Effects of intramuscular administration of tiletamine-zolazepam with and without sedative pretreatment on plasma and serum biochemical values and glucose tolerance test results in Japanese black bears (Ursus thibetanus japonicus)

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Objective—To establish a safe anesthetic protocol with little effect on blood biochemical values and IV glucose tolerance test (IVGTT) results in Japanese black bears (Ursus thibetanus japonicus).

Animals—16 captive female Japanese black bears (5 to 17 years of age).

Procedures—Bears were randomly assigned to 4 treatment groups (4 bears/group) in which various treatment combinations were administered via blow dart: tiletamine HCl and zolazepam HCl (9 mg/kg) alone (TZ), TZ (6 mg/kg) and acepromazine maleate (0.1 mg/kg), TZ (6 mg/kg) and butorphanol tartrate (0.3 mg/kg), or TZ (3 mg/kg) and medetomidine HCl (40 µg/kg). Glucose injection for the IVGTT was started 130 minutes after TZ administration. Blood samples were obtained before, at, and intermittently after glucose injection for measurement of biochemical variables as well as plasma glucose and serum insulin concentrations during the IVGTT. Rectal temperature, pulse rate, and respiratory rate were assessed every 15 minutes during the experiment.

Results—Induction and maintenance of anesthesia were safely achieved with little adverse effect on cardiopulmonary function when each of the 4 anesthetic regimens was used, although mild hypothermia was induced. No difference was evident between treatment groups in blood biochemical values. Blood glucose and insulin concentration profiles during the IVGTT were similar among the bears given TZ, with or without acepromazine or butorphanol, but hyperglycemia and hypoinsulinemia developed in bears given TZ with medetomidine.

Conclusions and Clinical Relevance—All 4 anesthetic regimens yielded chemical restraint without affecting clinical and biochemical values in bears, but medetomidine appeared to affect IVGTT results. For this reason, medetomidine should not be used when anesthetizing bears for IVGTTs. (Am J Vet Res 2012;73:1282–1289)

Japanese black bears (Ursus thibetanus japonicus), a subspecies of Asiatic black bears, undergo annual cycles in body mass characterized by fattening in autumn and weight loss in winter. During the hibernation period in winter, bears rely on fat stores as their main energy source,¹ and pregnant bears give birth and nurse cubs.² ³ Therefore, the accumulation of body fat in autumn is critical to the survival and reproduction of bears.

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these carbohydrates into body fat. However, the metabolic mechanisms involved in the regulation of body mass or body fat have yet to be elucidated.

The distinct seasonal fluctuation in the amount of body fat without adverse health effects in hibernating species makes such species ideal subjects for studying body mass regulation and obesity. Body mass gain is not simply a consequence of increased food intake. Various other factors, such as metabolic enzymes, hormones, and metabolic rate, are related to the circannual rhythm of energy balance and fat metabolism. To our knowledge, the IVGTT has never been performed in Japanese black bears, and few if any studies on pancreatic function have involved bears. To perform an IVGTT in bears, anesthesia must be maintained for a long period, and anesthetics may have undesirable effects on carbohydrate metabolism. However, there is little information about anesthesia in Japanese black bears, and there are few reports on the effects of anesthetics on carbohydrate and lipid metabolism in this species. Consequently, anesthetic protocols are needed that are safe and effective in bears and whether these protocols interfere with IVGTT results needs to be determined.

In bears, a 1:1 mixture of TZ is commonly used for anesthesia because it induces reliable anesthesia with predictable signs of recovery and has a wide margin of safety. The disadvantages of using TZ are the lack of specific antagonists or reversal agents and the finding that additional doses prolong recovery time. Despite the common use of TZ in bears, the effects of TZ on carbohydrate metabolism and insulin secretion in bears are unknown. Medetomidine HCl is a specific α2-adrenergic receptor agonist and is widely used in veterinary medicine as a sedative, analgesic, and muscle relaxant. The combination of TZ with medetomidine has been used for the immobilization of bears because medetomidine administration allows the dose of TZ required to achieve immobilization to be decreased and because the effects of medetomidine are readily and rapidly reversed with subsequent atipamezole administration. In many species, medetomidine administration yields undesirable effects such as hyperglycemia, hypoinsulinemia, and bradycardia. However, no reports exist of the effect of medetomidine on carbohydrate metabolism in bears. Acepromazine maleate and butorphanol tartrate are other sedatives that might be combined with TZ for anesthesia in bears, particularly because the effects of these agents on glucose metabolism are minimal in dogs. The purpose of the study reported here was to establish a safe and effective anesthetic protocol with minimal effects on blood biochemical values and IVGTT results in Japanese black bears.

Materials and Methods

Animals—Sixteen adult (5- to 17-year-old) non-pregnant female Japanese black bears kept in Ani Mataginosato Bear Park, Akita Prefecture, Japan, were used in the study. The bears hibernated in indoor rooms without feed (but with water) from December to mid-April. The study was conducted between May and June 2010. During the active season from May to November, all bears were provided with approximately 2 kg of dried corn/d with some fruits, vegetables, and commercial bear food pellets as supplements. Water was freely available at all times. The study was performed in accordance with the guidelines of the Animal Care and Use Committee of Hokkaido University.

Study design—The experiment was performed indoors during the morning, and bears were not fed after 4 pm the previous day. Bears were randomly assigned to 4 premedication groups (4 bears/group): TZ (9 mg/kg) alone with no premedication, acepromazine (0.1 mg/kg) followed by TZ (6 mg/kg), butorphanol (0.3 mg/kg) followed by TZ (6 mg/kg), or medetomidine (40 µg/kg) followed by TZ (3 mg/kg).

The TZ solution was prepared by reconstituting powdered TZ with 5 mL of diluent to achieve a concentration of 300 mg/mL. The doses of acepromazine and butorphanol were estimated from published information on dogs and cats. The doses for TZ alone and TZM were established through evaluation of previous studies on TZ and TZM use in Japanese black bears. The dose of TZ in combination with acepromazine or butorphanol was established on the basis of results of preliminary experiments that showed > 5 mg of TZ/kg would be necessary to immobilize the bears (unpublished data).

The dose of premedication was determined on the basis of estimated body weight and was subsequently administered IM via blow dart to bears assigned to treatments involving a premedication. The TZ was administered in a similar manner to all bears 30 minutes after premedication. When the effect of the TZ was insufficient, additional doses of TZ (approximately a third of the original dose) were given IM at approximately 45 minutes after the first dose of TZ was administered.

Once immobilization (anesthetic induction) was achieved, the bears were weighed and handled. Final doses of TZ were calculated on the basis of actual body weights. Catheters were inserted into both external jugular veins: one for anesthetic (TZ) infusion and glucose injection and the other for blood sample collection. The bears were kept immobilized throughout the experiment by continuous IV infusion of TZ dissolved in 500 mL of aceted Ringer’s solution at a rate of 1 to 3 mg/kg/h.

Recording and monitoring—Induction time was defined as the interval from TZ administration to the point at which bears moved to sternal or lateral re-
and total cholesterol concentrations and plasma ALT were obtained. Baseline plasma concentrations of glucose, triglycerides, total cholesterol, and heparin sodium was used as the anticoagulant to determine plasma ALT and AST activities. Blood samples were also placed in evacuated tubes at 60, 90, and 120 minutes after glucose injection and placed into evacuated tubes. Immediately after each sample was collected, approximately 1 mL of heparinized saline (0.9% NaCl) solution was used to flush the catheter.

After the IVGTT was finished, the continuous IV infusion of TZ was discontinued. Naloxone hydrochloride (0.04 mg/kg, IV; butorphanol antagonist) or atipamezole HCl (200 μg/kg, IM; medetomidine antagonist) was administered to bears in the TZB and TZM groups, respectively, to facilitate anesthetic recovery. Bears in the other groups received no additional treatment.

Blood sample processing—Evacuated tubes containing a mixture of sodium fluoride, heparin sodium, and EDTA-2Na as anticoagulant were used for plasma glucose determination. Tubes containing EDTA-2K were used to determine plasma triglycerides and total cholesterol concentrations, and heparin sodium was used as the anticoagulant to determine plasma ALT and AST activities. Blood samples were also placed in evacuated tubes without anticoagulant and allowed to clot. All samples were chilled on ice, then underwent centrifugation at 1,880 × g for 10 minutes within 10 (for plasma) or 30 (for serum) minutes after sample collection. Separated serum (for NEFA and insulin) and plasma (for glucose, triglycerides, total cholesterol, ALT, and AST assays) were stored at −30°C for approximately 2 weeks until assayed.

Blood biochemical analyses—Analyses other than glucose and insulin were analyzed in the serum or plasma harvested from blood samples obtained 60 minutes after the administration of TZ, with the exception of the sample for 1 bear in the TZB group. For that bear, the sample obtained 90 minutes after the TZ administration was used because the 60-minute sample could not be obtained. Baseline plasma concentrations of glucose and serum concentrations of insulin were analyzed in the samples obtained 60, 90, and 120 minutes after the administration of TZ. Plasma glucose, triglycerides, and total cholesterol concentrations and plasma ALT and AST activities were measured with an automatic blood analyzer. Serum NEFA concentrations were measured with a commercial kit. Serum insulin concentration was measured with an ELISA developed for dog insulin concentration measurement, with purified dog insulin used as the standard. The intra-assay and inter-assay coefficients of variation for the ELISA were 3.6% and 8.0%, respectively.

IVGTT analysis—Values of $t_{1/2}$ and the K were determined from the results of each IVGTT. To calculate the $t_{1/2}$, linear regression analysis of a semilogarithmic plot of plasma glucose concentration versus time between 10 and 30 minutes after glucose injection was used. The K value was calculated by use of the following formula: $K = (0.693/t_{1/2}) \times 100$. Areas under the glucose and insulin curves (AUC$_{glucose}$ and AUC$_{insulin}$) were calculated as incremental area above zero with statistical software. The AUC$_{glucose}$ and AUC$_{insulin}$ were determined from 10 minutes before (−10 minutes) to 10 minutes after glucose injection and from −10 minutes to 180 minutes after injection (end of the IVGTT).

Statistical analysis—Unless otherwise stated, statistical analyses were performed with statistical software. To analyze the effects of 4 anesthetic combinations on the selected blood variables (triglycerides, total cholesterol, and NEFA concentrations and AST and ALT activities), the differences in these variables among groups were evaluated via the Kruskal-Wallis test. Differences in body weight, total TZ dose, and induction time among groups were also evaluated with the Kruskal-Wallis test. When a statistical test revealed a significant difference, the Dunn multiple comparison test was performed. Differences in rectal temperatures, pulse rates, and respiratory rates among groups were evaluated similarly. Changes in those 3 variables over time (the changes from the values at 2 hours after TZ injection [10 minutes before glucose injection]) in each group were evaluated via the Steel multiple comparison test with the aid of statistical software.

To analyze the effects of 4 anesthetic combinations on IVGTT results, differences in baseline glucose and insulin concentrations and in values calculated from the results of each IVGTT (peak plasma glucose and serum insulin concentrations, $t_{1/2}$, K, AUC$_{glucose}$, and AUC$_{insulin}$) among groups were evaluated with the Kruskal-Wallis test followed by the Dunn multiple comparison test. Again, because there were no significant differences among the plasma glucose and serum insulin concentrations measured at the 3 points before glucose injection, the means of these 3 baseline values were used to represent the pretest values. To identify any significant changes from baseline values in the serial measurements of glucose and insulin concentrations performed during IVGTTs, the Steel multiple comparison test was used. Values of $P < 0.05$ were considered significant. All data are reported as mean ± SD.

Results

Animals—Body weights of the bears, doses of TZ required for anesthetic induction, doses of premedica-
tion, and induction times in each group were summarized (Table 1). No significant differences were detected in body weight among groups. No bears, except for 2 bears in the TZM group that became laterally or ster- singly recumbent, had signs of sedation for 30 minutes after premedication administration (acepromazine, butorphanol, or medetomidine). Administration of TZ caused the bears to become recumbent within a mean ± SD time of 13.1 ± 4.8 minutes (range, 6 to 26 minutes), with no significant differences among groups.

Within the TZ, TZA, and TZB groups, 2, 1, and 3 bears, respectively, needed additional doses of TZ to achieve immobilization; consequently, total doses of TZ for these groups were not significantly different. In contrast, sufficient anesthetic depth was achieved with a significantly lower dose of TZ for bears in the TZM group (3.6 ± 0.9 mg/kg) than for bears that received TZ alone (11.2 ± 2.0 mg/kg). Additional doses of TZ prolonged the induction time, but the mean induction time of each group was not significantly different.

Physiologic effects—Rectal temperatures before glucose injection (2 hours after TZ administration) were not significantly different among groups (Table 2), and no significant (P = 0.10) correlation was found between rectal and ambient temperatures at this point. For all groups, there were no significant changes in rectal temperatures over time, although temperatures decreased gradually in the TZA, TZB, and TZM groups (Figure 1). Mean rectal temperatures at the end of the experiment in each group were 35.8° ± 1.0°C for TZ alone, 35.8° ± 1.3°C for TZA, 35.3° ± 0.4°C for TZB, and 34.6° ± 1.0°C for TZM.

Pulse rates at 2 hours after TZ administration were significantly (P < 0.05) higher in the TZA (129 ± 23 beats/min) group than in the TZB (57 ± 29 beats/min) and TZM (47 ± 7 beats/min) groups. After the 2-hour measurement point, there were no significant changes in pulse rates over time in all groups. Respiratory rates at 2 hours after TZ administration were not significantly different among groups. After the 2-hour measurement point, some variability in respiratory rates was observed among groups, but there were no significant changes over time in any group. The TZB group had a lower respiratory rate than the other groups, and the mean respiratory rate throughout the experiment in the TZB group was 10 ± 3 breaths/min.

Induction and maintenance of anesthesia during the experiment were safely achieved in all bears. No bears died or developed noticeable physical problems. However, vomiting was observed during induction of anesthesia in 1 bear from the TZA group. Additionally, 2 and 3 bears that received TZ alone and TZA, respectively, had increased salivation during the experiment.

Hematologic effects—Baseline concentrations of plasma lipids and activities of hepatic enzymes did not differ significantly among the groups (Table 2). Baseline concentrations of plasma lipids and activities of hepatic enzymes did not differ significantly among the groups (Table 2).

### Table 1—Mean ± SD body weight, dose of TZ and premedication for anesthetic induction, and induction time in Japanese black bears (4/group) immobilized with TZ (9 mg/kg) alone (no premedication), acepromazine maleate (0.1 mg/kg) followed by TZ (6 mg/kg), butorphanol tartrate (0.3 mg/kg) followed by TZ (6 mg/kg), or medetomidine HCl (40 μg/kg) followed by TZ (3 mg/kg).

<table>
<thead>
<tr>
<th>Group</th>
<th>Additional dose required</th>
<th>Body weight (kg)</th>
<th>TZ dose (mg/kg)</th>
<th>Total dose of premedication (mg/kg)</th>
<th>Induction time (min)</th>
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<td></td>
<td></td>
<td>Initial</td>
<td>Additional</td>
<td>Total</td>
<td></td>
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<tr>
<td>TZ</td>
<td>Yes (n = 2)</td>
<td>71.3 ± 21.3</td>
<td>9.1 ± 0.9</td>
<td>21 ± 2.6</td>
<td>11.2 ± 2.0</td>
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<td></td>
<td>No (n = 2)</td>
<td>65.0 ± 17.0</td>
<td>8.7 ± 1.1</td>
<td>42 ± 1.6</td>
<td>12.8 ± 0.4</td>
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<td>TZA</td>
<td>Yes (n = 1)</td>
<td>66.0 ± 14.6</td>
<td>6.6 ± 0.9</td>
<td>0.5 ± 1.0</td>
<td>7.1 ± 1.1</td>
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<tr>
<td></td>
<td>No (n = 3)</td>
<td>63.7 ± 16.9</td>
<td>6.8 ± 1.0</td>
<td>6.8 ± 1.0</td>
<td>12.6 ± 1.1</td>
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<tr>
<td>TZB</td>
<td>Yes (n = 3)</td>
<td>72.0 ± 17.4</td>
<td>6.5 ± 0.5</td>
<td>2.7 ± 2.0</td>
<td>9.2 ± 1.9</td>
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<tr>
<td></td>
<td>No (n = 3)</td>
<td>78.3 ± 14.6</td>
<td>6.4 ± 0.5</td>
<td>3.6 ± 1.1</td>
<td>10.0 ± 1.2</td>
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<td>TZM</td>
<td>Yes (n = 1)</td>
<td>68.8 ± 6.6</td>
<td>3.3 ± 0.3</td>
<td>0.3 ± 0.6</td>
<td>3.6 ± 0.9*</td>
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<tr>
<td></td>
<td>No (n = 3)</td>
<td>70.3 ± 7.0</td>
<td>3.1 ± 0.2</td>
<td>—</td>
<td>3.1 ± 0.2</td>
</tr>
</tbody>
</table>

*Value differs significantly (P < 0.05) from the value for TZ alone.

— = Not applicable.

Acepromazine, butorphanol, and medetomidine were administered IM as premedications 30 minutes before TZ was administered IM. One hour after TZ administration, anesthesia was maintained with a continuous IV infusion of TZ (1 to 3 mg/kg/h). Induction time was defined as the interval from TZ administration to the point at which bears moved to sternal or lateral recumbency and no response could be elicited to auditory stimulation from clapping or shouting.

### Table 2—Mean ± SD baseline plasma triglycerides concentration, total cholesterol concentration, and AST activity and ALT activity and serum NEFA concentration in the bears in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>TZ alone</th>
<th>TZA</th>
<th>TZB</th>
<th>TZM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>3.5 ± 0.7</td>
<td>3.3 ± 0.7</td>
<td>3.5 ± 0.6</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.0 ± 1.8</td>
<td>7.7 ± 1.6</td>
<td>7.6 ± 0.8</td>
<td>8.0 ± 0.2</td>
</tr>
<tr>
<td>NEFA (mmol/L)</td>
<td>0.23 ± 0.21</td>
<td>0.77 ± 0.79</td>
<td>0.28 ± 0.22</td>
<td>0.29 ± 0.15</td>
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<tr>
<td>AST (U/L)</td>
<td>95.0 ± 9.1</td>
<td>105.3 ± 41.5</td>
<td>90.3 ± 16.7</td>
<td>100.3 ± 12.3</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>23.3 ± 6.0</td>
<td>21.8 ± 7.1</td>
<td>21.8 ± 7.8</td>
<td>23.0 ± 2.7</td>
</tr>
</tbody>
</table>
line concentrations of plasma glucose concentration were significantly higher in the TZM group (6.9 ± 1.2 mmol/L) than in the TZ-alone (4.5 ± 0.4 mmol/L), TZA (4.7 ± 0.7 mmol/L), and TZB (4.2 ± 0.1 mmol/L) groups (Table 3). Plasma glucose concentrations peaked imme-

diately after glucose injection (0 minutes) in all groups and then decreased gradually over time, but there were no significant differences in peak values among the groups (Figure 2). For bears in the TZ-alone, TZA, and TZB groups, plasma glucose concentrations decreased at a similar rate and returned to baseline concentrations within 60 minutes after glucose injection. In contrast, plasma glucose concentrations remained high until 90 minutes after glucose injection for bears in the TZM group.

The t_{1/2} and K values in the TZM group were significantly longer and slower, respectively, compared with the values in the TZ-alone group (P < 0.05). The AUC_{glucose} from 10 minutes before (–10 minutes) to 10 minutes after glucose administration and from –10 to 180 minutes was significantly (P < 0.05) higher in the TZM group than in the TZ-alone group (Table 3).

Baseline serum insulin concentrations were significantly lower in the TZM group (23 ± 17 pmol/L) than in the TZ-alone (108 ± 88 pmol/L), TZA (79 ± 32 pmol/L), and TZB (136 ± 87 pmol/L) groups. Insulin concentrations after glucose injection varied according to the type of premedication. For bears that received TZ alone or TZB, the peak insulin concentration was observed within 10 minutes after glucose injection (Figure 2). The insulin response after glucose injection was slightly delayed for bears in the TZA group, and the peak concentration was observed 30 minutes after glu-

cose injection. For these 3 groups, serum insulin concentrations returned to baseline values by 60 minutes after glucose injection. In contrast, the insulin response for the TZM group was delayed, with the peak concentration at 90 minutes after glucose injection. However, the peak insulin concentration was not significantly different from the peaks in the other 3 groups.

The AUC_{insulin} from –10 minutes to 10 minutes in the TZM group was approximately a twentieth of the values of the other 3 groups, with a significant difference evident between the TZB and TZM groups. However, there were no significant differences among the groups in AUC_{insulin} from –10 minutes to 180 minutes.

Discussion

The safety and efficacy of 4 combinations of anes-

thetics in Japanese black bears were evaluated in the present study. Results indicated that the 4 administered combinations of TZ with and without premedications yielded sufficient anesthesia without noticeable important physical problems. Approximately 7 and 9 mg of TZ/kg was necessary to induce anesthesia in bears pre-
mobilized with 0.1 mg of acepromazine/kg and 0.3 mg of butorphanol/kg, respectively.

The mean anesthetic induction time of 4 bears was considerably longer than that in previous reports of bears immobilized by TZ^{14} or TZM,^{19} in which the defi-
nition of induction time was similar to that used in the present study. Many factors, such as doses of drugs re-
ceived, physical condition of the bear, and sensitivity to the anesthetic used, may influence the induction time. A possible explanation for the prolonged induction time in the present study is that the drugs administered for initial immobilization may have been injected into fat tissue rather than in muscle. The target for the blow

Figure 1—Changes in mean ± SD rectal temperature (A), pulse rate (B), and respiratory rate (C) over time in bears (4/group) immobi-
lized with TZ (9 mg/kg) alone (no premedication; black cir-
cles), acepromazine maleate (0.1 mg/kg) followed by TZ (6 mg/ kg; white circles), butorphanol tartrate (0.3 mg/kg) followed by TZ (6 mg/kg; triangles), or medetomidine HCl (40 µg/kg) followed by TZ (3 mg/kg; squares). Acepromazine, butorphanol, and me-
edetomidine were administered IM 30 minutes before TZ was ad-

ministered IM in all groups. One hour after TZ administration, anesthesia was maintained with a continuous IV infusion of TZ (1 to 3 mg/kg/h). a,bValues with different letters are significantly (P < 0.05) different among groups at 2 hours after TZ administration.
The reference interval for resting pulse rates of bears is 60 to 90 beats/min, and pulse rates observed in the TZB group were within this range throughout the experiment. Pulse rates in the bears that received TZ alone were in agreement with those in a previous study involving bears that were slightly higher than the upper reference limit. In dogs, administration of TZ commonly yields an increase in pulse rate and appears to do the same in bears. Therefore, care will need to be taken to mitigate the hypothermia induced by the drug combinations used in the present study when administered in a cold environment.

The reference interval for respiratory rate in bears is 15 and 30 breaths/min, and respiratory rates observed in the TZB group were in agreement with those in a previous study involving bears. However, noticeable suppression of NEFA concentrations vary somewhat during the active season for bears, ranging from approximately 1 to 4 mmol/L, and the plasma triglycerides concentrations observed in the present study were low. Some reports exist of the suppression of plasma NEFA concentrations in captive wild American black bears anesthetized with a combination of ketamine and xylazine HCl, the values in the present study were low. Some reports exist of the suppression of plasma NEFA concentrations in American black bears. In contrast, administration of ketamine and xylazine administration in dogs, cats, and cattle has been shown to have a minimal adverse effect on the respiratory system in bears. However, when high doses of TZ are administered, significant respiratory depression develops. In the present study, a high dose of TZ was administered for anesthetic induction, and after initial immobilization, anesthesia was maintained by continuous IV infusion of TZ at a lower dose. Therefore, the lower respiratory rates evident in bears that received TZ alone or TZB early in the course of anesthesia may have been attributable to an initial high dose of TZ. The gradual increase in respiratory rate during anesthesia may have been due to a progressive reduction in blood TZ concentration. In contrast, the constantly low respiratory rates in the TZB group may have been attributable to the respiratory depressant effect of butorphanol.

Little information is available regarding blood biochemical values in Japanese black bears, and reference intervals have yet to be established. However, plasma total cholesterol concentration and AST and ALT activities in the present study were within the physiologic reference range for American black bears (Ursus americanus). Therefore, we suggest that effects of the drugs used on these variables were negligible. Previous studies have shown that blood triglycerides concentrations vary somewhat during the active season for bears, ranging from approximately 1 to 4 mmol/L, and the plasma triglycerides concentrations observed in the present study were within this range. Compared with reported blood NEFA concentrations in captive and wild American black bears anesthetized with a combination of ketamine and xylazine HCl, the values in the present study were low. Some reports exist of the suppression of plasma NEFA concentration by medetomidine and xylazine administration in dogs, cats, and cattle and by acepromazine administration in dogs. However, noticeable suppression of NEFA concentrations by these drugs was not evident in the study bears. Baseline glucose values in bears that received TZ alone, TZA, and TZB were within the physiologic reference interval for American black bears, and the baseline insulin concentrations were comparable with the summer concentrations in American black bears. In contrast, administration of α₂-adrenergic receptor agonists, such as xylazine and medetomidine, reportedly causes hyperglycemia in cats and rats by suppressing appetite and causing hyperglycemia.

<table>
<thead>
<tr>
<th>Variable</th>
<th>TZ alone</th>
<th>TZA</th>
<th>TZB</th>
<th>TZM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (mmol/L)</td>
<td>4.5 ± 0.4a</td>
<td>4.7 ± 0.7a</td>
<td>4.2 ± 0.1a</td>
<td>6.9 ± 1.2b</td>
</tr>
<tr>
<td>Peak (mmol/L)</td>
<td>26.4 ± 3.2</td>
<td>25.4 ± 3.4</td>
<td>28.3 ± 3.7</td>
<td>26.5 ± 2.5</td>
</tr>
<tr>
<td>t½ (min)</td>
<td>16.0 ± 6.0b</td>
<td>24.0 ± 10.4b</td>
<td>22.5 ± 13.5b</td>
<td>51.5 ± 8.1b</td>
</tr>
<tr>
<td>K (percentage/min)</td>
<td>3.2 ± 2.3</td>
<td>3.3 ± 1.5b</td>
<td>3.9 ± 1.8b</td>
<td>1.4 ± 0.2b</td>
</tr>
<tr>
<td>AUC (mmol/L/min)</td>
<td>1,741 ± 374a</td>
<td>1,613 ± 374a</td>
<td>1,444 ± 394b</td>
<td>513 ± 19b</td>
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<td>–10 to 10 min</td>
<td>1,206 ± 196a</td>
<td>1,508 ± 205b</td>
<td>1,420 ± 191b</td>
<td>2,564 ± 261b</td>
</tr>
</tbody>
</table>

*Values with different superscript letters differ significantly (P < 0.05) among groups.*
insulin secretion by the pancreas, and results of the present study suggested that medetomidine had the same effect in Japanese black bears. To the authors’ knowledge, the IVGTT has never before been performed in Japanese black bears, yet anaesthesia is necessary to perform any medical examination on bears. For these reasons, the usual response to the IVGTT in nonanesthetized bears is unknown. Anesthesia is necessary to perform any medical examination on bears. For these reasons, the usual response to the IVGTT in nonanesthetized bears is unknown. In nonanesthetized dogs with a usual response to the IVGTT, plasma glucose concentration returns to the baseline concentration within 60 minutes after glucose injection and the insulin response is observed within 5 minutes. The serum insulin concentration also returns to the baseline concentration by 60 minutes after glucose injection. Although some variability in insulin response to glucose injection was observed in the present study, results of the IVGTTs in bears that received TZ alone, TZA, or TZB were comparable with the usual response in nonanesthetized dogs. Moreover, the t1/2 and K values of the bears in these 3 groups were similar to the values of healthy dogs (mean ± SD t1/2, 26 ± 9 minutes; K, 2.76 ± 0.91%/min). Therefore, we suggest that the effect of TZ alone, TZA, or TZB on the IVGTT performed in Japanese black bears is minimal.

The longer t1/2, slower K, and higher AUC glucose in the TZM group suggested a reduction in glucose tolerance, compared with tolerance in the other groups. The insulin response to glucose injection in the TZM group was noticeably suppressed, particularly during the early phase of the IVGTT, as indicated by the noticeable decrease of AUC insulin from 10 minutes before to 10 minutes after glucose injection. In dogs, the suppression of insulin secretion by medetomidine is sustained for approximately 2 to 3 hours. In the present study, serum insulin concentration increased gradually from 30 minutes after glucose injection (approx 3 hours after medetomidine administration). The suppression of insulin secretion by medetomidine may be sustained for approximately 3 hours in bears as occurs in dogs.

In the present study, induction and maintenance of anesthesia with TZ, with and without premedications, were safe and effective in Japanese black bears, with little adverse effects on cardiopulmonary function. However, the hypothermia that results from anesthesia needs to be addressed when bears are anesthetized in cold environments. Administration of TZ alone, TZA, or TZB appeared to have little effects on baseline values of serum or plasma biochemical variables and the results of IVGTTs. We therefore conclude that these 3 combinations can be used for chemical immobilization to allow performance of IVGTTs in Japanese black bears. In contrast, medetomidine, which appeared to have hyperglycemic and hypoinsulinemic effects, is inappropriate as a premedication in bears when evaluating glucose metabolism controlled by insulin.

![Figure 2](image_url)

**Figure 2**—Mean ± SD plasma glucose (black circles) and serum insulin (white circles) concentrations before and after IV glucose injection (0.5 g/kg) in TZ-anesthetized bears (4/group) immobilized with TZ (9 mg/kg) alone (no premedication; A), acepromazine maleate (0.1 mg/kg) followed by TZ (6 mg/kg; B), butorphanol tartrate (0.3 mg/kg) followed by TZ (6 mg/kg; C), or medetomidine HCl (40 µg/kg) followed by TZ (3 mg/kg; D). The 0-minute time point represents the point of glucose injection during an IVGTT. Values with different letters are significantly (P < 0.05) different from the baseline value (mean of the 3 values measured before glucose injection but after continuous IV infusion of TZ began).

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a. Zoletil 100, Virbac Laboratory, Carros, France.
b. A.C.P. 10 Injection 100 ml, Delvet Pty Ltd, Asquith, NSW, Australia.
c. Vetorphale, Meiji Seika Kaisha Ltd, Tokyo, Japan.
e. SurfFlash I.V. Catheters, TERUMO Co, Tokyo, Japan.
f. SOLACET E Terumo Corp, Tokyo, Japan.
g. Naloxone hydrochloride IV injection 0.2 mg, Daiichi Sankyo Co Ltd, Tokyo, Japan.
References


