

Saul S. Gilbert, Helen J. Burgess, David J. Kennaway and Drew Dawson
Am J Physiol Regulatory Integrative Comp Physiol 279:1980-1987, 2000.

You might find this additional information useful...

This article cites 23 articles, 8 of which you can access free at:

<http://ajpregu.physiology.org/cgi/content/full/279/6/R1980#BIBL>

Medline items on this article's topics can be found at <http://highwire.stanford.edu/lists/artbytopic.dtl> on the following topics:

Physiology .. Sleep
Medicine .. Hypothermia

Updated information and services including high-resolution figures, can be found at:

<http://ajpregu.physiology.org/cgi/content/full/279/6/R1980>

Additional material and information about *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* can be found at:

<http://www.the-aps.org/publications/ajpregu>

This information is current as of November 22, 2009 .

The American Journal of Physiology - Regulatory, Integrative and Comparative Physiology publishes original investigations that illuminate normal or abnormal regulation and integration of physiological mechanisms at all levels of biological organization, ranging from molecules to humans, including clinical investigations. It is published 12 times a year (monthly) by the American Physiological Society, 9650 Rockville Pike, Bethesda MD 20814-3991. Copyright © 2005 by the American Physiological Society. ISSN: 0363-6119, ESN: 1522-1490. Visit our website at <http://www.the-aps.org/>.

Attenuation of sleep propensity, core hypothermia, and peripheral heat loss after temazepam tolerance

SAUL S. GILBERT,¹ HELEN J. BURGESS,¹ DAVID J. KENNAWAY,² AND DREW DAWSON¹

¹Centre for Sleep Research, The University of South Australia, The Queen Elizabeth Hospital, Woodville, South Australia 5011; and ²Department of Obstetrics and Gynaecology, The University of Adelaide, Adelaide, South Australia 5005, Australia

Received 29 March 2000; accepted in final form 20 July 2000

Gilbert, Saul S., Helen J. Burgess, David J. Kenaway, and Drew Dawson. Attenuation of sleep propensity, core hypothermia, and peripheral heat loss after temazepam tolerance. *Am J Physiol Regulatory Integrative Comp Physiol* 279: R1980–R1987, 2000.—If changes in thermoregulation mediate sleepiness induced by sedative/hypnotics, then a reduction in the soporific efficacy (tolerance) of these agents may be accompanied by a concomitant reduction in their thermoregulatory effects. We compared the thermoregulatory and soporific effects of acute temazepam (30 mg at 1400) in 11 young male subjects before and immediately after 7 consecutive days of temazepam (30 mg). Subjects lay supine (0800–2030), while foot (T_{ft}) and rectal (T_c) temperatures were recorded. Sleep onset latency (SOL) was measured hourly using 20-min multiple sleep latency tests. Relative to placebo, temazepam significantly reduced both T_c and SOL (-0.31°C and 14.1 min) while increasing T_{ft} (3.39°C). A significant tolerance developed after the week of temazepam, with a mean reduction in soporific efficacy of 4.0 ± 0.8 min. This was accompanied by a concomitant attenuation in both T_c (-0.16°C) and T_{ft} (1.44°C). Furthermore, SOL was temporally related to T_{ft} and the maximum rate of decline in T_c before and after tolerance. Together, these results indicate that the thermoregulatory system may be functionally involved in the regulation of sleepiness.

heart rate; multiple sleep latency test; human male

ACROSS A WIDE RANGE of experimental situations, changes in core body temperature have been consistently associated with inverse changes in sleep propensity (24). This association is highlighted by the differential changes in body temperature resulting from the administration of either sedative/hypnotics or alerting agents. In the case of somnogenic agents such as melatonin (1, 6), adenosine (21, 23), adenosine agonists (23), and benzodiazepines (7, 17), an extensive body of evidence has demonstrated that these agents increase sleepiness while decreasing core body temperature. In contrast, agents such as caffeine, amphetamines, nicotine, and cocaine decrease sleepiness and increase body temperature (11). More recently, the timing of maximum sleep propensity has been significantly correlated with the maximum rate of decline in core tem-

perature after the daytime administration of both melatonin and temazepam (8). In addition, another study revealed that the time taken to reach normal nocturnal sleep onset may be related to the degree of peripheral heat loss (13). In light of the apparent robustness of the relationship between sleep propensity and body temperatures, it has been suggested that the thermoregulatory system may be functionally involved in the soporific effect of sedative/hypnotic drugs (2, 5, 8, 13).

If this is the case, then it is possible that a change in the soporific efficacy of these agents might be accompanied by a concomitant alteration in their thermoregulatory effects. One way of testing this hypothesis is to determine if any changes in thermoregulation occur after the development of tolerance to a sedative/hypnotic drug. In the normal course of sedative/hypnotic drug treatment, a gradual, relative reduction in therapeutic efficacy is a common side effect. Such a reduction in efficacy is generally known as tolerance. In human subjects, tolerance to temazepam, one of the most commonly prescribed sleeping medications in Australia, has been observed after daily administration for ~ 1 wk (20). Tolerance to doses of 30–40 mg have been characterized by a decrease in the total sleep time and an increase in the latency to sleep onset (10, 20). However, as yet, to our knowledge no study has measured the thermoregulatory changes accompanying chronic temazepam use. By determining whether the relationship normally seen between sleep propensity and thermoregulatory outputs in normal subjects is altered in temazepam-tolerant subjects, it is hoped that our understanding of the underlying physiological processes involved in the soporific effects of sedative/hypnotic agents may be increased. Therefore, the present study aims to compare the thermoregulatory and soporific effects of daytime temazepam in subjects before and after the evening administration of this agent for 7 consecutive days.

METHODS

Subjects. Twelve young healthy male subjects aged between 18 and 27 (mean \pm SE = 23.1 ± 1.1) yr were to attend

Address for reprint requests and other correspondence: S. Gilbert, Centre for Sleep Research, The Queen Elizabeth Hospital, Woodville Rd, Woodville, South Australia, 5011 (E-mail: saul@bigfoot.com).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

the laboratory on two nonconsecutive 24-h sessions from 2100 to 2100 the following evening to perform the “experimental daytime protocols” (Fig. 1A). Unfortunately, one subject participated in the first two 24-h sessions only and withdrew because of illness unrelated to the drug treatment. This patient’s data are excluded from all analyses. The remaining 11 subjects also slept at the laboratory for 7 consecutive evenings immediately followed by a whole day at the laboratory on the 8th day on two separate occasions for the “week-long evening protocols” (Fig. 1B). Both experimental daytime protocols were separated by no less than 24 h and no more than 7 days for each subject, whereas both week-long protocols were separated by no less than 14 days and were completed within 30 days for each subject. Potential subjects were screened for current medical conditions, sleep disorders, and irregular sleep/wake schedules using a general health questionnaire and a 2-wk sleep diary. In between each experimental session, subjects were instructed to maintain a regular sleep schedule but otherwise could sleep when they desired. Subjects were excluded on the basis of existing medical illness or the use of drugs known to affect sleep or

thermoregulation. Subjects gave written informed consent to participate in the present study. The present study was approved by The Queen Elizabeth Hospital Human Ethics Committee and was performed according to the Declaration of Helsinki.

Experimental daytime protocol. Subjects abstained from caffeine, alcohol, and medications for 24 h before and during the experimental procedure. As alcohol is a potentially dangerous contraindication for temazepam use, blood alcohol concentrations (BAC) were measured in all subjects on arrival in the laboratory. BAC were estimated using a standard calibrated breathalyzer (Lion Alcolmeter S-D2, Wales), accurate to 0.005% BAC. All subjects recorded a 0.0% BAC.

For the overnight sleep in the laboratory, subjects were allowed to self-select the time of lights out, which was no later than midnight for any subject. At 0700 the following morning, subjects were woken and permitted to shower as well as eat a light breakfast before having the following electrodes attached. Each subject was fitted with a conventional montage of polysomnographic electrodes attached to the face and scalp (C4-A2, O1-A1, outer canthi electrooculogram, submental electromyogram) and connected to a Medilog MPA-2 (Oxford Medical Limited, Oxtun, UK). Temperature thermistors were placed at three body sites; left and right foot (499B, Sub-Zero Products, Cincinnati, OH) and rectal (Steri-Probe 491B, Cincinnati Sub-Zero Products) temperatures were recorded. Foot thermistors were placed on the arch of the foot sole and attached with Micropore surgical adhesive tape (3M). Rectal thermistors were self-inserted 10 cm into the rectum. An additional thermistor was placed on the head of the bed at the level of the mattress to record ambient temperature. All thermistors were connected to a custom temperature system (Strawberry Tree), which took samples every second and averaged each input into 30-s “bins.” For the duration of the experimental protocol, the entire laboratory was thermostatically maintained at 25°C by a Daiken FDY100B/RV100 air conditioner (Raymol, Woodville, Australia). The ambient temperature thermistors verified that the temperature at the level of the mattresses in all bedrooms was maintained between 24 and 26°C at all times. To further minimize intersubject and interconditional variations in thermoregulation, all subjects were instructed to wear light cotton shorts and t-shirts. In addition, both feet were covered with a light cotton sheet at all times.

From 0900 until 2030 (excluding multiple sleep latency tests), subjects lay in the supine position and were permitted to read or watch television. At 1400, either 30 mg of temazepam or placebo (1 g of glucose) was administered orally in a double-blind, counterbalanced design (Fig. 1). A light lunch, which did not contain hot food, was provided at 1530.

Week-long experimental protocol. For each subject, the first two experimental daytime protocols were followed by 7 consecutive days of either temazepam (30 mg) or placebo (1 g of glucose) administered orally at 2330 in a double-blind counterbalanced design (Fig. 1B). The aim of this week of temazepam administration was to facilitate the development of tolerance to the treatment agent. On the day immediately after the 7th night, the identical procedure performed in the day of the experimental daytime protocols was conducted to determine the effects that the week-long treatment had on sleep propensity and thermoregulation.

Measurement of objective sleep propensity. Sleep propensity was assessed hourly from 1100 to 2000 using the Multiple Sleep Latency Test (MSLT; adapted from Ref. 3). In an environment conducive to sleep (i.e., dark and quiet), subjects were instructed to lie still on their backs, close their eyes, and attempt to fall asleep. The instructions were re-

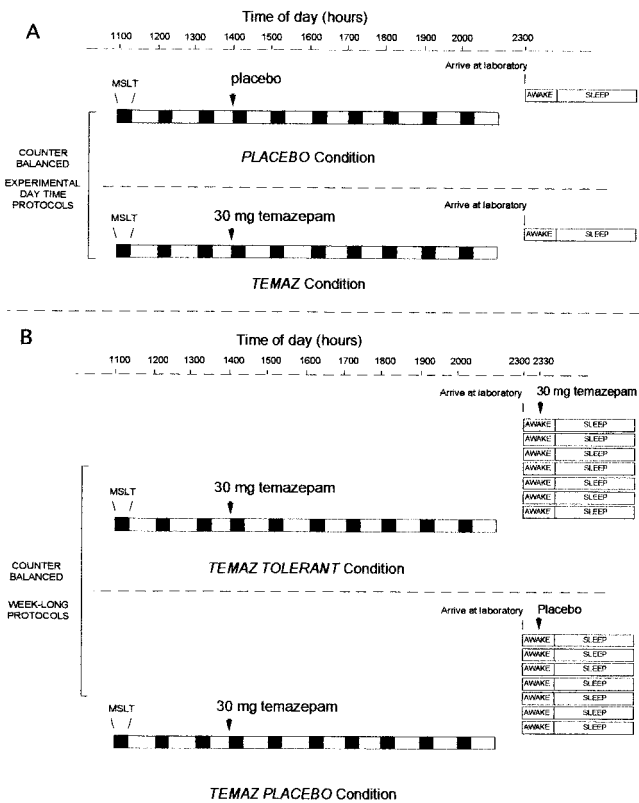


Fig. 1. A: 2 24-h experimental daytime protocols. Subjects slept overnight in the laboratory where, on the following day, rectal and foot temperatures (T_c and T_R , respectively) were recorded continuously while subjects lay supine in bed. Sleep onset latency was measured hourly from 1100 to 2000, and either placebo (1 g glucose) or 30 mg temazepam was administered orally at 1400 in a double-blind, counterbalanced fashion. B: 2 1-wk-long experimental protocols. Subjects slept at the laboratory for 7 consecutive nights where temazepam (30 mg) or placebo (1 g glucose) was given at 2330. Administration of these 2 agents was double-blind and counterbalanced. On the day immediately after the 7th night, T_c and T_R were recorded continuously while subjects lay supine in bed. Sleep onset latency was measured hourly from 1100 to 2000, and 30 mg temazepam was administered orally at 1400. MSLT, Multiple Sleep Latency Test.

peated for each subject, every test. Subjects were woken after they remained in stage 2 sleep for three consecutive, 30-s epochs (as measured via PSG). If the subject did not fall asleep after 20 min, the sleep latency was recorded as 20 min and the test was terminated.

Assessment of cardiac variables. Disposable, pregelled, Ag/AgCl electrocardiogram (ECG) spot electrodes (Meditrace 200, Graphic Controls) were attached to the torso in six positions. These included the jugular notch of the sternum, 4 cm under the left nipple, the right lateral side, the xiphoid process of the sternum, at the base of the neck over vertebrae C3/C4, and on the back over vertebrae T8/T9. All electrodes were attached to an Ambulatory Monitoring System (AMS 4.6, Vrije Universiteit, The Netherlands) that was secured on the bed, next to the subject. The AMS detected the ECG signal from the three electrodes positioned at the jugular notch of the sternum, 4 cm under the left nipple, and on the right lateral side (ground). This signal was recorded with an amplifier with a time constant of 0.3 s and 1-MW input impedance and through a band-pass filter of 17 Hz. Each R peak was detected with a level detector with automatic level adjustment (22), and a millisecond counter was read and reset to obtain the interbeat intervals that were stored continuously. In this way heart rate was determined. The remaining electrodes were used to determine cardiac impedance (this data is not reported here).

Heart rate was recorded in this way in only five subjects. For the remaining six subjects, heart rate was determined automatically using a Medilog MPA-2 (Oxford Medical Limited). Standard ECG electrodes (Oxford disposable electrodes) were attached to the right upper chest and left lower rib cage and connected to a head box, which was, in turn, connected to the Medilog MPA-2.

Statistical analyses. To minimize random variability and to minimize interconditional differences in raw scores, heart rate, skin, and rectal temperature data were expressed relative to the time of drug administration and averaged into 30-min bins (15 min before and 15 min after each time bin). In addition, data from both feet were combined and averaged. Hourly sleep propensity was defined as the sleep onset latency to stage 1 (3 consecutive 30-s epochs in stage 1). Descriptive statistics were also expressed relative to placebo to minimize circadian masking.

For sleep onset latency (SOL), a single average value was also calculated for each condition. To obtain a single value for each subject, each hourly value after 1400 was averaged. These values were subsequently combined and then averaged. The resulting value was used as the mean SOL for each condition.

The thermoregulatory and soporific effects of the placebo and all three temazepam conditions reported in RESULTS were compared using a two-way within-groups (condition and time) repeated-measures ANOVA. The interaction effect was considered the most relevant because the 3-h period before administration (or baseline) was included in the above ANOVAs. As heart rate was recorded only during each MSLT trial in 6 of the 11 subjects, the mean heart rate value for this period was employed for statistical analysis. To determine times where significant differences between conditions occurred, planned means comparisons were conducted. All three temazepam conditions were compared separately with the placebo condition, and the temazepam conditions were also compared with each other. To account for possible violations in the covariance matrix resulting from large numbers of repeated measures, adjusted Greenhouse-Geisser (G-G) significance values were used to determine significance in the repeated-measures ANOVAs as well as the planned

comparisons. For all variables, data are expressed as means \pm SE.

The temporal relationship between SOL and the two thermoregulatory variables, core temperature and foot temperature, was examined using Pearson's product-moment correlation coefficient (r). For these analyses, the minimum SOL for each subject was correlated separately with both the rectal temperature and foot temperature value that occurred at the time of this minimum SOL value. Core body temperature, foot temperature, and SOL (placebo - treatment for all variables) were expressed relative to the time of drug administration (1400). For core body temperature, both the magnitude of temperature change, as well as the maximum rate of decline (MROD) in temperature were used. The rate of core temperature change was obtained using the algorithm: $dt^2 - dt/t^2 - tI$, where t is the time and dt is the data value at that time. Data are expressed as means \pm SE.

Intraindividual correlations were also obtained and were calculated for the pre- and posttolerant conditions separately. For each subject, the hour to hour (1500-2000) changes in SOL were correlated with the hourly changes in both thermoregulatory variables (core and foot temperatures) using Pearson's product-moment correlation coefficient. The temporal relationship between core and foot temperatures was also determined using these intraindividual correlations.

RESULTS

Pretolerant conditions. For all the measured variables, SOL, core body temperature, foot temperatures, and heart rate, planned comparisons revealed that values in both pretolerant conditions (referred to as "Temaz Placebo" and "Temaz" in Figs. 1-6) were not statistically ($G-G > 0.05$) different from each other at any time point. As can be seen in Fig. 1, the MSLT treatment day was preceded by 7 consecutive evenings of a placebo treatment in the Temaz Placebo condition. Because of its similarity to the protocol for the tolerant condition ("Temaz Tolerant"), Temaz Placebo was considered the more appropriate control. Therefore, whereas both pretolerant conditions will be illustrated in Figs. 1-6, Temaz Placebo only will be referred to as the pretolerant condition in the following results and in the DISCUSSION.

Raw baseline values for foot and core body temperatures. The raw core body temperatures at 1400 in all four conditions were quite similar, with mean temperatures \pm SD of 36.94 ± 0.33 , 36.93 ± 0.20 , 36.94 ± 0.30 , and $36.91 \pm 0.21^\circ\text{C}$ for placebo, Temaz, Temaz Tolerant, and Temaz Placebo conditions, respectively. Similarly, the raw foot temperatures at 1400 were also similar between all for conditions with means \pm SD temperatures of 29.25 ± 2.55 , 29.60 ± 2.77 , 29.15 ± 3.18 , and $29.25 \pm 3.06^\circ\text{C}$ for placebo, Temaz, Temaz Tolerant, and Temaz Placebo conditions, respectively.

SOL. The changes in the latency to stage one sleep (SOL) across the day (1100-2000) for all four experimental conditions are illustrated in Fig. 2A. Significant main effects for "condition" [$F(3,30) = 42.3$, $G.G < 0.0001$] and "time" [$F(9,90) = 32.3$, $G.G < 0.0001$] were obtained as well as a significant "condition by time" interaction [$F(27,270) = 11.2$, $G.G < 0.0001$]. SOLs in the placebo condition remained high for the duration of

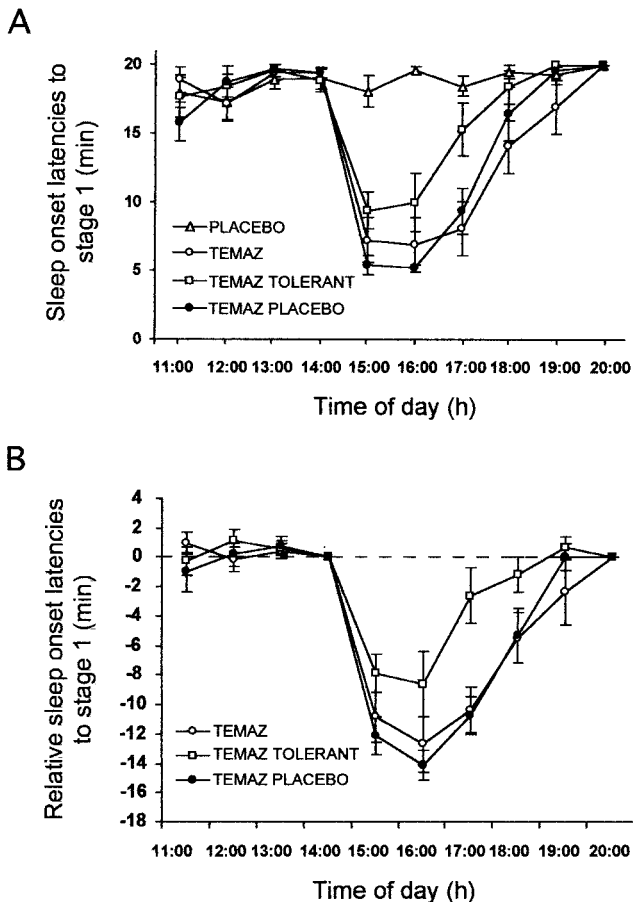


Fig. 2. *A*: effects of placebo and 30 mg of temazepam on the sleep onset latency to stage 1 before (Temaz Placebo and Temaz) and after (Temaz Tolerant) temazepam tolerance in 11 young males. *B*: the effects of 30 mg of temazepam on the sleep onset latency to stage 1 before and after temazepam tolerance, with values from the placebo condition subtracted from each experimental condition to remove the masking effect of normal circadian temperature changes. In both *A* and *B*, each condition is expressed relative to the time of pill administration (1400), and data are expressed as means \pm SE.

the protocol, with mean values of 18 min or above for the 6 h after treatment administration. Planned comparisons revealed that SOL before temazepam tolerance was significantly ($G-G < 0.05$) shorter than placebo for the first 4 h after treatment administration, but returned to baseline in the last 2 h (1900–2000). In the temazepam-tolerant condition, SOL was significantly ($G-G < 0.05$) shorter than placebo from 1500 to 1700 but was also significantly ($G-G < 0.05$) longer than the pretolerant condition from 1500 to 1800. SOL dropped sharply in the first hour after temazepam administration (1500) in all treatment conditions, with SOL before temazepam tolerance being maximally reduced 1 h later (1600) and 14.1 ± 1.0 min shorter than placebo (see Fig. 2*B*). In the temazepam-tolerant condition, the maximal reduction in SOL also occurred at 1600 but was only 8.6 ± 1.5 min shorter than placebo (see Fig. 2*B*). For the remaining 4 h, SOLs steadily increased and were equivalent to placebo by 1800.

The mean SOL before tolerance was 8.4 ± 2.0 min shorter than placebo, whereas in the tolerant condi-

tion, the mean SOL was only 4.4 ± 1.4 min shorter than placebo. As a result, the mean reduction in soporific efficacy as a result of temazepam tolerance was 4.0 ± 0.8 min.

Core body temperature. The changes in core body temperature from 1100 to 2000 for all four experimental conditions are illustrated in Fig. 3. Data are expressed relative to the time of treatment administration (1400). Significant main effects for condition [$F(3,30) = 42.6$, $G-G < 0.0001$] and time [$F(18,180) = 28.1$, $G-G < 0.0001$] were recorded as well as a significant condition by time interaction. [$F(54,540) = 8.48$; $G-G < 0.0001$]. In the placebo condition, a gradual increase in relative core temperature was observed across the day, increasing $0.40 \pm 0.06^\circ\text{C}$ in the 6 h after pill administration (see Fig. 3). In the pretolerant condition, core temperature remained significantly ($G-G < 0.05$) below placebo (from 1430 to 2000), with temperatures (relative to 1400 and to placebo) being reduced by $0.31 \pm 0.05^\circ\text{C}$ 3 h after treatment administration (see Fig. 4). From 1700, core temperature paralleled the change in core temperature seen in the placebo condition (see Fig. 3).

Although core temperature changes in the temazepam-tolerant condition were temporally similar to the core temperature changes observed in the pretolerant condition (see Fig. 3), planned comparisons revealed that core temperature, relative to placebo, was significantly ($G-G < 0.05$) attenuated compared with the pretolerant condition at all times after pill administration (see Fig. 4). Core temperature in the tolerant condition was, relative to placebo, maximally reduced at 1500 at which point core temperature reached $-0.16^\circ\text{C} \pm 0.03$ (see Fig. 4).

Foot temperatures. The changes in foot temperature for the 4 h before and 6 h after placebo and temazepam administration are illustrated in Fig. 5. As was observed for both SOL and core body temperature, significant main effects for condition [$F(3,30) = 8.7$, $G-G < 0.05$] and time [$F(18,180) = 11.8$, $G-G < 0.05$] were obtained as well as a significant condition by time interaction [$F(54,540) = 6.2$, $G-G < 0.05$].

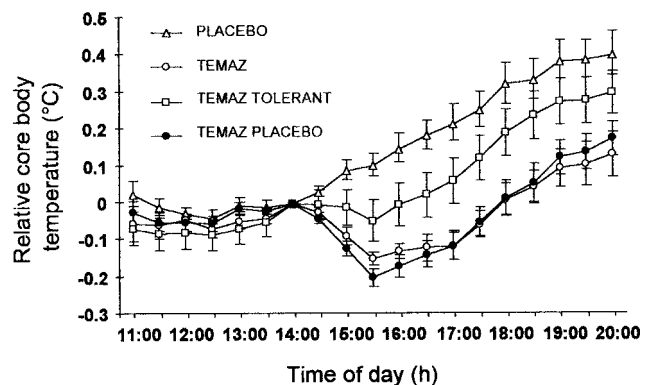


Fig. 3. Time course of the mean (\pm SE) changes in T_c in response to placebo and a 30 mg temazepam dose before and after temazepam tolerance in 11 young males. Each condition is expressed relative to the time of acute temazepam administration (1400).

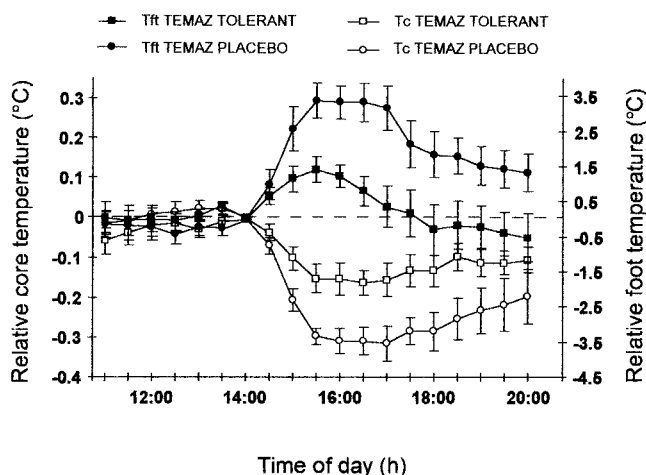


Fig. 4. Changes in mean (\pm SE) T_{re} and T_c in response to a 30-mg dose of temazepam before and after temazepam tolerance in 11 young males. Each condition is expressed relative to the time of acute temazepam administration (1400), and, to remove the masking effect of normal circadian temperature changes, the values from the placebo condition at each time point have been subtracted (for each subject separately) from corresponding times in each experimental condition.

In the placebo condition, foot temperatures increased 2.32°C between 1600 and 1730 and then remained fairly consistent for the final 2.5 h but reached a maximum at 1800 of $2.48 \pm 0.79^\circ\text{C}$. Planned comparisons revealed that foot temperatures in the pretolerant condition remained significantly ($G\text{-}G < 0.05$) above placebo for the 6 h after temazepam administration with a maximum temperature, relative to placebo, of $3.39^\circ\text{C} \pm 0.49$. In the temazepam-tolerant condition, foot temperatures were significantly ($G\text{-}G < 0.05$) higher than placebo for the first 2 h only (1430–1630) but were significantly ($G\text{-}G < 0.05$) lower than the pretolerant condition from 1500 to 2000. The maximum temperature, relative to placebo, was $1.44^\circ\text{C} \pm 0.38$, which, similar to the pretolerant condition, occurred at 1530.

Heart rate. The changes in heart rate for placebo, temazepam-tolerant, and both pretolerant experimental conditions are illustrated in Fig. 6. Although the

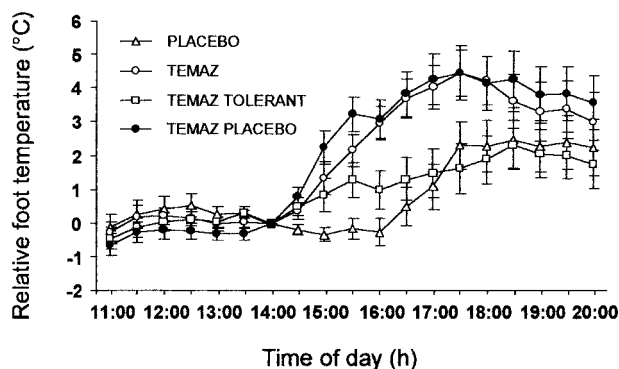


Fig. 5. Time course of the mean (\pm SE) changes in T_{ft} in response to placebo and a 30-mg temazepam dose before and after temazepam tolerance in 11 young males. Each condition is expressed relative to the time of acute temazepam administration (1400).

main effect of time was significant [$F(9,90) = 18.9$, $G\text{-}G < 0.0001$], the main effect of condition was not significant [$F(3,10) = 1.5$, $G\text{-}G > 0.05$]. In addition, although the condition by time interaction gave a significant P value ($P = 0.03$), the effect was nonsignificant after the $G\text{-}G$ adjustment was made [$F(27,270) = 1.71$, $G\text{-}G = 0.08$]. However, a clear trend was observed in the tolerant condition (see Fig. 6) where, from 1600 to 1800, heart rate was an average of 6.2 beats/min above both placebo and pretolerant conditions.

Temporal relationship between core body temperature and foot temperatures. The temporal relationship between changes in core temperature and changes in foot temperature is illustrated in Fig. 4. For both treatment conditions, there was a mean negative linear intraindividual relationship between the changes in core temperature and foot temperature after temazepam administration. For the first 3 h after administration, the mean correlation coefficient was -0.58 ± 0.08 (means \pm SE) before tolerance and -0.48 ± 0.08 in the tolerant condition. However, this relationship was reduced when all 6 h were analyzed with mean correlation values of 0.20 ± 0.12 and 0.20 ± 0.13 (for pre- and posttolerant conditions, respectively).

Temporal relationships between SOL and core body temperature. With both pre- and posttolerant conditions taken together, a significant ($P < 0.05$) linear relationship between minimum SOL and the associated “rate of core temperature decline” value was obtained with an r value of -0.44 . The temporal association between sleep propensity and core body temperature was also calculated within each individual and is displayed in Table 1. Before the development of tolerance, the intraindividual relationship between SOL and core temperature within each subject approximated a positive linear trend with a mean (\pm SE) r value of -0.25 ± 0.11 . A similar trend was found in tolerant subjects with a mean r value of -0.22 ± 0.10 . However, higher mean r values were obtained when sleep propensity was correlated with the MROD in core temperature with intraindividual correlations of

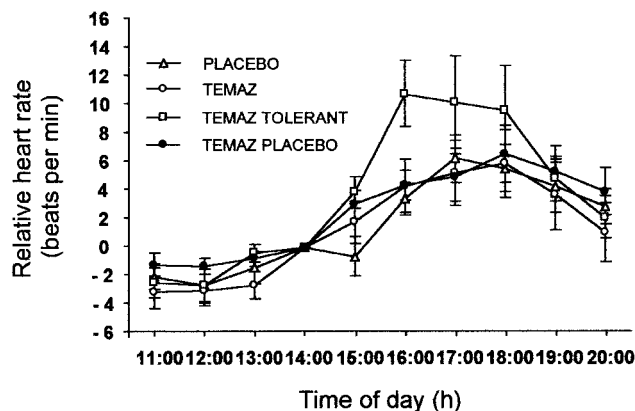


Fig. 6. Time course of the mean (\pm SE) changes in heart rate in response to placebo and a 30-mg temazepam dose before and after temazepam tolerance in 11 young males. Each condition is expressed relative to the time of acute temazepam administration (1400).

Table 1. Illustrates the temporal, intra-individual relationship between changes in SOL and changes in both T_{ft} and the T_c MROD for each subject separately using Pearson's product-moment correlation coefficient

Subject	T_c MROD vs. SOL		T_{ft} vs. SOL	
	Pretolerant	Tolerant	Pretolerant	Tolerant
01	-0.20	-0.54	0.61	0.67
02	0.29	-0.21	0.63	0.55
03	-0.76	-0.78	0.60	0.71
04	-0.74	-0.83	0.34	0.48
05	-0.59	-0.23	0.52	0.36
06	-0.91	-0.69	0.50	0.74
07	-0.47	-0.20	0.88	0.87
08	-0.70	-0.95	0.89	0.65
09	-0.77	-0.58	0.85	0.77
10	-0.59	-0.49	0.12	0.17
11	-0.83	-0.60	0.66	0.45
Means \pm SE	-0.57 ± 0.11	-0.55 ± 0.08	0.60 ± 0.07	0.58 ± 0.06

Relationships are illustrated for each subject both before (Pretolerant) and after (Tolerant) temazepam tolerance. SOL, sleep onset latency; T_{ft} , foot temperature; T_c , core temperature; MROD, maximum rate of decline.

-0.57 ± 0.11 and -0.55 ± 0.08 for pre- and posttolerant conditions, respectively.

Temporal relationships between SOL and foot temperatures. Combining both pre- and posttolerant conditions, a significant ($P < 0.05$) positive linear relationship was obtained between minimum SOL and the associated foot temperature magnitude with an r value of 0.48. In addition, the correlational analysis examining the intraindividual relationship between SOL and foot temperature magnitude also yielded a positive linear relationship with mean (\pm SE) r values of 0.60 ± 0.07 and 0.58 ± 0.06 for pre- and posttolerant conditions, respectively. These values for individual subjects are displayed in Table 1.

DISCUSSION

In the present study, administration of a 30-mg dose of temazepam for 7 consecutive days was sufficient to produce a mild soporific tolerance in young healthy males. Notably, however, this reduction in soporific efficacy was accompanied by a significant and concomitant attenuation of core hypothermia as well as peripheral heat loss. Moreover, sleep propensity was significantly related to both foot temperature and the maximum rate of decline in core temperature both before and after temazepam tolerance. That is, despite the changes in SOL and core and foot temperatures that occurred after the development of temazepam tolerance, the relationships between these variables remained the same. These findings support the idea that the thermoregulatory system may be functionally involved in the regulation of sleep propensity.

The soporific efficacy of temazepam after 7 consecutive days of 30 mg of this agent was significantly attenuated by a mean value of 4.0 ± 0.8 min relative to placebo. The magnitude of this attenuation was greater than reported in a previous study (20), where a

mean increase in SOL of 1.8 min was observed in young males after 8 consecutive days of an identical 30-mg temazepam dose. However, this discrepancy is likely to reflect the fact that, in the present study, the mean SOL included only the averages after 1400.

In the placebo condition, changes in foot temperature (used as an indirect measure of peripheral heat loss; see Ref. 15) reached a maximum of 2.5°C at 1830, revealing a clear circadian increase in heat loss. In the pretolerant condition, foot temperatures increased earlier (within 30 min of administration) and continued to increase until 1600 where temperatures paralleled, but remained higher than, the placebo condition. Although the mechanism by which temazepam may induce heat loss is not clear, it is thought that diazepam, a similar benzodiazepine, may act directly on peripheral receptors controlling vasodilation (5). Because temazepam is a metabolite of diazepam, it is possible that temazepam-related heat loss may occur via a similar mechanism.

Although foot temperatures in the tolerant condition were initially increased above placebo, the degree of heat loss was always attenuated compared with that seen before the development of tolerance (see Fig. 4). Similarly, the maximal reduction in core temperature in tolerant subjects (relative to placebo) was half that recorded before the development of tolerance (-0.16 ± 0.03 vs. $0.31 \pm 0.05^\circ\text{C}$). This is the first study to demonstrate such a clear shift in thermoregulatory function associated with temazepam tolerance. More importantly, however, the attenuation in both these thermoregulatory variables occurred concomitantly with the reduction in soporific efficacy in the tolerant condition, highlighting the possibility that the thermoregulatory system may be functionally involved in the regulation of sleepiness.

This hypothesis was further supported by the fact that changes in foot temperatures were temporally associated with changes in sleepiness. A significant ($P < 0.05$) correlation of 0.48 was observed between sleep propensity and peripheral heat loss when both pre- and posttolerant conditions were combined. More importantly, mean intraindividual correlations between these variables before temazepam tolerance ($r = 0.60 \pm 0.07$) were equivalent to the mean intraindividual correlations after tolerance ($r = 0.58 \pm 0.06$). The slightly weaker relationship observed in the former correlation (0.48) is likely to reflect the masking effect of between-subject variability.

Interestingly, these results parallel those obtained by Kräuchi and colleagues (14) after an examination of the thermoregulatory and soporific changes after both evening melatonin administration and a carbohydrate-rich meal. Despite the fact that these treatments had different thermoregulatory effects, for all manipulations, SOL was always shorter when heat loss was greater. Moreover, the strength of the association between these variables is almost identical for both studies [0.47 vs. 0.48 for Kräuchi et al. (13) and the present study, respectively]. Although a causal link between these variables cannot be supported by these correla-

tions, the robustness of this relationship is striking and is worthy of further research. Therefore, although it is well established that changes in sleep-state affect thermoregulation (e.g., Ref. 18), it may be that changes in thermoregulation equally affect mechanisms governing sleep.

Although it has been suggested that heat loss, rather than a decrease in core temperature, may be the important factor involved in the initiation of sleepiness (13, 16), these findings have been based on studies measuring the magnitude of core temperature decline. As documented previously, for daytime temazepam as well as melatonin administration (8), it was the maximum rate of decline in core temperature and not the magnitude that was the better correlate of sleep propensity. Similar results were found in the present study, with a significant ($P < 0.05$) correlation of -0.44 observed between SOL and the maximal rate of decline in core temperature across both experimental conditions. Moreover, stronger mean intraindividual correlations of -0.57 ± 0.11 and -0.55 ± 0.08 (for pre- and posttolerance conditions, respectively) were obtained between SOL and the maximal rate of decline in core temperature compared with -0.25 ± 0.11 and -0.22 ± 0.10 for SOL and the magnitude of core temperature decline. Interestingly, Kräuchi and colleagues (13) also reported a similar correlation between the magnitude of core temperature decline and maximum sleep propensity with a correlation of 0.26. It is possible that, because the core is well insulated, the lower correlation observed between SOL and core temperature magnitude reflects the relatively long lag-time between the initiation of core temperature changes and the measurement of these changes.

The possibility that these findings are simply the result of a change in posture is reasonable, because it has been demonstrated that the cardiovascular changes associated with lying down results in the redistribution of heat from the core to the periphery (14) and that this is accompanied by an increase in sleep propensity. However, in the present study, subjects remained supine for the duration of the experimental protocol and only sat semirecumbent for the lunch meal. Alternatively, as has been speculated by previous researchers (9, 13), the physiological signal for the series of thermoregulatory events leading to heat loss, hypothermia, and sleepiness may be the anticipation of sleep itself. In the present study, however, sleep would also have been anticipated during the MSLTs in the placebo condition, yet the "thermoregulatory cascade" did not follow.

Changes in foot temperatures were significantly associated with changes in core body temperature (Fig. 4). As peripheral heat loss is the principal mechanism determining the hypothermic effects of other soporific agents such as melatonin (2, 12), it is possible that a similar thermoregulatory pathway exists for temazepam. Indeed, there was a significant intraindividual relationship between heat loss and hypothermia with individual decreases in core temperature being closely associated with increases in foot temperatures. Inter-

estingly, for both pre- and posttolerant groups, this relationship was only significant for the first 3 h after temazepam administration, with correlations of -0.58 ± 0.08 and -0.48 ± 0.08 . When all 6 h after drug administration were analyzed, the average correlation within each subject was not as strong, with values of 0.20 ± 0.12 and 0.20 ± 0.13 (for pre- and posttolerant conditions, respectively). It is possible that the reduced strength of the relationship between heat loss and hypothermia after 1700 indicates that heat loss was no longer driving the changes in core temperature.

As changes in core temperature reflect changes in heat production as well as heat loss, the role that heat production played in the thermoregulatory and soporific effects of temazepam was also examined. As with previous studies (8, 14, 15), heart rate, an indirect, noninvasive measure of heat production, was used. In the pretolerant condition, the gradual increase in heart rate across the afternoon was equivalent to the placebo condition. Although temazepam has been shown to increase heart rate at doses ranging from 5 to 30 mg (7, 17), it is notable that, in this study as well as a previous study in our laboratory (8), acute temazepam administration did not appear to have significant cardiac effects. However, this discrepancy may reflect different experimental protocols used.

Although heart rate was not significantly altered after temazepam tolerance, heart rate was elevated above placebo in the tolerant condition by an average of 6 beats/min from 1600 to 1800 (Fig. 6). As the variance in this condition was relatively high, it is possible that there was insufficient power in the present study to detect cardiac changes of this magnitude. Nevertheless, this tolerance-related increase in heart rate is notable because it is in the opposite direction to the changes observed in all the other measured variables. The heart is controlled centrally via both parasympathetic and sympathetic inputs, whereas the vessels regulating skin blood flow are predominantly controlled by the sympathetic nervous system. Therefore, it is possible that temazepam may differentially affect these two branches of the autonomic nervous system in temazepam-tolerant subjects. Alternatively, temazepam may act peripherally to induce heat loss while acting centrally to mediate heat production.

Regardless of the mechanism, the robustness of the associations between sleep propensity and thermoregulatory variables such as peripheral heat loss and hypothermia over such a wide array of experimental manipulations is remarkable and indicates the possibility of underlying physiological processes. Nevertheless, more research needs to be performed before a more comprehensive understanding of this relationship is reached. In addition, future studies should look toward a better understanding of possible mechanisms of action and/or sites where hypnotic/soporifics could influence both thermoregulation and sleep propensity. In this way, through gaining a better understanding of the physiological processes underlying sleep, more ap-

appropriate treatment of sleep disorders may be achieved.

Perspectives

Previous research has focussed on the possible role that thermoregulation (in particular distal vasodilation) plays in normal nocturnal sleep onset and in the soporific effects of melatonin and certain sleeping pills. The present study extends this body of research by demonstrating that thermoregulatory changes may also be linked to the soporific attenuation associated with the development of tolerance in the sleeping pill temazepam. As such, the results highlight the physiological effects associated with the chronic use of one of the most commonly prescribed sleeping pills. As chronic temazepam use is extremely common in the elderly, it would be useful to determine if similar physiological processes (observed here in young subjects) also occur in this older group of individuals. Moreover, if thermoregulatory variables such as heat loss do in fact mediate temazepam-induced sleepiness in the elderly, then the use of classical benzodiazepines as treatment for age-related sleep onset insomnias could be replaced by nonaddictive therapies that manipulate the thermoregulatory system directly.

This work was supported by grants from the Australian Research Council, the National Health and Medical Research Council, and The Queen Elizabeth Hospital Research Foundation.

REFERENCES

1. **Cagnacci A, Elliott JA, and Yen SS.** Melatonin: a major regulator of the circadian rhythm of core temperature in humans. *J Clin Endocrinol Metab* 75: 447–452, 1992.
2. **Cagnacci A, Soldani R, and Yen SS.** Hypothermic effect of melatonin and nocturnal core body temperature decline are reduced in aged women. *J Appl Physiol* 78: 314–317, 1995.
3. **Carskadon MA and Dement WC.** Effects of total sleep loss on sleep tendency. *Percept Mot Skills* 48: 495–506, 1979.
4. **Cote P, Noble J, and Bourassa MG.** Systemic vasodilation following diazepam after combined sympathetic and parasympathetic blockade in patients with coronary heart disease. *Cathet Cardiovasc Diagn* 2: 369–380, 1976.
5. **Dawson D and Encel N.** Melatonin and sleep in humans. *J Pineal Res* 15: 1–12, 1993.
6. **Dollins AB, Zhdanova IV, Wurtman RJ, Lynch HJ, and Deng MH.** Effect of inducing nocturnal serum melatonin concentrations in daytime on sleep, mood, body temperature, and performance. *Proc Natl Acad Sci USA* 91: 1824–1828, 1994.
7. **Ford GA, Hoffman BB, and Blaschke TF.** Effect of temazepam on blood pressure regulation in healthy elderly subjects. *Br J Clin Pharmacol* 29: 61–67, 1990.
8. **Gilbert SS, van den Heuvel CJ, and Dawson D.** Daytime melatonin and temazepam in young adult humans: equivalent effects on sleep latency and body temperature. *J Physiol (Lond)* 514: 905–914, 1999.
9. **Van den Heuvel CJ, Noone JT, Kennaway DJ, and Dawson D.** Changes in sleepiness and body temperature precede nocturnal sleep onset: evidence from a polysomnographic study in young men. *J Sleep Res* 7: 159–166, 1998.
10. **Van der Kleijn E.** Effects of zopiclone and temazepam on sleep, behaviour, and mood during the day. *Eur J Clin Pharmacol* 36: 247–251, 1989.
11. **Kleitman N.** *Sleep and Wakefulness*. Chicago: University of Chicago Press, 1963.
12. **Kräuchi K, Cajochen CD, Mori Graw P, and Wirz-Justice A.** Early evening melatonin and S-20098 advance circadian phase and nocturnal regulation of core body temperature. *Am J Physiol Regulatory Integrative Comp Physiol* 272: R1189–R1196, 1997.
13. **Kräuchi K, Cajochen C, Werth A, Wirz-Justice E.** Warm feet promote the rapid onset of sleep. *Nature* 401: 36–37, 1999.
14. **Kräuchi K, Cajochen C, and Wirz-Justice A.** A relationship between heat loss and sleepiness: effects of postural change and melatonin administration. *J Appl Physiol* 83: 134–139, 1997.
15. **Kräuchi K and Wirz-Justice A.** Circadian rhythm of heat production, heart rate, and skin and core temperature under unmasking conditions in men. *Am J Physiol Regulatory Integrative Comp Physiol* 267: R819–R829, 1994.
16. **Kräuchi K, Werth E, Wüst D, Renz C, and Wirz-Justice A.** Interaction of melatonin with core body cooling: sleepiness is primarily associated with heat loss and not with a decrease in core body temperature (Abstract). *Sleep* 22: S285, 1999.
17. **Matejcek M, Neff G, Abt K, and Wehrli W.** Pharmacoelectroencephalogram and psychometric study of the effect of single doses of temazepam and nitrazepam. *Neuropsychobiology* 9: 52–65, 1983.
18. **Parmeggiani PL.** Interaction between sleep and thermoregulation: an aspect of the control of behavioral states. *Sleep* 10: 426–435, 1987.
19. **Pleuvry BJ, Maddison SE, Odeh RB, and Dodson ME.** Respiratory and psychological effects of oral temazepam in volunteers. *Br J Anaesth* 52: 901–905, 1980.
20. **Roehrs T, Kribbs N, Zorick F, and Roth T.** Hypnotic residual effects of benzodiazepines with repeated administration. *Sleep* 9: 309–316, 1986.
21. **Salin-Pascual RJ, Franco M, Garcia-Ferreiro R, Vazquez J, Suarez J, Sanchez L, and Jimenez-Anguiano A.** Differences in sleep variables, blood adenosine, and body temperature between hypothyroid and euthyroid rats before and after REM sleep deprivation. *Sleep* 20: 957–962, 1997.
22. **Thakor NV, Webster JG, and Thompkins WJ.** Optimal QRS detector. *Med Biol Eng Comput* 21: 343–350, 1983.
23. **Ticho SR and Radulovacki M.** Role of adenosine in sleep and temperature regulation in the preoptic area of rats. *Pharmacol Biochem Behav* 40: 33–40, 1991.
24. **Zulley J, Wever R, and Aschoff J.** The dependence of onset and duration of sleep on the circadian rhythm of core body temperature. *Pflügers Arch* 391: 314–318, 1981.