Comparison of the analgesic efficacy of oral ABT-116 administration with that of transmucosal buprenorphine administration in dogs

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Objective—To evaluate the analgesic efficacy of ABT-116, a transient receptor potential cation channel vanilloid subfamily V member 1 antagonist, and compare it with that of buprenorphine by measurement of mechanical and thermal nociceptive thresholds in dogs.

Animals—Six 7- to 8-month-old dogs (3 males and 3 females).

Procedures—In a crossover study design, all dogs received ABT-116 (30 mg/kg, PO) and buprenorphine (0.03 mg/kg, orotransmucosally), with each treatment separated by 1 week. Physiologic variables were recorded prior to and 1, 6, and 24 hours after drug administration. Thermal (thoracic) and mechanical (dorsolateral aspect of the radius [proximal] and dorsopalmar aspect of the forefoot [distal]) nociceptive thresholds were assessed prior to (baseline) and 15 minutes and 1, 2, 4, 6, 12, 18, and 24 hours after treatment.

Results—Buprenorphine administration resulted in higher overall thermal and proximal mechanical nociceptive thresholds, compared with ABT-116. Distal mechanical nociceptive thresholds after treatment were higher than baseline values for both treatments, but the magnitude of change was greater for buprenorphine at 1 hour after administration. Whereas HR and RR sporadically differed from baseline values after ABT-116 administration, rectal temperature increased from a baseline value of 39 ± 0.2°C (mean ± SD) to a peak of 40.6 ± 0.2°C at 6 hours.

Conclusions and Clinical Relevance—In dogs without inflammation or nerve injury, PO administration of ABT-116 did not consistently result in an increase in nociceptive thresholds. However, clinically relevant increases in rectal temperature were identified after ABT-116 administration. (*Am J Vet Res* 2012;73:476–481)

In recent years, TRPV1 has received considerable attention as a novel therapeutic target of pain. This receptor is believed to be a component of the pain pathway because it is reportedly activated in response to noxious thermal and chemical stimuli.[1,2] In vivo studies[3–5] have demonstrated that SC, IP, and PO administration of TRPV1 antagonists can alleviate and prevent neuropathic pain and thermal and mechanical hyperalgesia in rodents.

The TRPV1 is a calcium-permeable, nonselective cation channel that is expressed at sensory nerve terminals and in brain tissue, dorsal root ganglia, trigeminal ganglia, and some nonneuronal tissues, such as skin and urinary bladder tissue.[6,7] This receptor is also believed to function as a molecular integrator of several noxious stimuli, including capsaicin, heat, acid, anandamide, polyamines, and lipoxygenase by-products.[8–11] The activity of TRPV1 is modulated by inflammatory mediators such as bradykinin, extracellular ATP, prostaglandins, nerve growth factor, glutamate, and activated phospholipase C.[7,12–13]

Abbreviations

HR	Heart rate
RR	Respiratory rate
TRPV1	Transient receptor potential cation channel vanilloid subfamily V member 1

Various TRPV1 antagonists have been evaluated in experimental studies for their ability to block the actions of TRPV1 and provide analgesia. Examples include A-425619, A-784168, AMG8163, AMG9810, benzimidazole compound 7, N-(4-tert-butylphenyl)-4-(3-chloropyridin-2-yl)piperazine-1-carboxamide, capsazepine, JNJ-17203213, and JYL1421.[11] Most of these antagonists reverse pain-associated responses to mechanical and thermal stimuli in rodents with inflammatory conditions such as those associated with carrageenan- or complete Freund adjuvant–induced thermal or mechanical hyperalgesia at the plantar surface of the hind paw.[7,14] A major adverse effect of most TRPV1 an-

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tagonists in many animal species, including humans, is an increase in body temperature.[7,11,15–17] Although the mechanism of hyperthermia is unclear and still under investigation, it has been hypothesized that TRPV1 receptors are involved in thermoregulation.[18–20]

ABT-116 is a TRPV1 antagonist under evaluation as an analgesic in dogs. An experimental study[a] in rodents demonstrated a potent analgesic effect of ABT-116 in the treatment of pain attributable to osteoarthritis, acute inflammation, and bone cancer. The purpose of the study reported here was to evaluate the analgesic efficacy and identify any adverse effects of PO ABT-116 administration in dogs. We also sought to compare the analgesic efficacy of ABT-116 with that of buprenorphine.

Materials and Methods

Animals—Three male and 3 female 7- to 8 month-old healthy Walker hounds weighing between 16 and 23 kg were used. Dogs were housed individually; fresh water and commercial dry dog food were provided ad libitum. The dogs had daily interaction with study personnel for socialization. They were also familiarized with the nociceptive devices and the study environment for 1 week prior to the start of the study. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Colorado State University.

Experimental protocol—A balanced crossover design was used. A small meal (approx half a can[b]) was given on the morning of each trial, 1 to 2 hours before treatment, as recommended by the manufacturer of ABT-116.[a] Equal numbers of dogs were treated in 1 of 2 sequences: ABT-116 (30 mg/kg, PO) followed by buprenorphine liquid (0.03 mg/kg, orotransmucosally) and buprenorphine followed by ABT-116. Treatments were separated by a washout period of 1 week, which represented a minimum of 5 half-lives for each drug as recommended.[21] The half-life of ABT-116 in dogs administered the same oral dose as used in the present study is reportedly 16 hours[a]; the half-life of buprenorphine administered as in the present study is 5.5 hours.[22] The dose of ABT-116 was selected on the basis of the manufacturer's recommendation to achieve plasma concentrations with demonstrated efficacy in rodents.[a] The buprenorphine dose was selected on the basis of prior data[22] and clinical experience. Buprenorphine was selected as the comparison drug in part because of its measurable analgesic efficacy with mechanical nociceptive threshold testing in a previous study.[22] Evaluators were unaware of the treatments administered.

Dogs were observed for their behavior during undisturbed and interactive moments, and the presence of urine, feces, vomit, and saliva was recorded prior to (baseline) and 15 minutes and 1, 2, 4, 6, 12, 18, and 24 hours after drug administration. Venous blood samples were obtained for PCV and total protein concentration determination, and rectal temperature, HR, and RR were recorded prior to and 15 minutes (HR and RR only) and 1, 6, and 24 hours after drug administration. Blood samples were also collected at fixed time points for subsequent analysis of ABT-116 plasma concentrations (results not reported here). Heart rate was measured by means of femoral pulse palpation and RR by observation of thoracic excursions, both of which were measured over a 30-second interval. Rectal temperature was measured after all other behavioral and physiologic evaluations were performed to avoid the potential influence of thermometer insertion on these variables.

Assessment of analgesic efficacy—Analgesic efficacy was assessed by use of a thermal nociceptive threshold device and 2 mechanical nociceptive threshold devices. Dogs were familiarized with these devices for 1 week prior to the study, during which repeatability of the response time and the response characteristics of each dog were also recorded. All devices were calibrated on the morning of each trial, and the calibrations were checked twice daily. For the device used on the distal aspect of the forelimb, known weights were used for calibration.

For threshold measurement, the laterodorsal aspect of each dog's thorax was clipped of hair and the thermal threshold device,[c] which consisted of a thermal probe, was applied there. This device was used to measure skin temperature before drug administration (baseline) and was then remotely heated at a fixed rate. The highest temperature at which the dog first responded (eg, turned its head toward the stimulus or attempted to bite the device) was considered the threshold temperature. The difference between baseline skin and the threshold was calculated for each measurement.

One[d] of the 2 mechanical nociceptive threshold devices used in the study reported here was applied to the proximal aspect of the forelimb, specifically the dorsolateral aspect of the radius (proximal mechanical threshold), of each dog. The device consisted of a blunt-ended probe attached to a force sensor that could be remotely activated to increase force at a fixed rate. The threshold was recorded as the force at which the dog first responded. The second mechanical nociceptive threshold device[e] was a manually applied C-clamp equipped with a calibrated 1-cm^2 force transducer connected to an electronic recorder capable of recording the peak force or pressure at which the dog first responded. The clamp was used to apply force manually in a dorsopalmar manner just distal to the large foot pad over the metacarpal bones (distal nociceptive threshold). Values were subsequently converted from pounds per centimeter^2 to Newtons.

At each measurement time point, thermal and nociceptive thresholds were determined 2 to 3 times. Attempts were made to have 2 to 3 measurement values within 10% of each other, and the mean of these values was used in subsequent analyses.

To minimize tissue damage in the absence of a response to noxious stimulation, cutoffs of 60°C, 20 N, and 20 lb/cm^2 were set for devices used to determine the thermal, proximal mechanical, and distal mechanical nociceptive thresholds, respectively. All threshold measurements were obtained prior to (baseline) and 15 minutes and 1, 2, 4, 6, 12, 18, and 24 hours after drug administration, which was after behavioral and physiologic data had been obtained.

Statistical analysis—Data were summarized as mean ± SD by use of statistical software.[f] An ANCOVA and

ANOVA with repeated-measures were used. The repeated-measures factor was time, and the between-subject factor was treatment identity (ABT-116 vs buprenorphine). Baseline data were included as a covariate in each ANCOVA, which was performed to compare the overall adjusted posttreatment effects, interaction, and change over time between the 2 drugs. Because baseline data were handled as a covariate in the ANCOVA, comparisons between baseline data and data at the other time points were analyzed by use of ANOVA. Pairwise comparisons between treatments at each time point and between times for each treatment were examined by use of *t* tests. Residuals from the ANCOVA and ANOVA were evaluated and confirmed to be approximately normally distributed and independent. Values of $P < 0.05$ were considered significant for all analyses.

Results

Animals—No differences were evident between treatments for any baseline observations (behavior and activity, PCV, blood total protein concentration, rectal temperature, HR, RR, and thermal, proximal mechanical, and distal mechanical thresholds) for the 6 dogs evaluated. Five dogs had diarrhea between 2 and 4 hours after ABT-116 administration, and one of these dogs also developed transient vomiting 2 hours after ABT-116 administration. Salivation (drooling) and sedation of variable duration and magnitude were observed when dogs received buprenorphine.

Table 1—Mean ± SD values of physiologic variables in 3 male and 3 female 7- to 8-month-old healthy Walker hounds after administration of ABT-116 (30 mg/kg, PO) and buprenorphine (0.03 mg/kg, orotransmucosally).

Variable	ABT-116	Buprenorphine
PCV (%)		
Baseline	46.2 ± 2.6	47.5 ± 0.5
1 h	46.8 ± 3.4	47.8 ± 1.3
6 h	44 ± 1.9*	45.8 ± 1.2*
24 h	42.2 ± 2.2*	43.2 ± 2.2*
Total protein (g/dL)		
Baseline	6.0 ± 0.3	6.1 ± 0.1
1 h	6.2 ± 0.3	6.1 ± 0.2
6 h	5.8 ± 0.2	5.9 ± 0.4
24 h	5.9 ± 0.2	5.9 ± 0.2
HR (beats/min)		
Baseline	115 ± 12	117 ± 9
1 h	109 ± 10	105 ± 13
6 h	120 ± 14	95 ± 13*†
24 h	116 ± 5	109 ± 13
RR (breaths/min)		
Baseline	27 ± 3	27 ± 4
1 h	19 ± 2*	19 ± 4*
6 h	22 ± 3	19 ± 4*
24 h	22 ± 6*	19 ± 4*
Rectal temperature (°C)		
Baseline	39.0 ± 0.3	39.0 ± 0.2
1 h	40.1 ± 0.6*	38.8 ± 0.6†
6 h	40.6 ± 0.2*	38.5 ± 0.6†
24 h	40.0 ± 0.4*	39.0 ± 0.2†

*Value differs significantly ($P < 0.05$) from the respective baseline value. †Value differs significantly ($P < 0.05$) between treatments at indicated time point.

Physiologic effects of treatment—Dogs did not differ significantly in RR, PCV, and blood total pro-

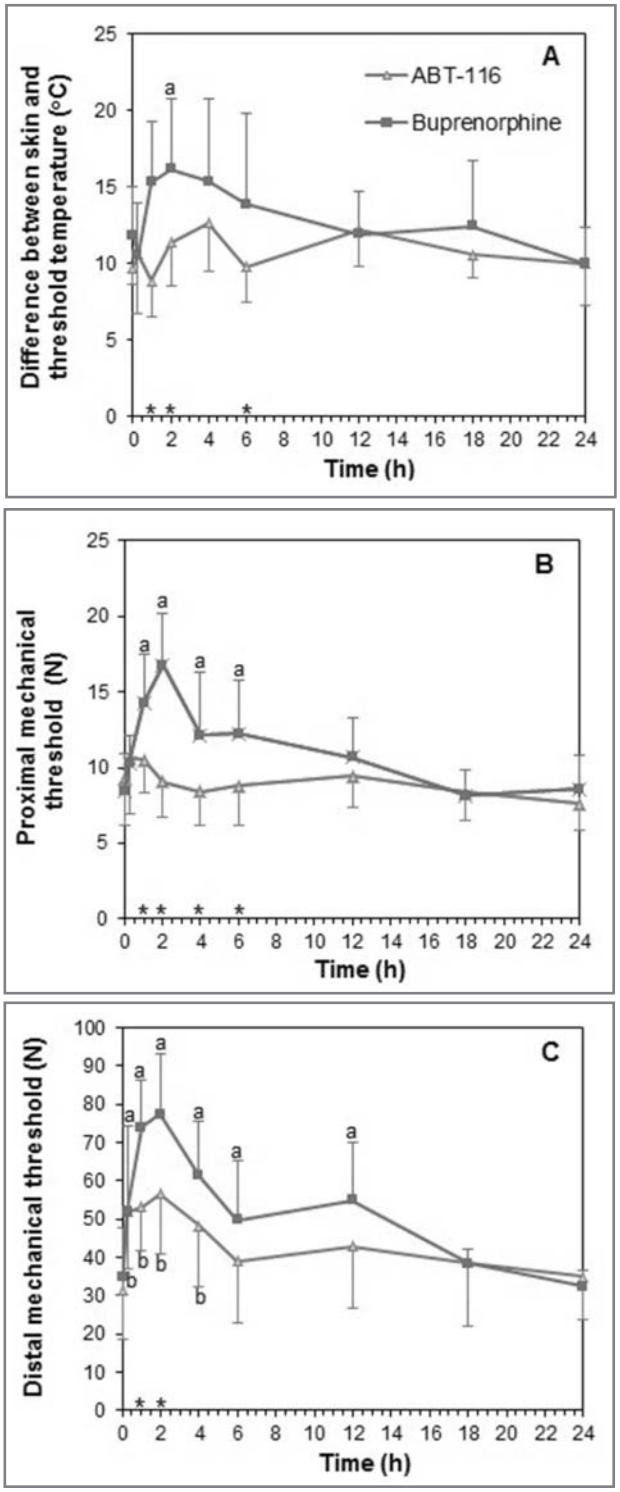

Figure 1—Mean ± SD values for thermal (A), proximal mechanical (B), and distal mechanical (C) nociceptive thresholds in 3 male and 3 female 7- to 8-month-old healthy Walker hounds after administration of ABT-116 (30 mg/kg, PO) or buprenorphine liquid (0.03 mg/kg, orotransmucosally). Time 0 on the x-axis represents values measured at baseline prior to drug administration. [a,b]Values with different letters differ significantly ($P < 0.05$) from the corresponding baseline value. *Value differs significantly ($P < 0.05$) between treatment groups at the indicated time point.

tein concentration after ABT-116 and buprenorphine treatments. However, PCV was significantly lower than baseline values 24 hours after buprenorphine ($P < 0.001$) and ABT-116 ($P < 0.001$) were administered. The RR was significantly lower than baseline values at 1 ($P = 0.001$), 6 ($P = 0.002$), and 24 ($P = 0.002$) hours after buprenorphine treatment and 1 ($P = 0.001$) and 24 ($P = 0.042$) hours after ABT-116 treatment (**Table 1**).

Relative to baseline data, significantly higher overall values for HR ($P = 0.033$) and rectal temperature ($P < 0.001$) were recorded for dogs after treatment with ABT-116, compared with the values after buprenorphine treatment. Heart rate was lower than the baseline value at 6 hours after buprenorphine administration ($P = 0.001$). After ABT-116 treatment, dogs had a significantly ($P < 0.001$) higher rectal temperature at 1, 6, and 24 hours, compared with the value before treatment.

Behavioral responses—Responses to the measuring devices differed by individual dog. Some turned their head toward the stimulus and, in some situations, attempted to bite the device, whereas others attempted to move away from the stimulus or had a skin twitch (thermal) or forelimb lift (mechanical).

Nociceptive thresholds—Higher overall thresholds were evident for buprenorphine versus ABT-116 with the thermal ($P = 0.035$) and proximal mechanical ($P = 0.017$) devices. The proximal nociceptive thresholds did not differ significantly from baseline at any point after ABT-116 treatment, whereas these values were higher than the baseline value at 1 ($P < 0.001$), 2 ($P < 0.001$), 4 ($P = 0.023$), and 6 ($P = 0.019$) hours after buprenorphine treatment. No differences in thermal thresholds were detected after ABT-116 treatment, whereas after buprenorphine administration, the thermal threshold at 2 hours was significantly ($P = 0.025$) higher than that at baseline (**Figure 1**).

In contrast, when pressure was applied to the forepaw, no significant differences were observed in the overall response between treatments. However, the time course of the response differed. In the ABT-116 group, distal mechanical nociceptive thresholds were higher than baseline values at 15 minutes ($P < 0.001$) and 1 ($P = 0.001$), 2 ($P < 0.001$), and 4 ($P = 0.022$) hours. After buprenorphine treatment, increases in this threshold were observed at every time point throughout the 12-hour measurement (Figure 1).

Discussion

The purpose of the present study was to evaluate the analgesic efficacy and adverse effects of ABT-116 and compare them with those of buprenorphine in dogs by use of 3 nociceptive testing modalities. Clinically relevant behavioral and physiologic observations were also recorded. Given the doses and testing methods used in the study, buprenorphine provided superior analgesia to that of ABT-116 at all time points when nociceptive thresholds were measured. Other than the changes in the mechanical nociceptive threshold of the distal aspect of the forelimb that persisted for 4 hours after ABT-116 administration, a change in nociceptive thresholds was not demonstrated after ABT-116 administration. This is in contrast to manufacturer-reported efficacy of this compound following testing in rodents in various models of inflammatory pain and might be explained by differences in test species or might be a result of the model itself.

The dose of ABT-116 used in the study was selected to yield plasma concentrations equivalent to those shown to be efficacious in rodents.[a] Plasma ABT-116 concentrations measured in the study dogs after a dose of 30 mg of ABT-116/kg was administered were in the reported target range for rodents, but we were unable to conclusively demonstrate analgesic efficacy. Because a dose-response assessment was not performed, one cannot determine whether a 30 mg/kg dose achieves an analgesic plasma concentration in dogs. However, evidence of adequate drug absorption was supported by other observations. For example, rectal temperature increased after ABT-116 administration in the present study, as has been reported for effective doses of ABT-116 in rats.[a]

Although it is possible that the drug has concentration-dependent effects, we postulate that it is more likely that drug actions differ between species, as has been shown in previous studies. For example, when the TRPV1 antagonist capsazepine was evaluated for its antinociceptive effects, guinea pigs responded favorably but rats and mice did not.[3] Another indicator that drug actions may differ among species is the differences in the magnitude of body temperature changes across species. In the present study, a significant increase in rectal temperature from baseline (39°C) was recorded after ABT-116 administration, with the highest individual temperature reaching 41.1°C. This was observed 1 hour after drug administration, and the highest mean (all dogs) rectal temperature was 40.6°C at 6 hours. Conversely, the drug manufacturer reports a maximum temperature increase of 1°C in conscious rodents receiving analgesic doses of ABT-116.[a] Other studies[15–17] found temperature increases after TRPV1 antagonist administration in rats, dogs, and monkeys, but the magnitude of the temperature change differed.

Another explanation for the differences in observed analgesic efficacy might be related to the model used to test efficacy. In the present study, oral ABT-116 administration did not prevent the response to a short-term noxious thermal stimulus. These findings are in agreement with reported results for the TRPV1 antagonists A-425619, SB705498, N-(4-tert-butylphenyl)-4-(3-chloropyridin-2-yl)piperazine-1-carboxamide, and AMG9810, which similarly do not have a significant effect on short-term thermal pain in rats.[14,23] However, these findings are in contrast to other TRPV1 antagonist studies[3–7,14–16] in rodents in which, through the use of an inflammatory or neuropathic pain model, antinociceptive effects of TRPV1 antagonists in response to thermal noxious stimuli were achieved.

A component of inflammation appears to be important in a model designed to evaluate the effects of TRPV1 antagonists. This is not surprising given that the TRPV1 receptor is sensitized by inflammatory mediators such as bradykinin, substance P, nerve growth factor, and prostaglandin E_2.[24–30] These mediators may activate TRPV1 directly (bradykinin) or activate protein kinases A and C, which can phosphorylate and

thus sensitize the receptor.[12,13,30–36] Had the analgesic efficacy of ABT-116 been evaluated by use of an inflammatory model in the present study, efficacy may have been detected in response to thermal threshold testing.

Despite the lack of efficacy of ABT-116 in response to thermal stimulation, we were able to demonstrate an analgesic effect, albeit short-lived, with the mechanical threshold test of the distal aspect of the forelimb in dogs. The reasons for this finding remain unclear, particularly considering the response differed from responses during the mechanical threshold test of the proximal aspect. Given the unequal duration of the threshold increase even in the buprenorphine group between 2 mechanical threshold tests (between 1 and 6 hours for the proximal test and between 15 minutes and 12 hours for the distal test), it is likely the sensitivity of the 2 tests differed. The device used for the proximal measurements has been used to demonstrate an analgesic effect of buprenorphine administration in cats[37] and the effect of butorphanol in cats and dogs.[38] Nonetheless, to our knowledge, the present study is the first in which the forelimb mechanical testing device was used in dogs that received buprenorphine; therefore, no data are available for comparison. However, results obtained by use of the distal mechanical nociceptive stimulus are similar to those reported for the same dose and route of buprenorphine administration in dogs; in that study, [22] evidence of analgesia was observed between 15 minutes and 8 hours after drug administration. Whether there is an inflammatory component associated with application of this device is unclear.

Findings of previous studies[39–41] suggest that TRPV1 receptors are not involved in the mechanosensory pathway in mice, whereas TRPV4 receptors are. However, ABT-116 has no activity at TRPV4[a] in that species; therefore, that mechanism cannot be considered as an explanation of the antinociceptive response to the mechanical noxious stimulus for the drug.

Adverse effects were observed after administration of both drugs in the present study. Sedation and salivation (drooling) were of similar magnitude and duration to what was reported after this dose of buprenorphine was orotransmucosally administered to dogs in another study.[22] Packed cell volume decreases in both groups in our study were likely due to blood sample collection and administration of heparinized saline (0.9% NaCl) solution to flush the catheter after sample collection for PCV and total protein concentration measurements; additionally, dogs were provided water during the study. Adverse effects after ABT-116 were primarily related to rectal temperature and to the gastrointestinal tract (diarrhea). These adverse effects had not been previously reported for either drug. Although no direct evidence exists, it is plausible that the hyperthermia induced the gastrointestinal signs because a high body temperature can damage the cell membranes and open the tight junctions, causing an increase in gastrointestinal permeability and gastrointestinal barrier disruption.[42–44] An alternative explanation is that TRPV1 antagonists may interrupt usual gastroprotective function of the TRPV1 receptors found in gastric epithelial cells by disrupting the mucus layer that surrounds the gastric epithelial lining.[45–49] This in turn may result in irritation of the stomach and duodenal lining and cause transient diarrhea or vomiting.

At the dose evaluated in the present study, ABT-116 administration led to a transient increase in the mechanical nociceptive threshold at the distal aspect of the forelimb in dogs but had no effect on thermal nociceptive thresholds. Changes in nociceptive thresholds following buprenorphine were observed with all testing modalities used. Hyperthermia and adverse gastrointestinal effects were observed after ABT-116 administration. These results suggest that at a dose of 30 mg/kg, orally administered ABT-116 is unsuitable as a treatment for acute, noninflammatory pain in dogs. Additional studies are needed to assess this compound's analgesic efficacy when inflammation exists.

a. ABT-116, Abbott Animal Health, North Chicago, Ill: unpublished data, 2009.
b. Hill's Pet Nutrition Inc, Topeka, Kan.
c. Thermal threshold testing system serial TCM054, Topcat Metrology Ltd, Ely, Cambridgeshire, England.
d. Mechanical threshold testing system serial TCM052, Topcat Metrology Ltd, Ely, Cambridgeshire, England.
e. Self-built C-clamp, Colorado State University, Fort Collins, Colo.
f. SAS/STAT software, version 9.2, SAS Institute Inc, Cary, NC.

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