The Effects of Blood Glucose Concentration on the Shivering Threshold in Rabbits

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BACKGROUND: Hyperglycemia is common in critically ill and surgical patients, as are core temperature disturbances. The effect of hyperglycemia on thermoregulatory defenses remains unknown. We determined the effect of blood glucose concentration on the shivering threshold in rabbits.

METHODS: Twenty-seven rabbits lightly anesthetized with isoflurane were randomly assigned to infusions of (1) saline, (2) insulin titrated to produce blood glucose concentrations 60 to 100 mg/dL, or (3) 50% dextrose titrated to produce blood glucose concentrations 200 to 300 mg/dL. Core temperature was reduced at a rate of 2 to 3°C/h by perfusing water at 10°C through a plastic tube positioned in the colon. Cooling continued until shivering was observed by an investigator blinded to treatment or until esophageal (core) temperature reached 34°C. Core temperatures at the onset of shivering defined the threshold. All analyses were conducted using SAS version 9.3 (SAS Institute Inc., Cary, NC).

RESULTS: Rabbits given saline shivered at 37.2 ± 0.5°C (mean ± SD). Rabbits given insulin shivered at 36.3 ± 1.1°C. Rabbits given dextrose shivered at 38.0 ± 0.6°C. The shivering threshold increased as a function of blood glucose concentration: shivering threshold (°C) = 0.009 [blood glucose concentration (mg/dL)] + 35.6, r² = 0.53. The shivering threshold thus increased approximately 1°C for each 100 mg/dL increase in blood glucose concentration.

CONCLUSIONS: Hyperglycemia increases the threshold for shivering, whereas hypoglycemia lowers the threshold on rabbits. (Anesth Analg 2015;121:525–31)

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hivering is akin to intense exercise and thus represents a substantial metabolic load. Intense shivering, as can occur after hypothermic anesthesia, roughly doubles basal metabolic rate. Energy for low-intensity shivering in humans is largely derived from lipids (53%), but muscles switch to carbohydrates (65%) with vigorous shivering,1 using glucose mostly derived from muscle glycogen.2,3 When available, ingested glucose is used for shivering heat production in preference to gluconeogenesis in cold-exposed humans.4

The metabolic cost of shivering alters substrate use. The reverse may also be true. For example, hyperglycemia stimulates production of proinflammatory cytokines5 that raise the central thermoregulatory set point (i.e., causing fever).6–8 A potential mechanism is that hypoglycemia excites warm-sensitive neurons and inhibits cold-sensitive neurons in rats.9 Furthermore, fructose ingestion increases absolute heat production in cold-exposed humans.10,11 In contrast, limited data in humans suggest that hypoglycemia may impair cold defenses12–15 and contribute to hypothermia in exhausted hikers.16

Perioperative hyperglycemia commonly follows the stress of surgical tissue injury. Conversely, perioperative hypoglycemia may result from aggressive efforts to control blood sugar. These perioperative glycemic perturbations may place surgical patients at increased risk if blood glucose concentration influences thermoregulatory responses. We therefore determined the effect of hypoglycemia and hyperglycemia on the shivering threshold. Specifically, we tested the hypothesis that hypoglycemia reduces the shivering threshold, whereas hyperglycemia increases the threshold in rabbits.

METHODS

With the approval of our committee on animal research, we studied 27 male Japanese white rabbits weighing an average of 3.4 kg (range, 2.9–3.8 kg). Daytime core temperature in these rabbits is usually approximately 39°C. Ambient temperature was maintained near 24°C throughout the study. To exclude the effects of circadian variation on body temperature, studies began about 10:00 AM and typically ended near 3:00 PM.

Protocol

Animal preparation was performed as previously described.17,18 In brief, the animals were anesthetized by inhalation of 2.5% to 4% isoflurane and 67% nitrous oxide in oxygen. Intubation of the trachea was performed with a 3-mm endotracheal tube after application of topical anesthesia with 8% lidocaine spray. After endotracheal intubation,
the rabbits were allowed to breathe spontaneously. Nitrous oxide was discontinued and the end-tidal concentration of isoflurane adjusted to 0.2 minimum alveolar anesthetic concentration (0.4%) in 100% oxygen (NORMAC AA-102; GE Healthcare, Andover, MA).

A catheter was inserted in the marginal ear vein and 3 to 4 mL/kg/h lactated Ringer’s solution was infused throughout the study. A catheter was inserted into a femoral artery for blood gas analysis and blood glucose determination.

Shortly before each study, the animals were randomly assigned 1:1:1 to 1 of the 3 infusion regimens through a computer-generated randomization list as follows: (1) saline, (2) insulin titrated to produce blood glucose concentrations 60 to 100 mg/dL, or (3) 50% dextrose titrated to produce blood glucose concentrations 200 to 300 mg/dL. The target blood glucose concentration in the insulin group was 60 mg/dL ≤ blood glucose ≤ 100 mg/dL, and the target in the dextrose group was 200 mg/dL ≤ blood glucose ≤ 300 mg/dL. Saline was infused at 1 mL/kg/h. Insulin (2 units/mL) was infused at 0.5 to 1.5 mL/kg/h, and 5 to 10 units of insulin was injected under the skin as needed to produce blood glucose concentrations 60 to 100 mg/dL. Dextrose 50% was infused at 0.5 to 2 mL/kg/h to produce blood glucose concentrations 200 to 300 mg/dL. The total fluid infusion rate was adjusted to be 4 mL/kg/h by infusion of lactated Ringer’s solution.

Each target infusion was maintained for ≥30 minutes before blood glucose measurements. Between 120 and 150 minutes after starting the drug or saline infusion, core temperature was reduced at a rate of 2 to 3°C/h by perfusing water at 10°C through a plastic tube positioned in the colon. The study ended when shivering occurred or core temperature reached 34°C. At the end of the experiment, each animal was killed by KCl infusion, as requested by the local animal care committee.

Measurements
We defined shivering as rhythmical muscular contraction, accompanied by piloerection. Core temperature at the initial observation of shivering defined the threshold. Shivering was assessed by an observer blinded to the treatment and blood glucose concentrations. Core temperatures were recorded from the distal esophagus (MGA 3–219; Nihon Kohden, Tokyo, Japan). The accuracy of this device is ±0.1°C. Arterial blood was sampled for blood gas analysis and blood glucose determination (i-STAT; Abbott Point of Care Inc., Princeton, NJ) in each rabbit at the time of shivering defined the threshold. All analyses were considered statistically significant. Thus, 0.8 degrees was determined by our preliminary experiments.

The core temperature that triggered sustained vigorous shivering defined the shivering threshold. All analyses were conducted using SAS version 9.3 (SAS Institute Inc., Cary, NC). Data are expressed as means ± SDs; P < 0.05 was considered statistically significant.

RESULTS
Baseline characteristics in each group before cooling are shown in Table 1. Except for blood glucose concentration, baseline characteristics were similar among the groups before cooling.

Values in each group at the time of shivering are shown in Table 2. Except for mean arterial blood pressure, hemodynamic and respiratory values were similar at the time of shivering, as was the level of sedation assessed using bispectral index (BIS).

The individual and average shivering thresholds for each of the 3 groups are shown in Figure 1. The (mean ± SD) shivering threshold in the saline group was 37.2 ± 0.5°C. The shivering threshold in the insulin group was 36.3 ± 1.1°C. The shivering threshold in the dextrose group was 38.0 ± 0.6°C. There were significant differences in the shivering threshold between each group, except for the saline versus dextrose groups. The data suggest that the shivering threshold is correlated to blood glucose concentration (Table 2, Fig. 1). We regarded the lack of differences between saline and dextrose as type II error.

A regression of individual blood glucose concentrations versus individual shivering thresholds is shown in Figure 2. The shivering threshold increased as a function of blood glucose concentration: shivering threshold (°C) = 0.009 [blood glucose (mg/dL)] + 35.6; r² = 0.53. The shivering threshold thus increased approximately 1°C for each 100 mg/dL increase in blood glucose concentration.

DISCUSSION
Thermal input from the skin surface and deep tissues is integrated and interpreted by central control systems, which, in turn, activate warm and cold defenses. Behavioral responses, such as seeking shelter, are the most effective compensations for environmental thermal perturbations. Autonomic defenses against excessive warming include panting, active precapillary vasoconstriction, and sweating. Autonomic defenses against excessive cooling include arteriovenous shunt constriction, nonshivering thermogenesis, and shivering. Each defense can be characterized by its threshold (triggering core temperature), gain (intensity increase per incremental further deviation in core temperature), and maximal response intensity.20

Typically, the thresholds for warm defenses are clustered just above normal core temperature, whereas the thresholds for cold defenses are clustered just below normal core temperature.21 Temperatures between the lowest warm threshold and the highest cold threshold define the interthreshold range. Fever is characterized by a synchronous increase in all thermoregulatory thresholds (i.e., “set point” increase). In contrast, general anesthesia22,23 and related drugs, including opioids,24,25 reduce cold-response thresholds without minimally altering warm-response thresholds, thus increasing the interthreshold range.26
effective.\textsuperscript{31,32} Even during general anesthesia, for instance, vasoconstriction thresholds change synchronously.\textsuperscript{29} Vasoconstriction usually prevents additional hypothermia,\textsuperscript{30} it is highly likely that hypoglycemia reduces the vasoconstriction threshold, which is typically about 34.5°C during general anesthesia. To the extent that hypoglycemia reduces the vasoconstriction threshold in humans as well as rabbits, unwarmed patients will get colder before protecting themselves by activating thermoregulatory defenses.

Shivering can be induced during general anesthesia, but only by aggressive, deliberate cooling. Clinically, shivering is more common postoperatively when anesthetic-induced impairment of thermoregulatory control dissipates.\textsuperscript{25} Here, the problem is the mirror image to the intraoperative period: higher thresholds augment thermoregulatory defenses, including shivering. Hyperglycemia is thus likely to worsen postoperative shivering.

Hyperglycemia generally excites warm-sensitive neurons and inhibits cold-sensitive neurons in rats.\textsuperscript{34} Hypoglycemia may also reduce the set-point of temperature in humans.\textsuperscript{35–38} Reducing temperature is a physiologically reasonable response because hypoglycemia reduces glucose demand. However, our rabbits were not hypoglycemic as our low-est glucose concentrations were 88 ± 6 mg/dL. Fever generally results when inflammatory cytokines secreted by immune cells stimulate production of prostaglandin E2. Prostaglandin E2 penetrates the blood–brain barrier and raises the thermoregulatory set-point.\textsuperscript{39–42} Transient hyperglycemia caused by glucose loading also stimulates

### Table 1. Baseline Characteristics Before Cooling

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Insulin</th>
<th>Dextrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>77 ± 12</td>
<td>73 ± 14</td>
<td>84 ± 9</td>
</tr>
<tr>
<td><em>P values</em></td>
<td>0.8</td>
<td>Versus saline</td>
<td>0.5</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>309 ± 39</td>
<td>315 ± 25</td>
<td>282 ± 44</td>
</tr>
<tr>
<td><em>P values</em></td>
<td>0.9</td>
<td>Versus saline</td>
<td>0.3</td>
</tr>
<tr>
<td>Respiratory rate (breaths/min)</td>
<td>65 ± 8</td>
<td>69 ± 10</td>
<td>62 ± 12</td>
</tr>
<tr>
<td><em>P values</em></td>
<td>0.6</td>
<td>Versus saline</td>
<td>0.9</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.50 ± 0.08</td>
<td>7.44 ± 0.08</td>
<td>7.51 ± 0.09</td>
</tr>
<tr>
<td><em>P values</em></td>
<td>0.3</td>
<td>Versus saline</td>
<td>0.96</td>
</tr>
<tr>
<td>Pa\textsubscript{CO\textsubscript{2}} (mm Hg)</td>
<td>27 ± 4</td>
<td>30 ± 11</td>
<td>26 ± 4</td>
</tr>
<tr>
<td><em>P values</em></td>
<td>0.7</td>
<td>Versus saline</td>
<td>0.96</td>
</tr>
<tr>
<td>Pao\textsubscript{2} (mm Hg)</td>
<td>490 ± 40</td>
<td>478 ± 42</td>
<td>522 ± 34</td>
</tr>
<tr>
<td><em>P values</em></td>
<td>0.8</td>
<td>Versus saline</td>
<td>0.2</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>152 ± 20</td>
<td>92 ± 11*</td>
<td>271 ± 39†</td>
</tr>
<tr>
<td><em>P values</em></td>
<td>&lt;0.001</td>
<td>Versus saline</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Base excess (mmol/L)</td>
<td>−1.8 ± 3.7</td>
<td>−4.4 ± 2.7</td>
<td>−1.9 ± 3.5</td>
</tr>
<tr>
<td><em>P values</em></td>
<td>0.2</td>
<td>Versus saline</td>
<td>0.997</td>
</tr>
<tr>
<td>Esophageal temperature (°C)</td>
<td>39.3 ± 0.7</td>
<td>39.7 ± 1.0</td>
<td>39.4 ± 0.8</td>
</tr>
<tr>
<td><em>P values</em></td>
<td>0.6</td>
<td>Versus saline</td>
<td>0.9</td>
</tr>
<tr>
<td>BIS</td>
<td>87 ± 12</td>
<td>94 ± 3</td>
<td>90 ± 8</td>
</tr>
<tr>
<td><em>P values</em></td>
<td>0.2</td>
<td>Versus saline</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Results are baseline characteristics before cooling. Data are reported as means ± SDs. *P values were adjusted by Tukey method based on the assumption of normal distribution and equal variances among the groups. MAP = mean arterial blood pressure; Pa\textsubscript{CO\textsubscript{2}} = partial pressure of carbon dioxide; Pao\textsubscript{2} = partial pressure of arterial oxygen; BIS = bispectral index.

*P < 0.05 compared with saline group.
†P < 0.05 compared with insulin group.

Blood glucose concentrations vary considerably among surgical patients, as well as within a given operation.\textsuperscript{25} Variations exceeding 100 mg/dL are not unusual, even within a given patient. Our results indicate that, at least in rabbits, such variations change the shivering threshold by approximately 1°C, a clinically important difference. Our results extend previous work by identifying specific shivering thresholds at each blood glucose concentration.

In humans, the shivering threshold is about a 1°C below the vasoconstriction threshold.\textsuperscript{23} With only exceptions (e.g., after meperidine administration),\textsuperscript{26} the shivering and vasoconstriction thresholds change synchronously.\textsuperscript{28} Presumably, this also is the case when thresholds are modified by alterations in blood glucose concentration. It is thus likely that hypoglycemia reduces the vasoconstriction threshold as well as the shivering threshold. Conversely, hyperglycemia presumably increases both the vasoconstriction and shivering thresholds. To the extent that our results can be extrapolated to humans, slightly elevated blood glucose levels (e.g., >140 mg/dL) might increase the shivering thresholds, whereas pathologically low blood glucose levels might decrease the shivering thresholds in surgical patients, at least compared with typical glucose management.

Although vasoconstriction is largely restricted to arteriovenous shunts in the fingers and toes,\textsuperscript{30} it is highly effective.\textsuperscript{31,32} Even during general anesthesia, for instance, vasoconstriction usually prevents additional hypothermia, even during very large operations.\textsuperscript{33} Unwarmed surgical patients having prolonged major surgery may reach the vasoconstriction threshold, which is typically about 34.5°C during general anesthesia. The extent that hypoglycemia reduces the vasoconstriction threshold in humans as well as rabbits, unwarmed patients will get colder before protecting themselves by activating thermoregulatory defenses.

Shivering can be induced during general anesthesia, but only by aggressive, deliberate cooling. Clinically, shivering is more common postoperatively when anesthetic-induced impairment of thermoregulatory control dissipates.\textsuperscript{26} Here, the problem is the mirror image to the intraoperative period: higher thresholds augment thermoregulatory defenses, including shivering. Hyperglycemia is thus likely to worsen postoperative shivering.

Hypoglycemia generally excites warm-sensitive neurons and inhibits cold-sensitive neurons in rats.\textsuperscript{34} Hypoglycemia may also reduce the set-point of temperature in humans.\textsuperscript{35–38} Reducing temperature is a physiologically reasonable response because hypoglycemia reduces glucose demand. However, our rabbits were not hypoglycemic as our lowest glucose concentrations were 88 ± 6 mg/dL. Fever generally results when inflammatory cytokines secreted by immune cells stimulate production of prostaglandin E2. Prostaglandin E2 penetrates the blood–brain barrier and raises the thermoregulatory set-point.\textsuperscript{39–42} Transient hyperglycemia caused by glucose loading also stimulates
production of cytokines: interleukin-6, tumor necrosis factor-α, etc. Hyperglycemia caused by glucose loading may have similarly raised the set-point in our rabbits. The rabbit model we used is well established and has been used in many previous studies. An obvious limitation is that rabbits are not humans. However, thermoregulatory defenses are highly stereotypical and generally well conserved across species. Although the magnitude of the relationship (slope in Fig. 2) likely differs in humans, it would be quite surprising if blood glucose concentration has no effect on thermoregulatory response in humans. Now that we have established the relationship in rabbits, a reasonable next step would be to quantify the effect of hypoglycemia and hyperglycemia on the shivering threshold in humans.

Another limitation of our study is that the shivering threshold was not evaluated objectively, but by an observer. Thus, we cannot completely exclude that a least a part of shivering was nonthermoregulatory. There may be a role for electromyography to objectively evaluate the shivering threshold. However, our efforts to do so have not been successful.

Glucose modifications in our study were induced acutely. Whether hyperglycemia sustained for weeks, months, or years also increases the shivering threshold remains unknown. Similarly, very acute changes in glucose concentration (e.g., over a few minutes) may provoke even more profound effects. It is also possible that the mechanism by which blood glucose concentrations are modified matters. For example, the hyperglycemia of diabetes may well have different thermoregulatory effects than hyperglycemia resulting from administration of exogenous glucose or hyperglycemia induced by surgical stress.

Our study was a parallel group design. We could not use a crossover design because there were concerns about drop-outs and carryover effect. Inserting a catheter into a femoral artery is the best way to collect blood from a rabbit during cooling without disturbing it. It is almost impossible to insert a catheter into a femoral artery without a skin incision on rabbits. Our surgical instruments were not sterile. Our animal care committee thus insisted that we kill the rabbits after each study.

To date, few studies have been reported evaluating the use of BIS assessments in veterinary practice, despite its extensive validation and use in humans. However, rabbits have been used to study the EEG effects of anesthetics, and EEG has been used to evaluate the depth of anesthesia when using injectable combinations in this species. Although the BIS algorithm of humans many not be applicable to that of rabbits, BIS values may at least roughly help determine anesthetic depth in rabbits. Furthermore, BIS values correlate with the Observers’ Assessment of Alertness/
Sedation score in patients given midazolam for sedation. Nonetheless, hypnotic depth in our rabbits, as evaluated with bispectral monitoring, should thus be considered only a rough estimate of anesthetic effect.\textsuperscript{18} Shivering is one of the several thermoregulatory cold defenses. Nonshivering thermogenesis is the predominant protection in rats and similarly sized mammals. Whether nonshivering thermogenesis is equally affected by glucose concentration remains unknown. However, nonshivering thermogenesis contributes little, if anything, to metabolic heat production in humans\textsuperscript{44,45} after infancy.\textsuperscript{46} More importantly, we did not evaluate warm defenses, such as panting or sweating. It is thus impossible to determine from these data whether the observed glycemia-induced increase in the shivering threshold represents a set point increase (synchronous increase in all thermoregulatory thresholds as with fever) or results from a widening of the interthreshold range (as with general anesthesia).

In summary, in rabbits, the shivering threshold increased approximately linearly with increasing blood glucose concentration throughout the physiologic range and even extending to concentrations exceeding 300 mg/dL. The effect was substantial, with each 100 mg/dL increase in blood glucose increasing the shivering threshold by approximately 1°C. To the extent these results apply to humans, hypoglycemia may promote intraoperative hypothermia, whereas hyperglycemia may aggravate postoperative shivering.

**DISCLOSURES**

**Name:** Hirofumi Ino, MD.  
**Contribution:** This author helped design the study, conduct the study, analyze the data, and write the manuscript.  
**Attestation:** Hirofumi Ino has seen the original study data, reviewed the analysis of the data, and approved the final manuscript, and is the author responsible for archiving the study files.

**Name:** Taishi Masamune, MD, PhD.  
**Contribution:** This author helped design the study, conduct the study, analyze the data, and write the manuscript.  
**Attestation:** Taishi Masamune has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.

**Name:** Hiroaki Sato, MD, PhD.  
**Contribution:** This author helped design the study and analyze the data.  
**Attestation:** Hiroaki Sato has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.

**Name:** Katsumi Okuyama, MD, PhD.  
**Contribution:** This author helped design the study.  
**Attestation:** Katsumi Okuyama approved the final manuscript.

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**Contribution:** This author helped design the study, conduct the study, and analyze the data.  
**Attestation:** Keiichi Wada has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.

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**Contribution:** This author helped design the study.  
**Attestation:** Hironobu Iwashita approved the final manuscript.

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**Contribution:** This author helped write the manuscript.  
**Attestation:** Tadahiko Ishiyama approved the final manuscript.

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**Contribution:** This author helped design the study.  
**Attestation:** Takeshi Oguchi approved the final manuscript.

**Name:** Daniel I. Sessler, MD.  
**Contribution:** This author helped write the manuscript.  
**Attestation:** Daniel I. Sessler approved the final manuscript.

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**Figure 1.** The shivering thresholds in each group. Circles show shivering threshold in insulin group, diamonds show shivering threshold in saline group, and triangles show shivering threshold in dextrose group. Circles with SD error bars indicate the mean shivering thresholds in each group. *P* < 0.05.

**Figure 2.** Regression of individual blood glucose concentrations versus individual shivering thresholds. The shivering threshold increased as a function of blood glucose concentration: shivering threshold (°C) = 0.009 [blood glucose concentration (mg/dL)] + 35.6; \( r^2 = 0.53 \). The shivering threshold thus increases approximately 1°C for each 100 mg/dL increase in blood glucose concentration.
The Effects of Glucose on the Shivering Threshold

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Contribution: This author helped design the study.

Attestation: Takashi Matsukawa approved the final manuscript.

The manuscript was handled by: Steven L. Shafer, MD.

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