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**TITLE**

EFFECT OF TRIAZOLM ON RESPONSE TO A COLD WATER IMMERSION IN HUMANS

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ORIGINAL RESEARCH

# Effect of Triazolam on Responses to a Cold-Water Immersion in Humans

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Managing alertness of soldiers during sustained operations is a source of serious concern for military unit commanders. A frequently employed strategy is to induce sleep before an operation, especially operations requiring prolonged travel. Sleep-inducing drugs could have an action on thermoregulation through their effect on alertness and a possible direct effect on the brain. The goal of this study was therefore to evaluate the effect of a commonly prescribed triazolam (Halcion®) on thermoregulatory responses to cold-water immersion. Eight subjects were immersed twice in 18°C water for up to 90 min in the morning; once after ingesting 0.25 mg triazolam (TRZ) the prior evening, and again after placebo (PLB) treatment. There were no significant differences between trials for mean duration of the immersion, the change in rectal temperature and mean skin temperature. Total metabolic heat production was similar for both conditions: 767 ± 107 vs. 781 ± 105 $kJ \cdot m^{-2}$ for TRZ and PLB, respectively. The results should be considered in light of a large variation among the subjects in sensitivity to TRZ, which was unrelated to biometrical characteristics such as surface area-to-mass ratio, lean body mass, % body fat, and physical fitness. Although not statistically significant, there was a trend for a smaller increase in plasma free fatty acid and glycerol concentrations after water immersion with TRZ. The results suggest that the ingestion of a single dose of triazolam 11 h prior to a cold-water immersion is not likely to accelerate the rate of onset of hypothermia. Individual sensitivity, however, may predispose some sensitive subjects to negative effects in this regard.

MANAGING VIGILANCE, alertness and fatigue of soldiers during military operations in the field is one of the more difficult problems for unit commanders, especially during long or multiple extended missions. Appropriate work/rest scheduling is the first measure to consider, but is not always possible. The pharmacological approach to this problem has two complementary components: psycho-stimulants to keep soldiers alert until the mission is finished and sleeping agents to facilitate rest at the desired time. There are ongoing studies of relatively new, safe psychostimulants (2,11), and the wide-spread advocation by the medical community of the use of sleep-inducing drugs with a short half-life, such as benzodiazepines, has resulted in these agents also being used within the military. Moreover, such drugs are frequently self-administered during transportation of personnel to an operation, especially by individuals predisposed to motion-sickness. For example, there are anecdotal reports of a high incidence of use of triazolam (Halcion®, Upjohn, Kalamazoo, MI), a widely used benzodiazepine, by some U.S. Special Warfare personnel traveling to or returning from the Persian Gulf during the recent war. Thus, it is feasible that an operational scenario could involve the use of a benzodiazepine by Navy special warfare personnel who may subsequently be required to operate in a cold water environment.

Although effects of benzodiazepines on the thermoregulatory system can be expected, via a direct effect on the brain, very few data are available for humans. Benzodiazepines have been reported to reduce core temperature in drug poisoning (8) and to impair metabolic response to cold in newborn infants when given to mothers at high dosages within 15 h prior to delivery (3). In rats, high dosages of diazepam (Valium®, Hofman-La Roche, Nutley, NJ) can induce hypothermia in neutral ambient conditions for longer than 4 h after the injection (19), and prevent the hyperthermia caused by handling (22). The mechanisms causing these actions have not been explained but could involve both myorelaxant and anxiolytic effects of diazepam. No well-documented data are yet available for triazolam (1,2,4-triazolo-benzodiazepine, Halcion®).

Pilot studies on two subjects (Jacobs and Martineau, unpublished), suggested that triazolam might accelerate the onset of hypothermia in cold-water exposed individuals. The mechanisms of action on the thermoregulatory system of such a drug, and whether its impact on temperature regulation can be generalized to a large population, are not known.

The aim of this study was therefore to determine whether a single dose of triazolam, at the normally prescribed acute dosage (0.25 mg) used by healthy subjects to induce sleeping (10), ingested 12 h before a cold-water immersion is likely to affect the onset of hypothermia.

## METHODS

### *Subjects*

After approval of the protocol by the institutional human ethics committee, eight healthy males volunteered

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to serve as subjects. They were informed about the nature of the study and the techniques to be employed, signed a statement of informed consent, and were medically cleared for participation. None of the subjects was a routine user of sleep-inducing medications, nor had anyone used such drugs within 1 month prior to the experiment. Their mean (±SEM) age, height, weight, and calculated body surface area (BSA) were 33.4 ± 2.7 yr, 177.1 ± 2.1 cm, 78.4 ± 1.6 kg, and 1.95 ± 0.03 $m^2$, respectively. Before the experiment, their relative body fat content (BF, % of total body weight) was determined with hydrostatic weighing techniques and averaged 13 ± 2%. Their maximal oxygen consumption measured ($\dot{V}O_{2max}$) during treadmill running was 50 ± 2 mL $O_2 \cdot min^{-1} \cdot kg^{-1}$. To familiarize the subjects, and in an attempt to diminish the experimental stress of the first session, before beginning the first experiments they all experienced an immersion in cold water using a protocol and measurements identical to those to be used during experiments.

*Experimental Protocol*

Each subject participated in two experiments conducted with a counterbalanced double-blind design: after ingestion of triazolam (TRZ) and after ingestion of placebo (PLB). Subjects lay in a supine position immersed to the neck in stirred chilled water (18°C) for up to 90 min during each experiment. The TRZ (0.25 mg) or PLB was ingested at 10:00 p.m. the night before the experiment. TRZ was put in an opaque gelatin capsule filled with starch (Metamucil®); PLB was prepared in a similar fashion but without the TRZ. Both immersions took place at the same time of day for a given subject. Experiments were separated by a minimum of 1 week to permit drug elimination between tests.

*Experimental Session*

The same procedures and instrumentation were employed for all experiments. Subjects reported to the laboratory at 7:30 a.m. on each test day for instrumentation. After they voided, subjects self-inserted their rectal probe (Sherigan, Argyle, NY) into the rectum 12 cm beyond the anal sphincter. Thereafter, wearing only a bathing suit, subjects were instrumented with 12 calibrated heat-flow transducers (Concept Engineering, San Diego, CA), and skin electrodes (Sensormedics model 650414) were attached over the chest for a bipolar ECG recording. An indwelling intravenous catheter was inserted into a superficial forearm vein.

Subjects then lay quietly in a supine position on a stretcher while covered with blankets for 20 min at approximately 23°C. Their resting metabolic rate was determined during this time with a metabolic cart consisting of an IBM PC with polarographic $O_2$ (S-3A/I) and infrared $CO_2$ (CD-3A) analyzers (Applied Electrochemistry, Pittsburgh, PA), a ventilation module (Interface Associates, Irvine, CA) and appropriate A/D computer interface (Turbofit®) and software (Vacumetrics, Ventura, CA). Just prior to the immersion blood was sampled through the catheter.

The blankets were then removed and the stretcher was raised and lowered into the water by a winch while the subject remained in the supine position. The position of the stretcher was quickly adjusted in the water tank so that the level of immersion was similar for all subjects. The arm in which the catheter had been inserted was supported in a sling above the water line. The subjects remained in the water until their rectal temperature reached 35.5°C or 90 min elapsed.

*Measurements, Calculations and Biochemical Assays*

The following variables were measured continuously and averaged every minute during the immersions: rectal temperature ($T_{re}$), mean skin temperature ($\bar{T}_{sk}$) and mean skin heat flow ($H_{sk}$, using the 12 points area-weighed system described by Hody, 1973), ventilatory exchanges ($\dot{V}_E$, $L \cdot min^{-1}$ BTPS), oxygen uptake ($\dot{V}O_2$, $L \cdot min^{-1}$ STPD), and respiratory exchange ratio (RER, calculated from $\dot{V}O_2$ and $CO_2$ output, $\dot{V}CO_2$ $L \cdot min^{-1}$ STPD). ECG was continuously recorded during the immersion. The rate of metabolic heat production ($\dot{M}$) was subsequently calculated from $\dot{V}O_2$ and RER as previously described (13,17), and the integration of $\dot{M}$ every min yielded the total heat production ($\Sigma M$) during the test; the heat-debt (S) was estimated using the change in mean body temperature ($T_b$) observed during the experiment (21).

Blood was collected just prior to the immersion ($t_0$), at min 30 ($t_{30}$) and 60 ($t_{60}$) during the immersion, and at the end of the immersion ($t_{90}$). Venous blood was analyzed for hemoglobin concentration (Hb, Sigma Diagnostics) and hematocrit (Hct, micro-centrifugation) before storage at −20°C for further analysis. Relative changes in plasma volume were calculated using Dill and Costill's algorithm (4). Free fatty acid (FFA) concentrations were determined enzymatically using a commercial reagent kit (Wako Chemicals), glycerol (Gol) concentrations were assayed fluorimetrically (1), and plasma glucose (Glc) levels were determined with a glucose analyzer (model 23A, Yellow Springs Instruments, Yellow Springs, OH). All metabolite concentrations are expressed after being corrected for changes in plasma volume.

*Statistics*

The Statview Package (Abacus Concept, 1988, CA) was used for all calculations. Data were analyzed with a two-factor (time and treatment) analysis of variance for repeated measures. When the F ratio was significant ($p < 0.05$), paired *t*-tests were used to locate significant differences between means. Data are expressed as means ± SEM.

## RESULTS

No side effects or abnormal morning sleepiness were reported by any of the subjects after either the TRZ or PLB nights. Six of the subjects completed 90 min of immersion for both experiments; in one subject $T_{re}$ reached 35.5°C at minute 90 and 80, and in another at minute 41 and 39, for TRZ and PLB, respectively. Nevertheless, the average duration of the immersion was not significantly different between treatments: 83.9 ± 6.1 min for TRZ and 82.4 ± 6.3 min for PLB.

Pre-immersion values for $\dot{V}_E$ and $\dot{V}O_2$ were not different between the treatments; 6.57 ± 0.20 vs. 6.38 ± 0.21 and 0.249 ± 0.007 vs. 0.241 ± 0.005 $L \cdot min^{-1}$ for TRZ and

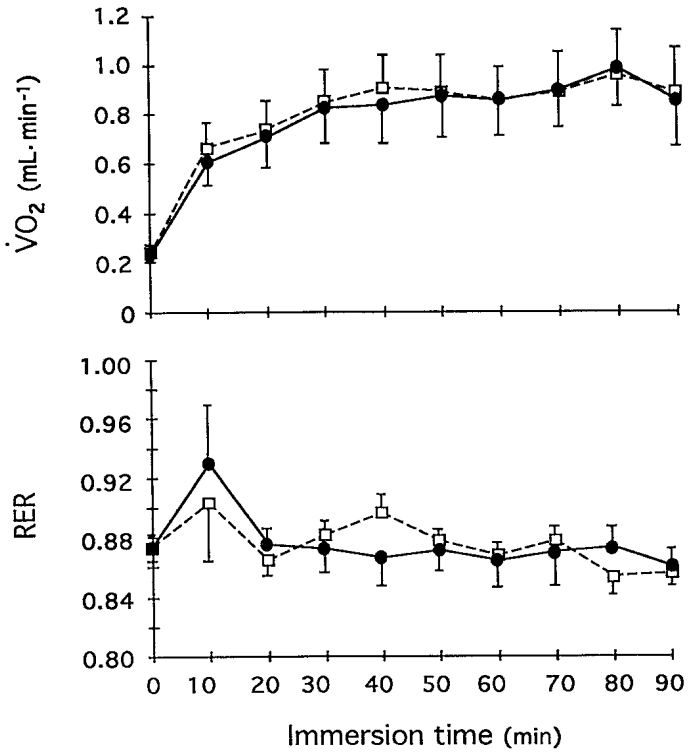


**Fig. 1.** Mean $\dot{V}O_2$ and RER during water immersion after treatment with triazolam (TRZ = filled circles) and placebo (PLB = open squares). From minute 10 until the end, mean $\dot{V}O_2$ was different from $t_0$ value for both experiments ($p < 0.01$). After a variable increase induced by the hyperventilation provoked by the immersion in cold-water, RER remained stable until the end of the test. There is no difference between the treatments.

PLB, respectively. $\dot{V}_E$ increased rapidly as soon as the subjects were immersed, and then continued to slowly increase, reaching a quasi steady-state during the second half-hour of immersion. The level of this plateau was not different between TRZ and PLB (19.8 ± 1.0 vs. 19.1 ± 0.9 L·min$^{-1}$, respectively). The kinetics of the changes in $\dot{V}O_2$ and RER are presented in **Fig. 1.** There were no inter-trial differences for either of these variables. $\dot{V}O_2$ increased quickly after the beginning of the immersion to reach a level significantly higher than resting values by the tenth minute of immersion and remained higher until the end of the experiment. The initial increase of $\dot{V}_E$ varied widely among the subjects, and this hyperventilation explains the increase in RER at the beginning of the immersion (Fig. 1). Thereafter, RER decreased to values similar to the pre-immersion values, where it remained relatively stable until the end of the immersion. As expected, metabolic heat production ($\dot{M}$) increased dramatically during the cold water exposure reaching 3.5 ± 0.8 times resting values during the first 40 min of immersion (**Fig. 2**). This increase varied widely among the subjects and was inversely related to their body fat content (**Fig. 3**). There were no significant differences between the treatments at any time of the immersion, and ΣM during TRZ and PLB were similar: 767 ± 107 and 781 ± 105 kJ·m$^{-2}$ (ns), respectively.

Pre-immersion $T_{re}$ and $\bar{T}_{sk}$ were not different between the treatments: 36.91 ± 0.06 vs. 36.86 ± 0.06, and 32.50 ± 0.20 vs. 32.26 ± 0.24°C for TRZ and PLB, respectively.

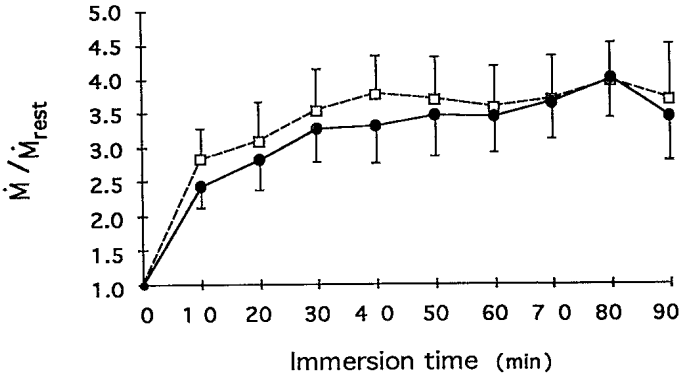


**Fig. 2.** The average ratio of the metabolic heat production ($\dot{M}$) to the resting value ($\dot{M}_{rest}$) during the immersions (TRZ = filled circles, PLB = open squares). For both treatments, $\dot{M}/\dot{M}_{rest}$ is significantly different from 1 from minute 10 until the end of the immersion. There is no difference between the treatments.

**Fig. 4** shows the changes in $T_{re}$ and $\bar{T}_{sk}$ during the water immersion; there were no differences between the treatments at any time. The cumulative S values by the end of the immersions were also similar, with values of −616 ± 14 and −608 ± 26 kJ·m$^{-2}$ for TRZ, and PLB, respectively.

The decrease in plasma volume observed during the cold test was significant at $t_{90}$, but not different between the treatments (−10 ± 1 vs. −10 ± 3 % for TRZ and PLB, respectively). $[Glc]_p$ remained stable throughout the immersions without any difference between trials; mean values were 4.34 ± 0.23 and 4.20 ± 0.16 mM at $t_0$, and 4.24 ± 0.22 and 4.44 ± 0.26 mM at the end of the immersion for TRZ and PLB, respectively. $[Gol]_p$ and $[FFA]_p$ increased along the water immersion (**Table I**). Because some blood samples were missing and one subject did not complete 90 min tests, data were available at $t_{90}$ in only four subjects (Table I). At this time, $[FFA]_p$ was different between TRZ and PLB: 0.568 ± 0.069 vs. 0.797 ± 0.053, $p < 0.05$, respectively (Table I).

## DISCUSSION

The results demonstrate that triazolam ingestion the night before a cold water immersion does not accelerate

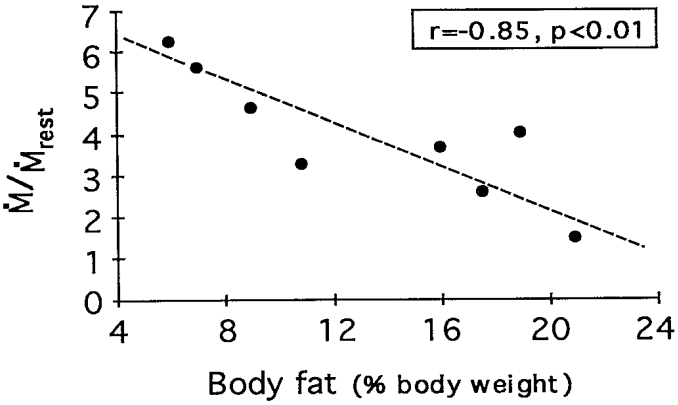


**Fig. 3.** Relationship between the average ratio of the metabolic heat production ($\dot{M}$) to the resting value ($\dot{M}_{rest}$) and the relative body fat content of the subjects (each point is the average value of $\dot{M}/\dot{M}_{rest}$ from the last 60 min of both experiments, the last 20 min for the subject who stopped early).

TABLE I. AVERAGE PLASMA CONCENTRATIONS OF GLYCEROL AND FREE FATTY ACIDS (FFA) DURING COLD WATER IMMERSION AFTER TRIAZOLAM (TRZ) AND PLACEBO (PLB) TREATMENTS.

| Time | Glycerol | | FFA | | (n) |
|---|---|---|---|---|---|
| | TRZ | PLB | TRZ | PLB | |
| $t_0$ | 0.052 ± 0.013 | 0.048 ± 0.010 | 0.443 ± 0.056 | 0.400 ± 0.044 | (6) |
| $t_{30}$ | 0.076 ± 0.013 | 0.083 ± 0.011 | 0.464 ± 0.052 | 0.482 ± 0.069 | (6) |
| $t_{60}$ | 0.118 ± 0.025* | 0.121 ± 0.021* | 0.666 ± 0.059* | 0.713 ± 0.082* | (5) |
| $t_{90}$ | 0.104 ± 0.032 | 0.130 ± 0.021* | 0.567 ± 0.069 | 0.797 ± 0.050*† | (4) |

$t_0$ to $t_{90}$ refers to minutes 0, 30, 60 and 90 of the test, n is the number of subjects. Units are mM. Statistical significance at $p < 0.05$: * = different from $t_0$; † = different from TRZ.

the onset of hypothermia. The average drop in $T_{re}$ observed during the cold-water immersion as well as the metabolic heat production were very similar to values previously reported for similar protocols (14,15,18). Only one subject experienced a dramatic fall in body temperature, although similar under both treatments, primarily due to his low body fat content.

Benzodiazepines are reported to have a short half-life, between 2 and 5 h (6) but triazolam has a half-life shorter than 3 h, with an average of 2.3 h (5); this is the main reason it is so extensively used. These pharmacokinetic parameters may vary widely among subjects (10). Because plasma peak concentration is reached up to 2 h after a single oral dose (5,10), plasma concentration of triazolam 11 h after ingestion is expected to be about 10% of the peak concentration. Moreover, several of its metabolites are still found in blood more than 12 h after ingestion (5). These metabolites are reported to be inactive (9) but their effects on thermoregulation are not known.

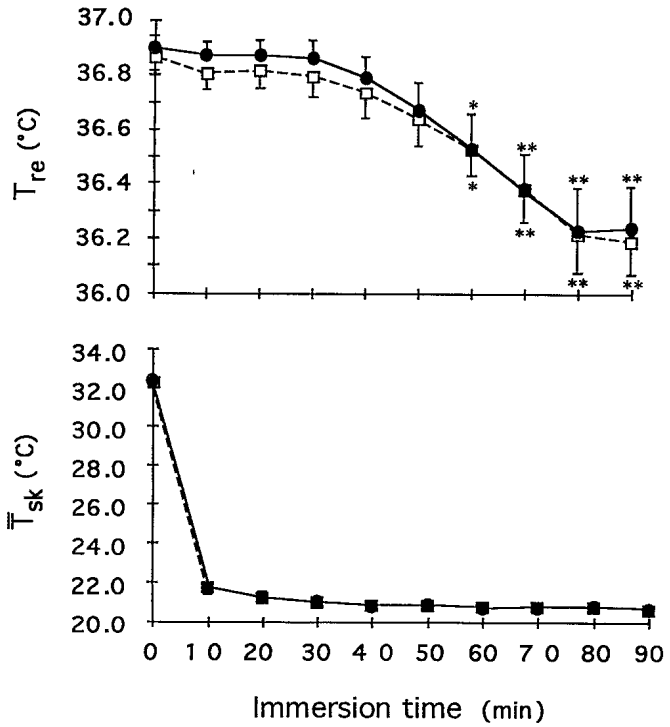


**Fig. 4.** Average $T_{re}$ and $\bar{T}_{sk}$ profiles observed during both immersions (TRZ = filled circles, PLB = open squares). Significantly different from $t_0$: * $p < 0.05$; ** $p < 0.01$. For $\bar{T}_{sk}$ each point is different from $t_0$ ($p < 0.01$). There is no difference between the treatments.

The only observed difference in the present study between TRZ and PLB was for the blood FFA concentrations (Table I). It is well established that cold exposure stimulates the secretion of powerful lipolytic hormones, such as catecholamines (9). Thus, we can speculate that the smaller increase in FFA after TRZ may be linked to a lower catecholamine production or decreased sensitivity during immersion after TRZ. A reduced availability of plasma FFA does not impair human temperature regulation during cold water immersion (14). Nevertheless, muscle metabolism during cold exposure could be so dependent on hormonal regulations (12,20) that reduced catecholamine production could result in a lower metabolic heat production by muscle (16). Such an effect would be consistent with both the lower $\Sigma M$ and S observed at the end of TRZ compared to PLB, although differences were not statistically significant.

We cannot preclude the possibility of varying levels of sensitivity to triazolam. Of the eight subjects, three showed a decreased $\dot{M}$, two showed an increased average rate of heat loss when treated with the TRZ, and two subjects who participated in pilot experiments prior to this investigation also demonstrated a more rapid decrease in $T_{re}$ after TRZ treatment. This sensitivity might be related to pharmacokinetic parameters not measured in this study.

In summary, the ingestion of a standard dose of 0.25 mg triazolam 11 h before cold water immersion did not accelerate the rate of onset of hypothermia. Nevertheless, we think that the potential effect of this drug in some subjects should be weighed carefully before using it the day prior to an operation involving cold-stress.

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