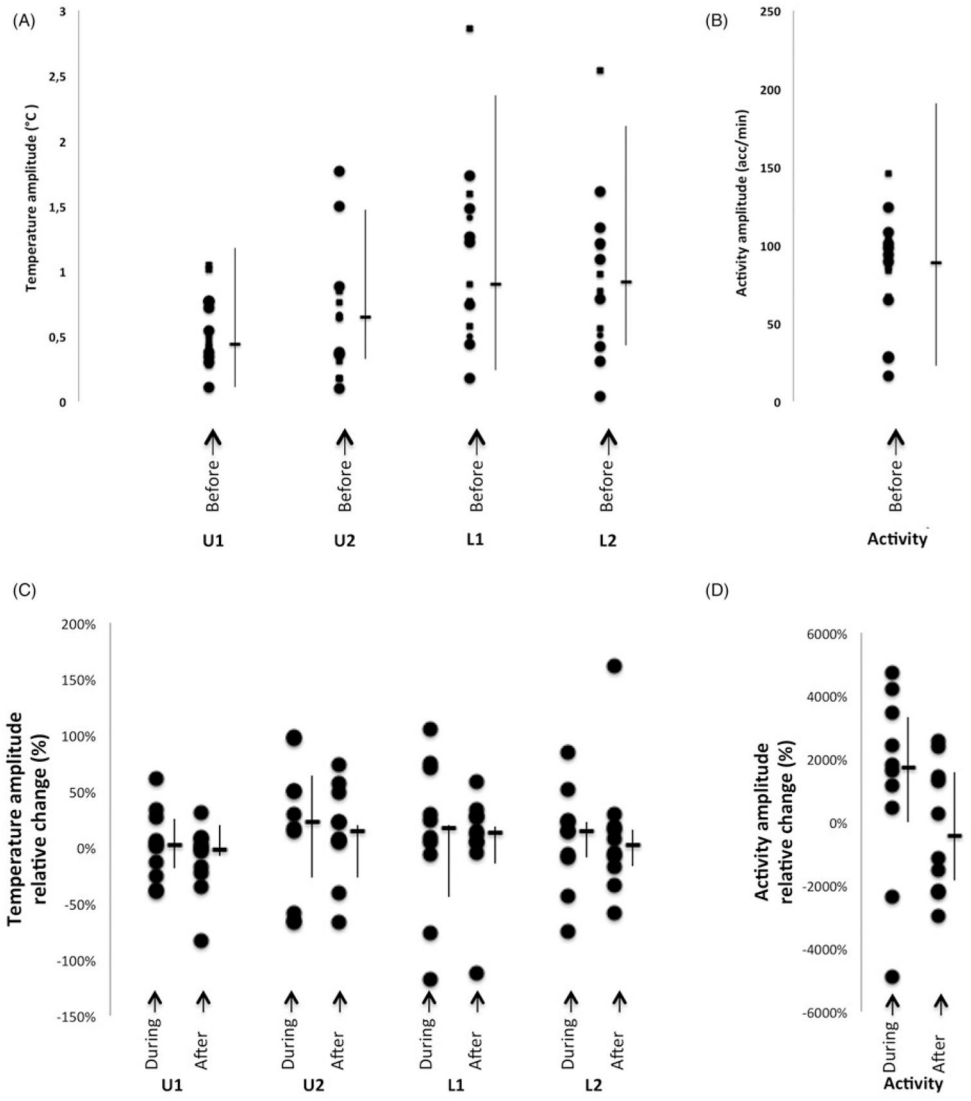

FIGURE 3.

Inter- and intra-patient variability over the three study spans regarding temperature and activity amplitudes. U1/U2, data from patches situated on the upper front chest; L1/L2, data from patches situated on the lower front chest, as shown on Figure 1. (A and C) Twenty-four-hour temperature amplitudes for each time series for each patient. In (A) are the patients' temperature amplitudes before chronotherapy from the four sites (U1/2 and L1/2) and in (C) are the patients' temperature amplitudes evolution during and after chronotherapy delivery. (B and D) Activity amplitude corresponding to each patient and each span is shown on the right of the temperature graph. Median and Interquartile are represented right next to each corresponding marked scatter.

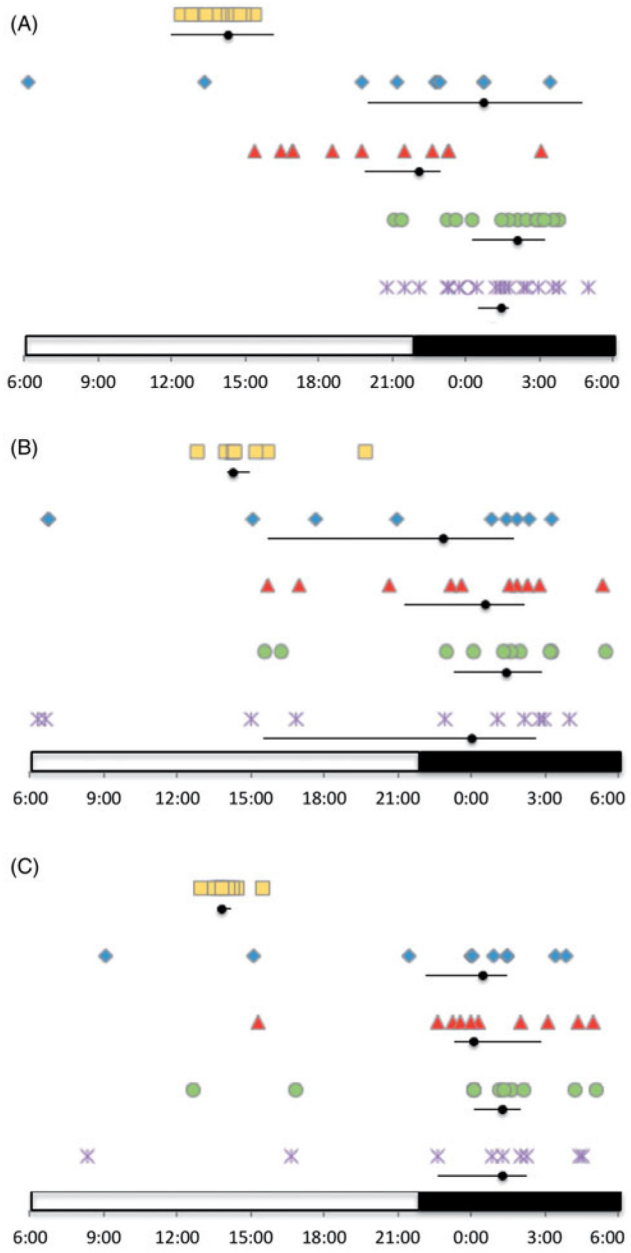

FIGURE 4.

Inter-patient differences in temperature and rest-activity acrophases at baseline, during and after chronotherapy. Upper thoracic sites (U1, blue lozenge; U2, red triangle), lower thoracic sites (L1, green round; L2, purple asterisk), activity (yellow square). (A) Baseline, (B) during, (C) after chronotherapy. Individual acrophases were spread over a range of ≈14 h for skin surface temperature and ≈3 h for rest-activity at each study span. Median and Interquartile ranges are represented in black right next to the corresponding marker scatter.

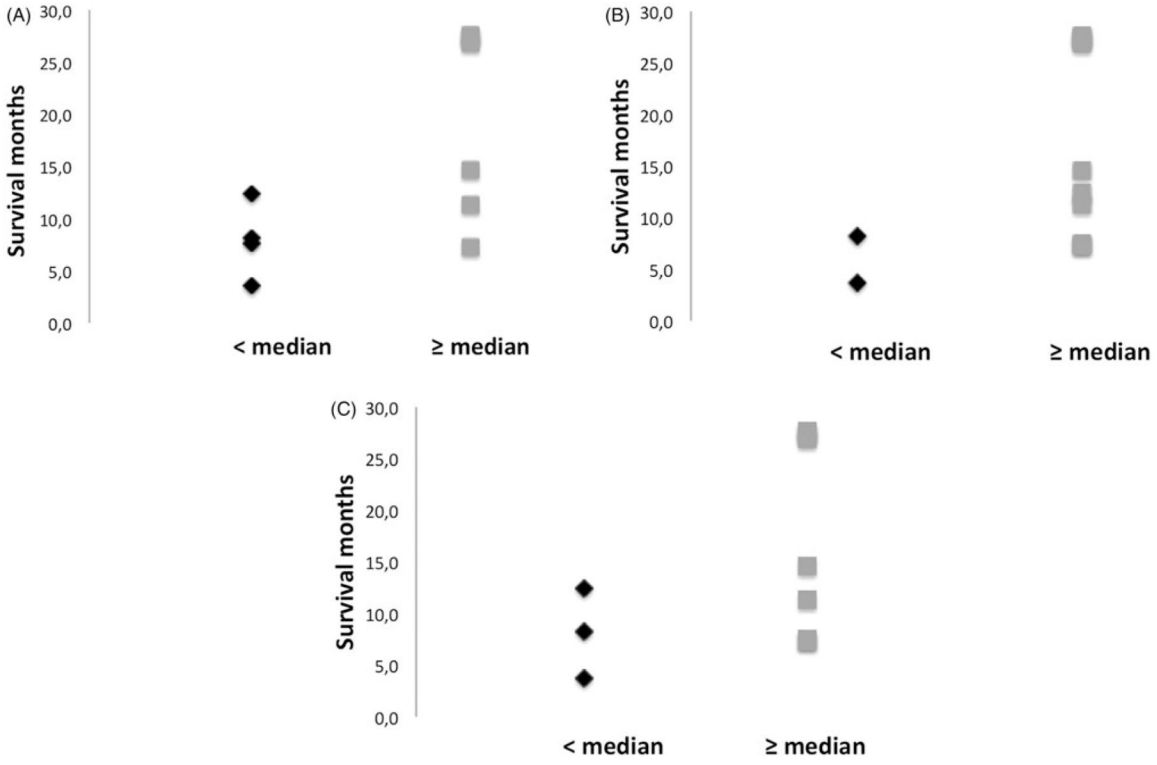

FIGURE 5.

Individual patient survival according to median value of highest skin surface temperature amplitude (among the four sites of recording). (A) At baseline, (B) during and (C) after chronotherapy. Higher was the individual skin surface temperature amplitude, longer was the survival.

TABLE 1

Characteristics of the 16 patients studied at baseline, and the 10 patients further studied during and after chronotherapy.

	At baseline	On treatment
No. patients	16	10
Gender		
Male	11 (69%)	7 (70%)
Female	5 (31%)	2 (20%)
Age (y)		
Median	71	71
Range	51–89	60–73
WHO performance status		
0	8 (50%)	3 (30%)
1	7 (89%)	6 (60%)
2	1 (6.2%)	1 (10%)
BMI (kg/m^2)		
<18.5 (underweight)	2 (12.5%)	2 (20%)
18.5–24.9 (normal)	10 (62.5%)	5 (50%)
25.0–29.9 (overweight)	4 (25%)	3 (30%)
Range	22.9	20.7
Median	16.5–26.1	16.5–26.1
Site of primary tumor		
Colon	11 (69%)	8 (80%)
Rectum	4 (25%)	1 (10%)
Other	1 (6.2%)	1 (10%)
No. metastatic sites		
1	10 (62.5%)	6 (60%)
2	5 (31.3%)	4 (40%)
>3	2 (12.5%)	0 (0%)
Organs involved		
Liver	13 (81%)	6 (60%)
Lung	10 (63%)	7 (70%)
Other	4 (25%)	3 (30%)
Chronotherapy treatments		
IFLO		4 (30%)
IFO		1 (10%)
IFL		1 (10%)
FLO		4 (40%)
Route		
Intravenous		9 (80%)
Hepatic artery infusion		1 (10%)
Monoclonal Antiodies		

	At baseline	On treatment
Bevacizumab		4 (40%)
Cetuximab		4 (40%)
Panitumumab		2 (20%)

WHO PS, World Health Organization performance status; I, Irinotecan; F, 5-fluororuacil; L, Leucovorin; O, Oxaliplatin; IFLO, Irninotacan, 5-fluorouracil, leucovorin, and oxaliplatin delivered on a circadian-based, chronomodulated schedule.

TABLE 2

Circadian parameters of skin surface temperature and rest-activity as quantified by Cosinor (τ = 24 h) for each study span.

	Baseline (16 pts)	During (10 pts)	After (10 pts)
Temperature			
Mesor	34.9 [34.2–35.4][a] (32.1–36.1)[b]	35.0 [34.7–35.5][a] (33.2–36.2)[b]	35.2 [34.8–35.5][a] (32.8–36.2)[b]
Amplitude	0.72 [0.43–0.72][a] (0.04–2.86)[b]	0.67 [0.31–0.87][a] (0.09–1.18)[b]	0.67 [0.38–0.93][a] (0.03–1.91)[b]
Acrophase	01:10 [22:35–3:07][a] (15:18–6:05)[b]	00:57 [16:56–2:20][a] (15:00–6:38)[b]	00:47 [22:49–2:06][a] (14:59–8:59)[b]
Rest-activity			
Mesor	100.9 [79.1–113.4][a] (42.2–160.9)[b]	82.7 [76.2–94.9][a] (58.2–133.0)[b]	88.3 [79.6–93.2][a] (47.5–136.6)[b]
Amplitude	89 [66.3–101.9][a] (16.6–146.1)[b]	60.2 [52.4–75.2][a] (11.9–112.9)[b]	77.8 [56.4–82.1][a] (28.1–130.6)[b]
Acrophase	14:12 [13:14–14:31][a] (12:19–15:18)[b]	14:13 [13:58–14:55][a] (12:44–19:38)[b]	13:42 [13:32–14:06][a] (12:51–15:24)[b]
I<O	97.6 [96.4–98.9][a] (75.6–99.9)[b]	94.6 [92.3–96.3][a] (85.8–98.9)[b]	96.7 [93.7–98.0][a] (77.7–99.7)[b]

[a] Median (25–75% quartiles).

[b] Range (minimum value–maximum value).

TABLE 3

Circadian parameters of skin surface temperature as quantified by Cosinor ($\tau = 24$ h) for each study span.

	Baseline (16 pts)	During (10 pts)	After (10 pts)
Upper chest patches			
Mesor	35.2 [34.4–35.6][a] [33.6–36.1][b]	35.3 [34.8–35.6][a] [34.2–36.2][b]	35.3 [34.9–35.8][a] [34.0–36.2][b]
Amplitude	0.47 [0.32–0.77][a] [0.10–1.77][b]	0.52 [0.18–0.68][a] [0.09–0.92][b]	0.42 [0.31–0.72][a] [0.04–1.55][b]
Acrophase	23:02 [19:42–2:05][a] [15:18–6:05][b]	00:13 [17:26–1:59][a] [15:02–6:38][b]	00:03 [22:57–2:10][a] [14:59–4:51][b]
Lower chest patches			
Mesor	34.7 [33.7–35.1][a] [32.1–35.9][b]	34.9 [34.6–35.3][a] [33.2–35.5][b]	35.1 [34.8–35.4][a] [32.8–35.5][b]
Amplitude	0.9 [0.54–1.30][a] [0.04–2.86][b]	0.83 [0.42–1.03][a] [0.2–1.18][b]	0.82 [0.57–1.05][a] [0.03–1.91][b]
Acrophase	1:41 [23:35–2:51][a] [20:43–4:56][b]	1:12 [16:41–2:51][a] [15:00–06:31][b]	1:08 [23:15–2:06][a] [12:51–15:24][b]

[a] Median (25–75% quartiles).

[b] Range (minimum value–maximum value).