The effect of an intravenous injection of branched chain amino acids on body temperature of cats undergoing general anesthesia

by

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Abstract

Perioperative hypothermia is a common complication in anesthetized cats. Nutrient induced thermogenesis (NIT) with intravenous administration of amino acids (AAs) is a technique used to increase endogenous heat production, which attenuates heat loss during general anesthesia. Branched chain amino acids (BCAAs) may be more effective than other AAs for NIT. The hypothesis tested was that cats receiving a single intravenous injection of BCAAs at 100 or 200 mg/kg prior to general anesthesia will have a body temperature at least 0.5℃ higher than cats receiving an injection of lactated ringer's solution (LRS). Ten research cats underwent general anesthesia three times with three different treatments; 3 ml/kg LRS, 100mg/kg BCAA (B100), or 200mg/kg BCAA (B200) solution immediately prior to induction of anesthesia. After induction, rectal and thoracic skin temperature were measured every 5 and 15 minutes with a temperature probe and thermograms, respectively. Blood samples were collected for the measurement of blood urea nitrogen (BUN), creatinine (Cre), glucose (BG) and plasma insulin (insulin) concentrations just prior to induction, at the end of the 90 minute period of anesthesia, and 24 hours after anesthesia induction. The differences between baseline and each time point on rectal (ΔTr) and thoracic skin (ΔTt) temperature during anesthesia were calculated. The trapezoid method was used for the calculation of AUC for ΔTr and ΔTt. The differences between baseline and each sampling point for BUN (ΔBUN), Cre (ΔCre), BG (ΔBG) and insulin (Δinsulin) were calculated. The normality of data was tested by D'Agositno-Pearson test. Parametric or non-parametric data were analyzed by one-way

repeated measures ANOVA with Tukey post-hoc test or Friedman test and Dunn's post hoc test. A value of $p < 0.05$ was set for significance.

There was no significant difference in AUC of ΔTr and ΔTt during anesthesia (p = 0.3675 and 0.9737, respectively). ΔBUN, ΔCre, ΔBG and Δinsulin did not differ between groups for any time points. However, the incidence of hypoglycemia after anesthesia tended to be higher in both B100 and B200 groups than those of LRS group. BCAAs-NIT did not reduce heat loss during anesthesia while likely to increase the risk of perioperative hypoglycemia in cats.

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List of Abbreviations

%	percentage
°C	Degree Celsius
Δ BUN	difference between each time point – baseline blood urea nitrogen
Δ Cre	difference between each time point – baseline blood creatinine
Δ Glu	difference between each time point – baseline blood glucose
Δ insulin	difference between each time point – baseline plasma insulin
ΔTr	difference between each time point – baseline rectal temperature
ΔTt	difference between each time point – baseline thoracic skin temperature
4EBP-1	Eukaryotic translation initiation factor 4E - binding protein 1
AAs	amino acids
AUC	area under curve
B100	branched chain amino acids 100 mg/kg group
B200	branched chain amino acids 200 mg/kg group
BCAAs	branched chain amino acids
BG	blood glucose
BUN	blood urea nitrogen
cm	centimeter
Cre	creatinine
CRI	constant rate infusion
CT	computed tomography
$ETCO_2$	end tidal carbon dioxide

ETiso	end tidal isoflurane
HR	heart rate
IM	intramuscular
insulin	plasma insulin
IPH	inadvertent perioperative hypothermia
IV	intravenous
IRS-1	insulin receptor substrate
g	gram
LRS	lactated ringer's solution
max	maximum
mg/kg	milligram/kilogram
min	minimum
ml	milliliter
ml/kg	milliliter/kilogram
ml/kg/hr	milliliter/kilogram/hour
mmHg	millimeter of mercury
n	number
NIT	nutrient induced thermogenesis
MAC	minimum alveolar concentration
MAP	mean arterial blood pressure
MRI	magnetic resonance imaging
mTOR	mammalian target of rapamycin
PKB	protein kinase b

RHEB ras homolog enriched brain

RT rectal temperature

RR respiratory rate

S6K1 ribosomal protein s6 kinase beta-1

SD standard deviation

SSI surgical site infection

SYS systolic arterial blood pressure

vWF von Willebrand factor

1. Introduction

Inadvertent perioperative hypothermia (IPH) is a common anesthetic complication in cats. More than 95% of cats become hypothermic during general anesthesia, when hypothermia is defined as body temperature < 37°C (Redondo *et al.* 2012a). Several adverse events that may affect patient outcome are associated with hypothermia, including an increase in surgical wound infection rate, delayed wound healing, arrhythmia, coagulopathy, increased recovery time from anesthesia, and longer hospital stays (Flores-Maldonado *et al.* 2001; Pietsch *et al.* 2007; Rajagopalan *et al.* 2008; Poucke *et al.* 2014; Yi *et al.* 2017; Sakata *et al.* 2020). In fact, minimizing loss of body heat by as little of 0.5 °C has demonstrated considerable benefits such as a decrease in intraoperative bleeding, lower infection rates and decreased shivering (Widman *et al.* 2002; Wu *et al.* 2015; Fujita *et al.* 2016) in people. Similarly, an improvement in body temperature of only 0.63°C results in a decrease in shivering and shortened recovery time from general anesthesia in dogs (Clark-Price *et al.* 2018). Thus, minimization of IPH will likely improve the perioperative care and outcome of cats undergoing anesthesia and surgery.

To date, several body heat loss reduction methods and systems have been investigated and used in clinical settings (Hale and Anthony 1997; Rigotti *et al.* 2015; Aarnes *et al.* 2017; Jourdan *et al.* 2017; Khenissi *et al.* 2017; Sakata *et al.* 2020). Only combination of a forced air blanket or electric warm pad and insulation layers decreased heat loss in cats

during general anesthesia (Hale and Anthony 1997; Sakata *et al.* 2020). Despite the effectiveness of these techniques, there are several limitations for use in routine clinical settings. Body contact area with heating devices is usually limited due to potential conflict with the surgical site and likely decreases the contribution of these devices to heat loss reduction (e.g. thoracic or abdominal surgery). The potential for surgical site contamination might be increased by the use of forced warm air blanket due to blowing contaminants from the floor onto the surgical field (Ackermann *et al.* 2018). For patients undergoing MRI or CT image acquisition, devices with ferrous metals will interact with magnetic fields, and blankets or materials that cover the patients may cause artifacts on the images and reduce diagnostic quality. Therefore, alternative methods that minimize IPH that can be used in any environment and do not interfere with surgical site are greatly needed.

Nutrient-induced thermogenesis (NIT) has been studied as a method to attenuate heat loss during anesthesia as an alternative warming method (Clark-Price *et al.* 2015, 2018; Wu *et al.* 2015; Takashima *et al.* 2016). The administration of amino acids (AAs) accelerated heat production in several species during general anesthesia. The mechanism of heat production is thought to occur through the stimulation of skeletal muscle protein synthesis via phosphorylation of insulin mTOR dependent translation factors 4EBP-1 and S6K1 (Yamaoka *et al.* 2006; Yamaoka 2008). AAs-NIT confers beneficial effects on anesthetized humans and animals, including shortening the recovery time from general anesthesia and decreasing the incidence of shivering. Of the different amino acids, the branched chain amino acids (BCAAs) leucine, isoleucine and valine likely play a dominant role in AAs-NIT. BCAAs appear to have a stronger anabolic effect on protein

synthesis compared to other amino acids thorough the activation of mTOR (Columbus *et al.* 2014). In a previous study in rats investigating heat production, an AAs solution without BCAAs had a significantly lower ability to increase body temperature than an AAs solution with BCAAs (Yamaoka *et al.* 2006). Thus, administration of a solution of BCAAs may be of greater benefit for minimization of hypothermia than other AAs solutions.

In previous studies using AAs-NIT, anesthetized dogs were delivered AA via a precision syringe pump by constant rate infusion (Takashima *et al.* 2016; Clark-Price *et al.* 2018). However, this technique requires an electronic precision syringe pump and monitoring of the infusion. Syringe pumps can be costly to purchase and may not be practical in some practice situations or environments (e.g. MRI). A method that delivers AAs as a single injection would greatly reduce the cost and simplify use. The objective of this study was to evaluate the effect of a single intravenous injection of BCAAs on heat loss during general anesthesia in healthy cats.

2. Literature Review

2.1. Basis of Thermoregulation

In homeotherms, body temperature is regulated within a narrow range for the optimization of metabolic activities by the hypothalamus (Kurz 2008). The anterior hypothalamic-preoptic area receives the afferent signals from peripheral thermoreceptors, and the signal is transferred to the posterior hypothalamic area (Boulant 2000). The signals are integrated in the posterior hypothalamus which maintains body temperature within a narrow ranges by mediating changes in vascular tone and metabolism (Kurz 2008; Díaz and Becker 2010). Afferent signals from peripheral receptors responding to a cold environment or decrease in body temperature are transmitted via A-delta nerve fibers to the hypothalamus (Bindu *et al.* 2017). The input is compared with the intrinsic threshold of the temperature settings at the thermoregulatory center in the hypothalamus (Bindu *et al.* 2017). The body will increase heat production and heat conservation activities when the input temperature is below the set threshold temperature.

The body temperature is divided into core and peripheral compartments by vascular tone (Grimm 2018). The blood flow in the peripheral compartment is exposed to the external environment and exchanges its heat by the existence of the temperature gradient between the peripheral tissue and externals (Díaz and Becker 2010). Core blood flow does not

communicate with the external environment (Díaz and Becker 2010). In a cold environment, peripheral vessels are constricted to minimize heat loss (Kurz 2008). Metabolically, the major part of thermogenesis depends on shivering and metabolism by muscle and the liver. Those metabolic responses are also activated by the efferent output from hypothalamus. However, general anesthesia depresses hypothalamus activities, leading to an increase in the threshold for hypothermia and obtunded reactions such as vasoconstriction and shivering (Díaz and Becker 2010).

2.2. Pathophysiology of IPH

IPH is a common anesthesia-related complication in small animals. The incidence of IPH, when defined as < 37℃, has been reported to be 85.6% and 96% in dogs and cats respectively (Redondo *et al.* 2012a, 2012b). In a recent study, 89% of dogs undergoing general anesthesia had a decrease in their perioperative body temperature as compared with their temperature immediately before or after anesthesia (Clark-Price *et al.* 2021). There are four major mechanisms through which body heat energy is transferred from the animal to the environment: radiation, conduction, convection, and evaporation (Grimm 2018). Heat loss through radiation is emission of heat in the form of infrared rays between surfaces. The magnitude of heat loss by radiation does not depend on the difference of heat between the surfaces of ambient temperature and patients, but depends on the emissivity of skin (Sessler and Todd 2000). In people, radiation has been considered as the major factor of heat loss during surgery (Sessler and Todd 2000). Heat loss via conduction and convection share a similar mechanism that heat energy is transferred from warmer to cooler sites. Conduction is heat transferred to the surrounding

environment via direct contact of the body with objects such as a surgical table. The difference of temperature between two adjacent surfaces proportionally changes the rate of heat transfer via conduction (Grimm 2018). Air flow surrounding patients entrains body heat from the body through convection, and the main determinant of heat loss is velocity of the air flow (Grimm 2018). Evaporation is heat loss thorough changing the form of body water to vapor or gas and releasing it into air. When vapor or gas leave the body, it accompanies heat energy to the external environment from the body as evaporation. Interaction of those four primary mechanisms combine to cause hypothermia during anesthesia.

In humans, body heat decreases during general anesthesia in 3 predictable phases. In the first phase (first 30 minutes), general anesthesia causes a rapid redistribution of body heat from a central compartment to peripheral tissues (Sessler and Todd 2000). Core body temperature is normally 2-4 ℃ higher than peripheral tissue temperature. The autonomic nerve systems regulate this temperature gradient. Anesthetic agents change the balance of the autonomic nervous systems resulting in peripheral vasodilation. This, in turn, causes a dramatic redistribution of body heat from the core to the periphery. The rate of temperature decline decreases in the second phase. This is because the major cause of heat loss converts from core to skin blood redistribution to environmental heat loss. Heat loss continues to exceed heat production during the second phase because heat generating mechanisms (increased metabolism and shivering) are suppressed by anesthesia. The second phase lasts up to 75 minutes after induction of general anesthesia. In the third phase, temperature decrease reaches a pseudo-equilibrium where heat production matches heat loss. However, the patient remains hypothermic overall.

Small animals such as cats have a relatively high body surface area to mass ratio (Hill and Scott 2004). As the size of the patients become smaller, the increase in the body surface area to mass ratio accelerates heat loss through conduction, convection, radiation (Koop and Tadi 2021). This characteristic possibly makes cats more prone to hypothermia during anesthesia compared to larger animals.

2.3. Clinical Importance for the Prevention of IPH

Several adverse events associated with IPH have been reported. Hypothermia is considered a risk factor of surgical site infection (SSI) and delayed wound healing (Flores-Maldonado *et al.* 2001; Sessler 2001; Pietsch *et al.* 2007). The postoperative SSI rate increases threefold as preanesthetic temperature difference reaches 1.9℃ between normothermic and hypothermic groups (Kurz *et al.* 1996). It is postulated that hypothermia induces multiple dysfunctions in immune function and in the coagulation cascade. Hypothermia may impair several functions of neutrophils, including migration to an infection site, phagocytosis, and oxidative killing of microbes. In an in-vitro study, those activities of neutrophils were decreased as body temperature dropped to 29℃ (Akriotis and Biggar 1985). Furthermore, a disturbance of platelet function and activity of coagulation factors were demonstrated during hypothermia, which can also increase the risk of wound dehiscence and infection after surgery (Pietsch *et al.* 2007). More specifically, the inhibition of release of thromboxane A_2 from activated platelets and down-regulation of glycoprotein Ib-IX complexes occurs in the hypothermic state (Michelson *et al.* 1994). Thromboxane A2 activates other platelets and promotes aggregation and glycoprotein Ib-IX complexes form a crosslink between vWF and

platelets. Disruption of coagulation cascade also increases the incidence of perioperative transfusion and coagulopathy (Widman *et al.* 2002; Rajagopalan *et al.* 2008).

Other complications associated with hypothermia include increased risk of perioperative arrythmia, especially atrial fibrillation, atrioventricular block, and ventricular fibrillation (Somerville 1960; Gurabi *et al.* 2018; Dietrichs *et al.* 2020). The etiology of hypothermia-induced arrhythmia has been speculated to be due to prolongation of conductance velocity, blocking of electrical pathways or re-entry in ventricular muscle (Dietrichs *et al.* 2020). Finally, postanesthetic shivering by hypothermia increases intracranial and intraocular pressures and oxygen consumption rate by 100 – 200% during anesthetic recovery (Ralley *et al.* 1988).

Minimizing the loss of body temperature by as little of 0.5 °C has considerable benefits to anesthetized humans (Widman *et al.* 2002; Wu *et al.* 2015; Fujita *et al.* 2016). A decrease of body temperature of 0.5℃ during surgery increases surgical site infection rate from 14.2% to 30.0% in patients undergoing esophagostomy (Fujita *et al.* 2016). Marianne et al. showed that a small difference in temperature, less than 0.5℃ between aggressive warming and normal warming groups, reduces surgical blood loss by 200 ml (Winkler *et al.* 2000). Also, hospitalization time is shortened significantly with small improvements in body temperature during anesthesia (Aoki *et al.* 2017). In a study in dogs, a difference of only 0.63 °C between treatment and control groups resulted in shortened recovery time from anesthesia and decreased shivering (Clark-Price *et al.* 2018). Thus, the reduction in the degree of IPH in cats would likely be of great benefit to feline medical care.

2.4. Conventional Patients Warming Methods and Devices

Several warming methods and systems have been investigated to prevent perioperative hypothermia in various species (Hale and Anthony 1997; Rigotti *et al.* 2015; Takashima *et al.* 2016; Aarnes *et al.* 2017; Khenissi *et al.* 2017; Clark-Price *et al.* 2018; Sakata *et al.* 2020). Pre-warming methods, administration of intravenous heated fluids, and the use of heat and moisture exchangers do not attenuate perioperative hypothermia in dogs and cats (Aarnes *et al.* 2017; Jourdan *et al.* 2017; Khenissi *et al.* 2017). The use of warm pads (heated water pad and an electric warm pad) and forced air blanket improved perioperative hypothermia in dogs (Clark-Price *et al.* 2016). Those devices attenuate heat loss through reduction of conduction and convection by the supplementation of external heat to patients reducing the temperature gradients. A combination of an electric warm pad and insulation layers has a minimal effect on the prevention of hypothermia in anesthetized cats undergoing oral hygiene procedure (Hale and Anthony 1997). Although effective in some cases, these warming devices have several limitations for use in routine clinical settings. Body contact area with heating devices may be limited by surgical site, especially in thoracic or abdominal surgery. Forced warm air blanket may increase surgical site infection by blowing contaminants onto the surgical site (Ackermann *et al.* 2018). Equipment with ferrous metal components cannot be used in an MRI suite due to the potential for interaction with the magnetic field. During other imaging studies such as computerized tomography (CT), blankets or materials that cover the patients may produce an image artifact and interfere with image acquisition and quality of the diagnostic modality. Other limitations include need for special devices, costs, maintenance and replacement blankets and water pads. Thus, alternative methods that can

be used in any environment, do not interfere with the surgical site, and are cost effective are greatly needed.

2.5. Nutrient-Induced Thermogenesis (NIT)

2.5.1 The mechanisms and clinical effectiveness of Amino acids (AAs)-NIT

Nutrient-induced thermogenesis (NIT), a metabolic technique that increases resting energy expenditure and produces additional body heat, has been more recently described (Carlson 1997). The magnitude of heat production varies according to the composition of the diet, administration route and rate of nutrients. Previous studies have confirmed that proteins and amino acids (AAs) have a higher thermic effect than carbohydrates and fats. The energy expenditure to energy contents ratio for proteins and AAs (30-40%) is greater than those for carbohydrate and glucose (5-10%), and fat (0-3%) (Carlson 1997). The mechanism of heat production associated with AAs is through two different pathways in skeletal muscle (Fujita *et al.* 2006; Yamaoka *et al.* 2006; Suryawan and Davis 2018). In one pathway, AAs are transported into skeletal muscle cells through amino acid transporters, which activate amino acid sensing components [e.g. Ras homolog enriched brain (RHEB)] in lysosomes (Suryawan and Davis 2018). The activated sensing components mobilize mammalian target of rapamycin (mTOR) complex1 (mTOR1) to the lysosome, thereby binding RHEB to mTOR and resulting in activation of mTOR1. Activated mTOR1 leads to enhance phosphorylation of insulin-mTOR-dependent translation factors 4E-BP1 and S6K1. This increases transcription of skeletal muscle protein synthesis, and heat is generated as a byproduct of the protein synthesis (Yamaoka

et al. 2006). In the second pathway, AAs stimulate insulin secretion from pancreatic islets (Fujita *et al.* 2006; Suryawan and Davis 2018). Released insulin binds to the insulin receptor on the surface of skeletal muscle cells and stimulates tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1). Tyrosine phosphorylate IRS-1 activates protein kinase B (PKB) and mTOR1, increasing the uptake of AAs and muscle synthesis in the skeletal muscle. The insulin release induced by AAs has a synergetic effect for heat production for AAs-NIT. In awake animals, thermosensors in the brain manage increases in body temperature by oxidative metabolism and heat production through NIT, in order to prevent hyperthermia (Selldén *et al.* 1994). General anesthesia obtunds these sensors in the brain and abolishes the efferent signals for the control of body temperature (Sessler 2008). In awake people with cervical spinal injury, intravenous administration of AAs increases body temperature more than in a control group (Aksnes *et al.* 1995). Central temperature regulation was uncoordinated in the spinal injury group due to interruption of the conduction pathway in the neck region, resulting in higher heat production than healthy people. This temperature regulation system can be eliminated to some extent in the anesthetized animals. Thus, thermic effects of NIT can occur unabated and attenuate heat loss during anesthesia, resulting in a better balance in heat loss to heat production. Administration of AAs has been shown to minimize heat loss in various species during general anesthesia and confers beneficial effects in anesthetized humans and animals (Selldén *et al.* 1994; Widman *et al.* 2002; Takashima *et al.* 2016; Aoki *et al.* 2017; Clark-Price *et al.* 2018). In fact, minimizing loss of temperature by as little of 0.5 °C has demonstrated considerable benefits to anesthetized humans with AAs-NIT (Aoki *et al.* 2017). These benefits include a decrease in intraoperative bleeding, infection rates and

shivering. In a study in dogs, a difference of only 0.63 °C between treatment of AAs-NIT and control groups resulted in a shortened recovery time from anesthesia and decreased shivering (Clark-Price *et al.* 2018). In terms of hemodynamic advantages, dogs receiving AAs-NIT treatment, regardless of the infusion rate of AAs-NIT, showed improvement of heart rate and mean arterial blood pressure compared with control dogs during isoflurane anesthesia (Takashima *et al.* 2016).

Potential disadvantageous effects of AAs-NIT should be considered for their utilization. The administration of AAs increases insulin secretion and reduces serum glucose concentration (Kuhara *et al.* 1991). Additionally, serum BUN may become elevated via more substrate entering the normal degradable pathway (ureagenesis) (Sellden and Lindahl 1998). In dogs, these effects are mild and temporal, and return to baseline concentrations within 24 hours (Clark-Price *et al.* 2018). However, evaluation of these effects have not been studied in cats.

2.5.2 Branched chain amino acids (BCAAs)

Branched chain amino acids (BCAAs), leucine, isoleucine, and valine, likely play a primary role in AAs-NIT (Yamaoka 2008). BCAAs appear to have a stronger anabolic effect on protein synthesis compared to other amino acids thorough the previously mentioned activation of mTOR (Blomstrand *et al.* 2006; Columbus *et al.* 2014). In support, the administration of BCAAs has been utilized in people to attenuate heat loss during anesthesia (Wu *et al.* 2015). The studies reported that BCAAs had the equivalent or slightly higher magnitude of thermogenesis and energy expenditure than those of other AAs. Furthermore, a previous study demonstrated that temperature in rats given an AAs

solution without BCAAs was significantly lower than rats receiving an AAs solution with BCAAs (Yamaoka 2008). Thus, BCAAs-NIT may be of greater benefit for minimization of hypothermia than other AAs-NIT.

2.5.3 The route and dose of BCAAs NIT

In previous studies evaluating AAs for perianesthetic hypothermia, anesthetized dogs were delivered AAs via a precision syringe pump by constant rate infusion (Clark-Price *et al.* 2015, 2018; Takashima *et al.* 2016). However, this technique requires an electronic, precision syringe pump and monitoring of the infusion. Syringe pumps can be costly to purchase and may not be practical in some practice situations or environments (e.g. MRI). A method that delivers AAs as a single injection would greatly reduce the cost and simplify use. This study will evaluate such a method.

A 10% AAs solution was administered to dogs at a rate of 8 mL/kg/hr and attenuated heat loss (Clark-Price *et al.* 2018). This infusion rate corresponded to a dosage of BCAAs of 196.8 mg/kg delivered over an hour. An effective dose as an infusion or as a single bolus dose in cats has not been determined. However, dietary protein requirements in cats are approximately twice that of dogs and thus cats may require a higher dosage of BCAAs to achieve a similar thermic effect (Legrand-Defretin 1994). Thus, in this study, we will evaluate two dosages of a single BCAAs injection (100 and 200 mg/kg) in cats for evaluation of effects on body temperature during anesthesia. This dosage will be administered as a single bolus over a period of 10 seconds instead of infused over an hour resulting in a higher plasma level of BCAAs and a greater delivery of BCAAs to the proposed site of action (skeletal muscle).

2.6. The Evaluation of Body Temperature

The distribution of body heat is not uniform throughout the body. Body temperature is roughly divided into peripheral and core temperature. Peripheral temperature is normally lower than core temperature by 2 – 4℃ and greatly influenced by ambient temperature and autonomic nerve tone while core temperature is consistently maintained (Sessler 2008). Therefore, core temperature measurement is usually recommended as an anesthetic monitoring tool for body temperature. The gold standard site to measure core body temperature is the pulmonary artery (Hayes *et al.* 1996). However, this requires invasive catheter placement within the pulmonary artery, which carries the risk of infection, arrythmia and cardiac irritation by the direct contact of the catheter with heart muscle. Instead, rectal temperature (RT) is utilized as the alternative method in clinical settings. RT has been validated with high agreement as a surrogate of core temperature even though there is a small temperature difference between them due to the influence of feces and air in the rectum (Southward *et al.* 2006). However, this difference can be minimized when the temperature probe is placed in the descending colon.

Thermography is the use of thermograms to study heat distribution in structures or regions and has been used as a research and diagnostic tool to measures body surface temperature in the field of anesthesiology in both human and veterinary medicine (Bruins 2018; Repac *et al.* 2020). It is a non-invasive method to visualize changes of skin temperature that correlates with heat production by increased metabolism of muscles (Adamson *et al.* 2008). For example, the skin temperature over the biceps femoris and gracilis muscles in dogs after 6 minutes of walking was significantly increased compared

with those of baseline skin temperature (Repac *et al.* 2020). BCAAs is thought to be predominantly metabolized in skeletal muscle, and thermography may be useful for detection of increased heat production by skeletal muscle after administration of BCAAs.

3. Objectives

1- Evaluate if an intravenous injection of BCAAs decreases heat loss during anesthesia without any adverse effects in healthy cats.

2- Determine if an intravenous injection of BCAAs improve hemodynamic parameters during anesthesia and recovery time from anesthesia in healthy cats.

4. Hypothesis

1. Healthy, anesthetized cats receiving a single intravenous injection of BCAAs will maintain a higher body temperature during anesthesia than control group cats.
2. Healthy, anesthetized cats receiving a single intravenous injection of BCAAs will have better hemodynamic parameter during general anesthesia and a shorter recovery time compared with control group cats.

5. Materials and Methods

5.1. Animals

Animals

This study was approved by the Auburn University Institutional Animal Care and Use Committee (Protocol # 2020-3718). A sample size of 10 cats per group was determined to be necessary to detect a difference between groups of 0.5 C with a sigma of 0.37 (Clark-Price et al. 2015), an alpha of 0.05, and power set to 0.80. Ten, university-owned, healthy, intact adult cats (5 female and 5 males), aged 3.3 ± 2.0 [mean ± SD] years of age, and weighing 3.5 ± 0.7 kg were enrolled in a triple cross over study. Each cat received each of three treatments, 3.0 ml/kg lactated ringer's solution (LRS), 100 mg/kg BCAA (B100), or 200 mg/kg BCAA (B200) in random order in a modified Latin-square design. Cats were considered health via no abnormalities on physical examination, complete blood count and serum chemistry at least 1 day prior to initiation of the study. Feed was withheld from the cats for 12 hours prior to the experiment, but access to water was ad libitum.

5.2. BCAAs solution

The BCAA solution was compounded no more than two weeks prior to use in the Auburn University College of Veterinary Medicine licensed institutional pharmacy following USP 797 guidelines for sterile compounding. Multiple attempts were made to create as concentrated a solution as possible. Through trial and error, a final solution that contained

65 mg/ml of BCAA was produced. The BCAA solution consisted of isoleucine 15 mg/ml, leucine 10 mg/ml, and valine 40 mg/ml.

5.3. Instrumentation

Cats were administered alfaxalone (4 mg/kg: Alfaxan® Multidose, Jurox inc, KS, USA) intramuscularly in the right femoral musculature and allowed to become sedated in a dedicated premedication/recovery cage. Once sedation was apparent (approximately 10 mintues), cats were carried into a dedicated, climate controlled study room and placed on a stainless steel table covered with a cotton bath towel. A 22-gage intravenous catheter (Terumo Surflo 22G × 1, Terumo, NJ, USA) was placed into the right or left cephalic vein after aseptic preparation. A calibrated, digital, scientific thermometer (Fischer Scientific™ Traceable Digital Thermometer, Fisher Scientific, NH, USA) was placed per rectum to a standardized distance of half the length from the base of the tail to the last rib of the individual cat. After the completion of the instrumentation, the assigned treatments, LRS, B100, or B200 was injected into the cat intravenously (IV) via the cephalic vein catheter over 10 seconds by the manual injection immediately before anesthesia induction. The investigator (HS) who recorded all variables and evaluated anesthetic depth and recovery time from general anesthesia was blinded to the treatment administered throughout the study.

5.4 General anesthesia induction and recovery

Immediately after BCAA injection, cats were induced with alfaxalone (2 mg/kg: Alfaxan® Multidose, Jurox Inc) IV, orotracheally intubated, and positioned in sternal

recumbency (Fig 1). General anesthesia was maintained with isoflurane (Isoflurane, Akorn, IL, USA) delivered in 100% oxygen with a rebreathing circuit for the use of pediatric patients (Pneupac® babyPAC, Smiths Medical Inc, MN, USA) for 90 minutes. The oxygen flow rate was fixed at 200 ml/kg/min throughout anesthesia for all cats. Cats were infused LRS (VetiVex, Dechra Pharmaceuticals, KS, USA) at 2 ml/kg/hr with a precision syringe pump (Medfusion® 3500, Smiths Medical Inc) that was confirmed for accuracy prior to beginning of the study. The rectal temperature recording was initiated immediately after anesthesia induction and then at 5 minutes interval during anesthesia and throughout the recovery period. The following physiological parameters were recorded at 5 minute intervals utilizing a multi-parameter monitor (Waveline Pro, Avante Animal Health, NC, USA) starting immediately after induction: heart rate (HR), respiratory rate (RR), hemoglobin oxygen saturation, indirect blood pressure, end-tidal partial pressure of carbon dioxide ($ETCO_2$) and end-tidal partial pressure of isoflurane (ET_{ISO}). Prior to the beginning of the study, accuracy of the gas analyzer was assessed with a standard calibration gas (Calibration Gas, Scott Medical Products, PA, USA). Isoflurane was adjusted to maintain adequate anesthetic depth and cardiovascular variables. Specifically, if either systolic or mean blood pressure was less than 90 or 60 mmHg, isoflurane concentration was decreased at approximately 0.2% increments ranging from the initial ET_{ISO} around 1.3% to 0.7% as the low-end to maintain normotension and prevent signs of inadequate anesthetic depth (blinking or strong palpebral reflex). Isoflurane administration was discontinued 90 minutes after anesthetic induction and cats were allowed to breathe room air during recovery.

Fig 1. The positioning of the cat during general anesthesia.

For recovery, cats were moved from sternal recumbency to right lateral recumbency and were extubated after the appearance of spontaneous swallowing or coughing motions without any external stimulation. Cats were then transferred to the premedication/recovery cage in the same recumbency on the bath towel and rectal temperature was recorded at 5 minute intervals for an additional 60 minutes. Recovery times, defined as the time from the end of isoflurane administration to extubation (Extubation), when the cats spontaneously moved to a sternal position and held their head up (Sternal), and when cats stood up or maintained sitting position (Standing) were also recorded.

5.5 Analysis for Images of Infrared Camera

The trunk of the cat was pictured using an infrared thermal imaging camera (Fluke TIX 580 Infrared Camera, Fluke Corporation, WA, USA) from a distance of approximately 60 cm above the cat to include the entire thoracic muscle in a thermographic image (Fig 2).

The images were taken every 15 minutes from immediately after anesthesia induction (base line: T0). The image was analyzed by camera specific software (SmartView, Fluke Corporation) to visualize skin temperature as color gradients. A region of interest was manually drawn and minimum, average, and maximum temperature were calculated (Fig 3).

Fig 2. Taking an image with the infrared camera for the assessment of skin temperature in the cat.

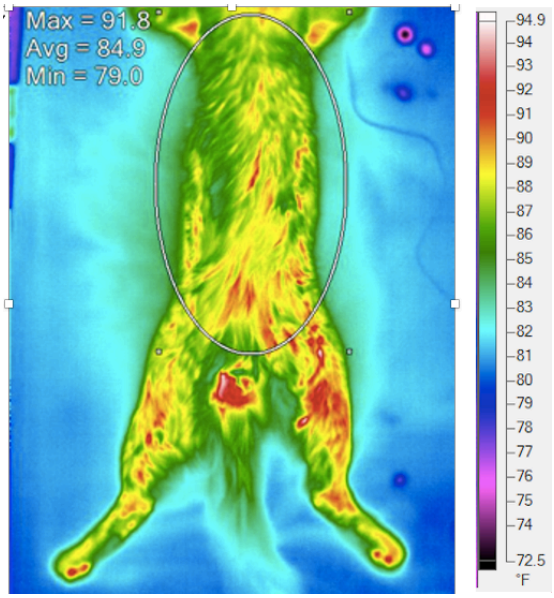


Fig 3. The thermal image of the trunk and maximum, minimum, and average body surface temperature (top on the left).

5.6 Measurement of Serum Insulin, Glucose, Urea Nitrogen, and Creatinine

During the study period, venous blood samples (3.5 ml) were collected from a jugular, cephalic or medial vein just prior to induction, at the end of the 90 minutes period of anesthesia, and 24 hours after anesthesia induction. Blood samples were centrifuged at 3000g for 10 minutes and serum was collected and analyzed for glucose, urea nitrogen, and creatinine immediately. Additional serum was stored at -80°C until the conclusion of the study, and then submitted to the Michigan State University Veterinary Endocrine Diagnostic Laboratory for insulin concentration analysis.

5.7 Statistical Analysis

Commercially available software (GraphPad Prism 9, GraphPad Software, CA, USA) was used for statistical analysis. The rectal temperature difference (ΔTr) and thoracic skin temperature difference (ΔTt) between the induction of anesthesia (T0) and each time point throughout the anesthesia and recovery periods (T5 – T150) were calculated (e.g. ΔTr T150 – T0). Similarly, the thoracic temperature difference (ΔTt) between the induction of anesthesia (T0) and each time point (T15, 30, 45, 60, 75, 90) was calculated (e.g. ΔTt T90 – T0). Area under the curve (AUC) for ΔTr and ΔTt between T0 and each time point, and cardiorespiratory valuables (heat rate, blood pressure, respiratory rate, and end-tidal CO_2) over time were calculated using the trapezoid method (Purves 1992) for comparison between groups. The data of ΔTr was separated into the anesthesia and recovery periods and analyzed individually. Cumulative MAC hours were calculated as recorded ET_{ISO} at 5 minute intervals divided by the MAC of isoflurane for cats (1.59%) (Barletta et al. 2016), and then that value was divided by 18 for each 5 minutes period

(1/18th of 90 minutes). These values were summed to give the cumulative MAC hours value for each cat. The differences between the baseline and each blood sampling point (post anesthesia and 24 hours after anesthesia) for BG (ΔBG), BUN (ΔBUN), creatinine (ΔCre) and insulin (Δinsulin) levels were calculated and analyzed. Normality of data was assessed with the D'Agositno-Pearson test. Parametric data were analyzed by one-way repeated measure ANOVA with a post-hoc Tukey test and non-parametric data were analyzed with a Friedman test and a post-hoc Dunn's test. A value of $p < 0.05$ was used for significance. Parametric data are presented as mean ± SD and non-parametric data are presented as median (range).

6. Results

Data for premedication/recovery and surgery room temperature, time from premedication to induction of anesthesia, duration of anesthesia, baseline rectal temperature, ΔTr (T0 to T90), ΔTr (T0 to T150), and recovery times are summarized in Table. 1. There were no statistically significant differences for any of those parameters among groups. Changes in ΔTr over time are displayed in Figure 3. There were no significant differences for AUC of ΔTr during anesthesia ($p = 0.3675$) or recovery ($p = 0.9689$) among groups. Changes in ΔTt over time was showed in Figure 4. ΔTt did not differ among groups. Data for heart rate, mean and systolic blood pressure, respiratory rate, and $ETCO_2$ are represented in Figure 5. There was no difference in AUC for heart rate ($p = 0.2344$), mean ($p = 0.5220$) or systolic ($p = 0.6470$) blood pressure, respiratory rate ($p = 0.2831$), or $ETCO_2$ ($p =$

0.8302) among groups. Cumulative MAC hours was 0.61 ± 0.03 for LRS, 0.6 ± 0.03 for A100 and 0.59 ± 0.04 for A200 and was not different among groups (p = 0.5879).

Data for BG, BUN, Cre, and insulin are summarized in Table 3. ΔBG, ΔBUN, ΔCre and Δinsulin did not differ among groups at any time points (Fig 6). All variables, except BG at 90 minutes after anesthesia induction, were within reference range for all sampling points. Eight cats were determined to be hypoglycemia (serum glucose ≦ 57 mg/dl) after anesthesia; 1 out of 10 cats for LRS (BG: 55 mg dl^{-1}), 4 out of 10 cats for B100 (BG range: 29 – 57 mg dl^{-1}), and 3 out of 10 for B200 (BG range: 37 – 55 mg dl^{-1}). BG for all hypoglycemic cats at post anesthesia returned to the reference rage without any interventions at 24 hours after anesthesia.

One cat in LRS group required additional alfaxalone 1 mg/kg IV for anesthesia induction since endotracheal intubation was not possible due to the inadequate anesthetic depth.

None of cats showed any evidence of phlebitis or pain on the cephalic catheter site after anesthesia.

Table 1. Mean ± standard deviation (SD) for premedication/recovery and surgery room temperature, baseline rectal temperature, rectal temperature difference between baseline and 60 minutes post extubation, time from premedication to anesthesia induction, duration of anesthesia, and recovery time from end of anesthesia to be sternal position and to standing. Median (min, max) for rectal temperature difference baseline and end of anesthesia, and recovery time from end of anesthesia to extubation. $p < 0.05$ was set significant.

	LRS	B100	B200	*p* value
Variables				
Premedication/recovery room temperature (℃)	20.9 ± 1.2	21.4 ± 1.3	21.0 ± 1.3	0.7268
Surgery room temperature (℃)	22.7 ± 0.8	22.7 ± 0.9	22.5 ± 0.9	0.8631
Baseline rectal temperature (℃)	38.2 ± 0.3	38.0 ± 0.4	38.1 ± 0.5	0.0677

Rectal temperature difference between baseline and end of anesthesia (℃)	-4.3 (-5.3 - -3.2)	-4.2 (-6 - -3.8)	-4.5 (-5.6 - -3.2)	0.7103
Rectal temperature difference between baseline and 60 minutes post extubation (℃)	-0.9 ± 0.8	-0.9 ± 0.8	-0.9 ± 0.6	0.8762
Time from premedication to anesthesia induction (minutes)	25.1 ± 4.3	23.9 ± 2.0	26.6 ± 6.5	0.4361
Duration of anesthesia (minutes)	92.9 ± 1.5	92.4 ± 1.2	92.5 ± 1.4	0.6406
Time to extubation (minutes)	1 (0.5 -15)	1 (0.4 - 6)	1 (0.6 - 8)	0.1448
Time to sternal (minutes)	27.1 ± 13.4	22.5 ± 8.9	24.9 ± 10.0	0.6033
Time to standing (minutes)	34.8 ± 12.4	30.2 ± 10.8	33.3 ± 10.7	0.5562

Table 2. Mean ± SD or Median (min, max) for blood glucose (BG), plasma insulin (insulin), blood urea nitrogen (BUN), and creatinine (Cre) for LRS, B100, and B200 in baseline, 90 minutes and 24 hours after anesthesia induction.

	Time point			
Variables	Baseline	90 minutes after anesthesia induction	24 hours after anesthesia induction	Reference range
LRS BG (mg/dl)	93.3 ± 8.4	80.2 ± 18.0	87.3 ± 9.1	58 - 116
B100 BG (mg/dl)	91.3 ± 13.6	66.9 ± 27.1	100.9 ± 26.7	
B200 BG (mg/dl)	94.6 ± 13.8	69.3 ± 26.1	88.1 ± 10.5	
LRS Insulin (μIU/ml)	4.39 ± 2.18	1.49 ± 0.63	4.60 ± 1.14	0.1 - 8.0
B100 Insulin (μIU/ml)	2.84 ± 1.26	1.58 ± 0.59	5.47 ± 2.09	
B200 Insulin (μIU/ml)	3.83 ± 2.13	1.40 (0.90 - 3.40)	4.85 (1.50 - 14.80)	
LRS BUN (mg/dl)	23.1 ± 3.1	22.4 ± 2.5	22.1 ± 3.3	5.0 - 30.0
B100 BUN (mg/dl)	24.0 ± 4.3	25.0 ± 3.5	21.9 ± 2.5	
B200 BUN (mg/dl)	25.2 ± 3.3	24.0 ± 3.5	22.4 ± 4.3	
LRS Cre (mg/dl)	0.97 ± 0.17	0.85 ± 0.13	0.93 ± 0.17	0.0 - 2.0
B100 Cre (mg/dl)	0.92 ± 0.12	0.90 (0.60 - 0.90)	0.90 ± 0.1	
B200 Cre (mg/dl)	0.92 ± 0.11	0.78 ± 0.1	0.85 ± 0.13	

Fig 3. Changes in mean ± SD of rectal temperature difference between each time point and baseline temperature for LRS (n = 10, black circles), B100 (n = 10, black triangles), and B200 (n = 10, black squares) minutes during general anesthesia (a) and recovery from general anesthesia (b).

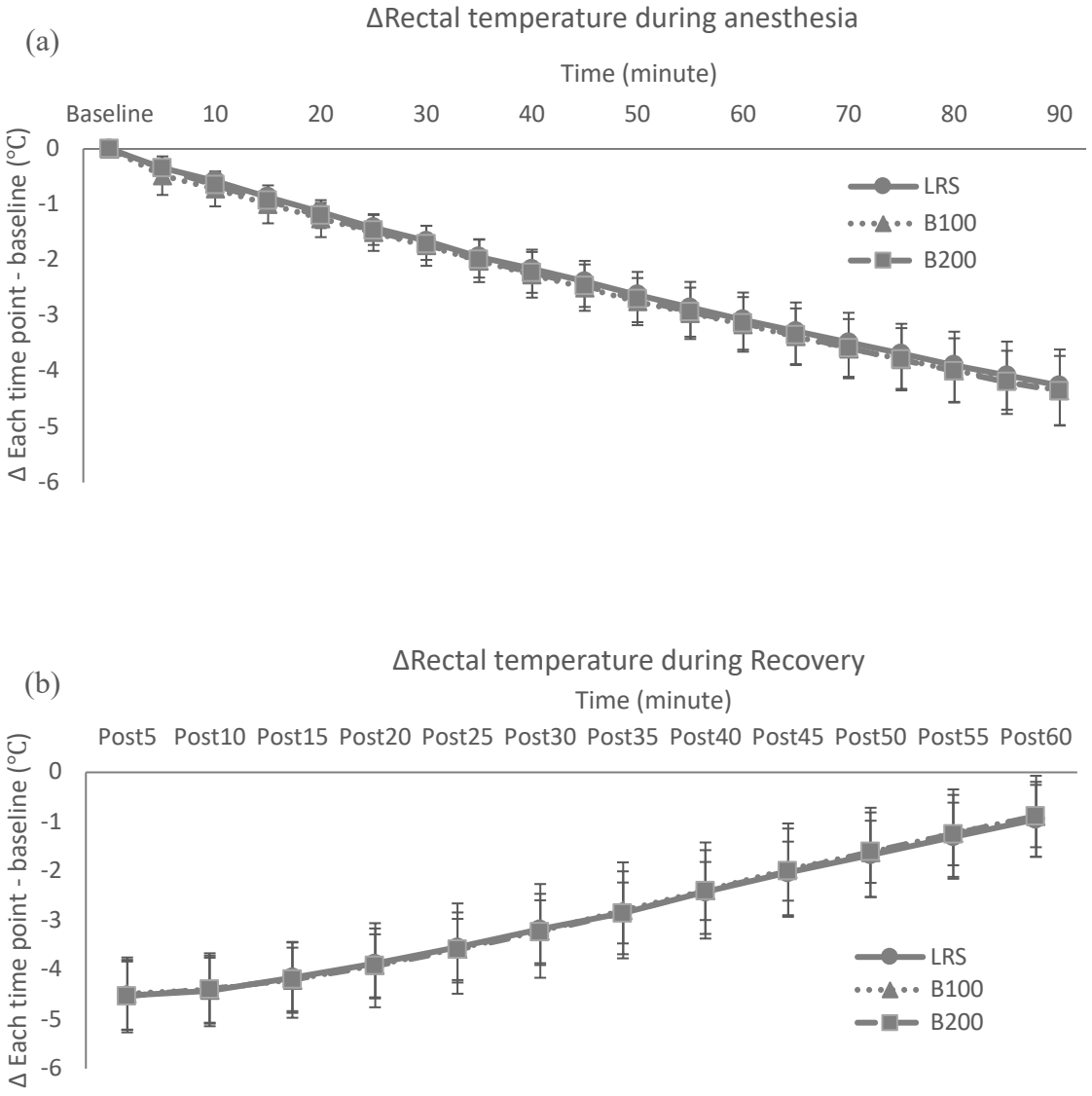


Fig 4. Changes in mean ± SD of thoracic skin temperature difference between each time point – baseline temperature for LRS (n = 10, black circles), B100 (n = 10, black triangles), and B200 (n = 10, black squares) during general anesthesia.

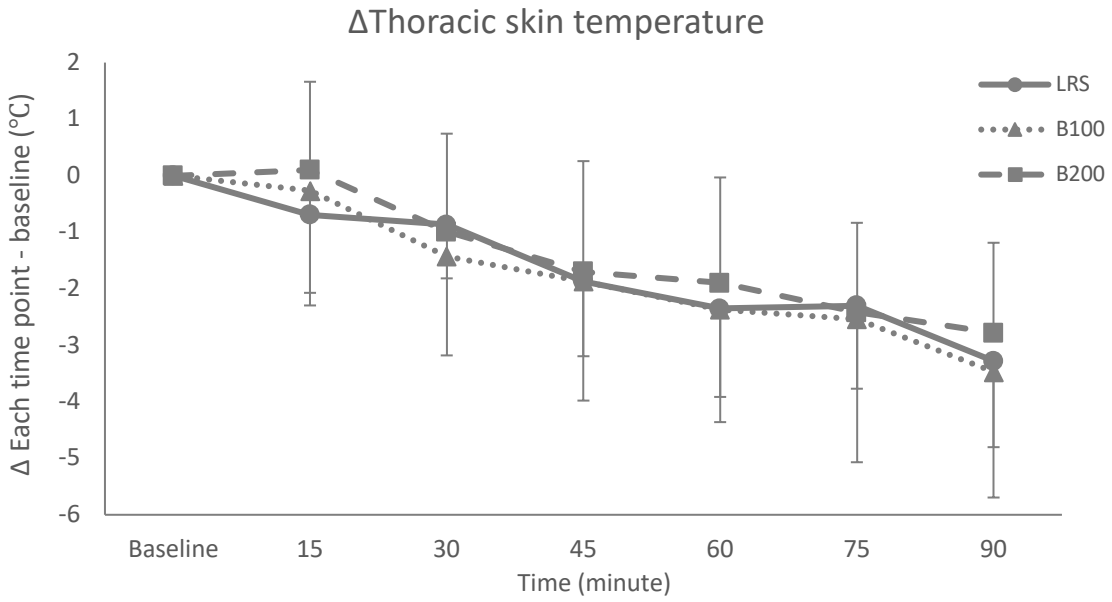
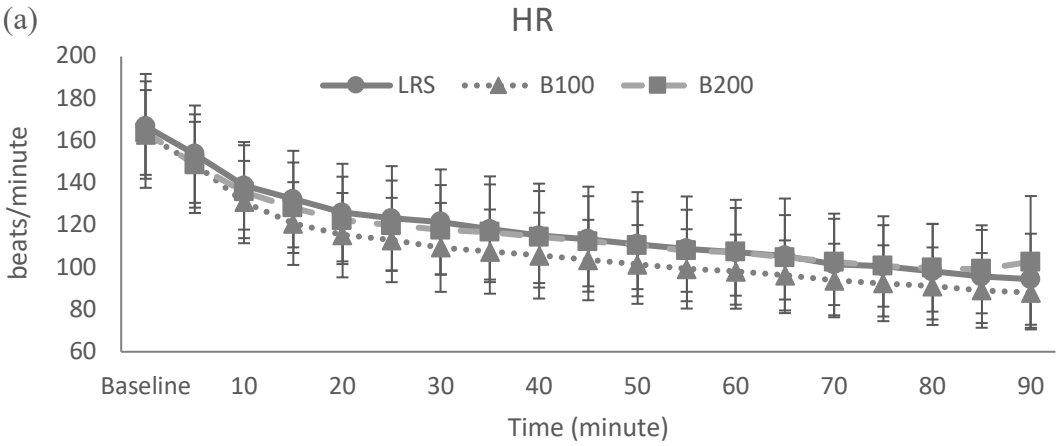
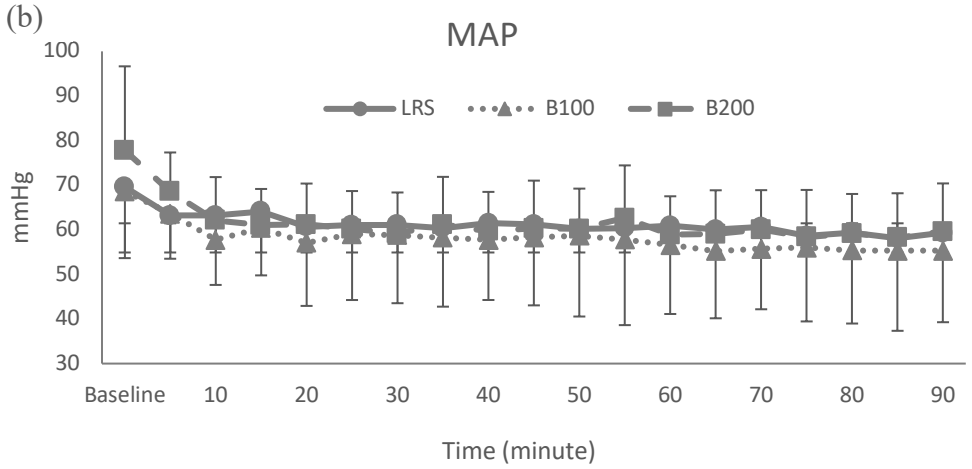


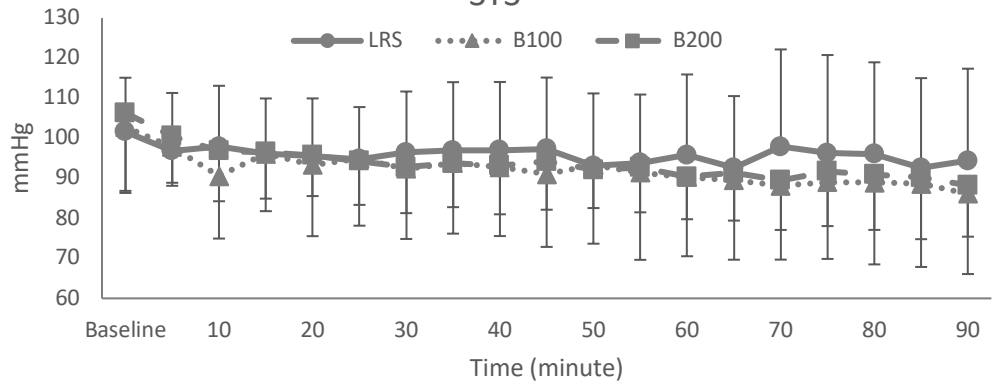
Fig 5. Changes in mean ± SD of heart rate (HR) (a), mean arterial blood pressure (MAP) (b), systolic arterial blood pressure (SYS) (c), respiratory rate (RR) (d), and end tidal CO_2 ($ETCO_2$) (e) over time for LRS (n = 10, black circles), B100 (n = 10, black triangles), and B200 (n = 10, black squares) during general anesthesia.



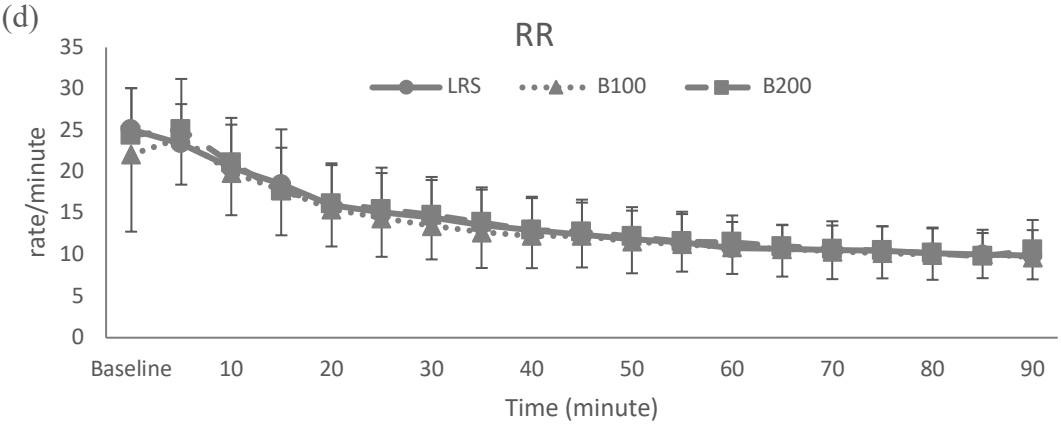
(b)


MAP
LRS
B100
B200
mmHg
100
90
80
70
60
50
40
30
Baseline
10
20
30
40
50
60
70
80
90
Time (minute)

(c)


SYS
LRS
B100
B200
mmHg
130
120
110
100
90
80
70
60
Baseline
10
20
30
40
50
60
70
80
90
Time (minute)

(d)


RR
LRS
B100
B200
rate/minute
35
30
25
20
15
10
5
0
Baseline
10
20
30
40
50
60
70
80
90
Time (minute)

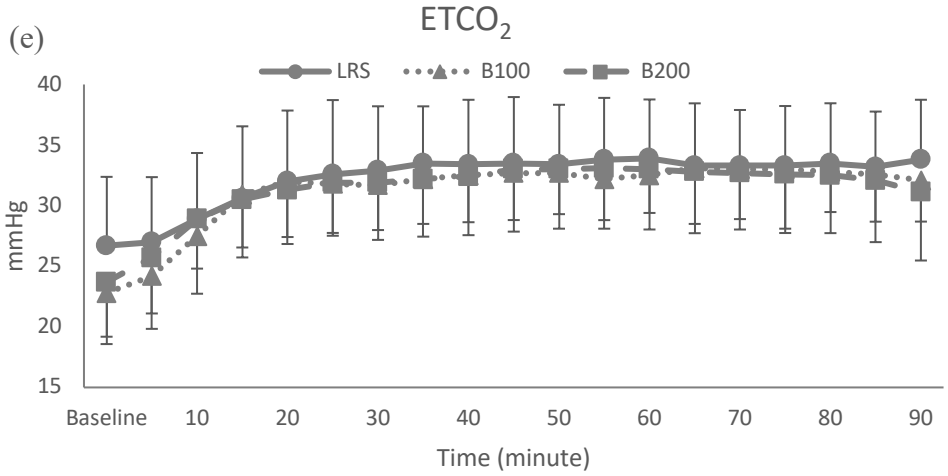
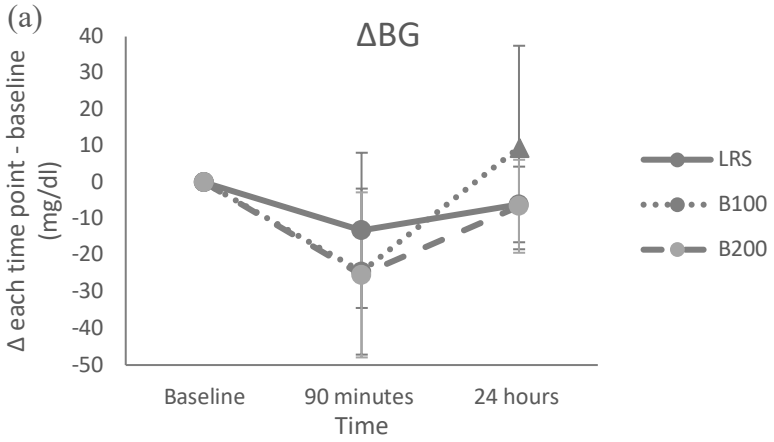
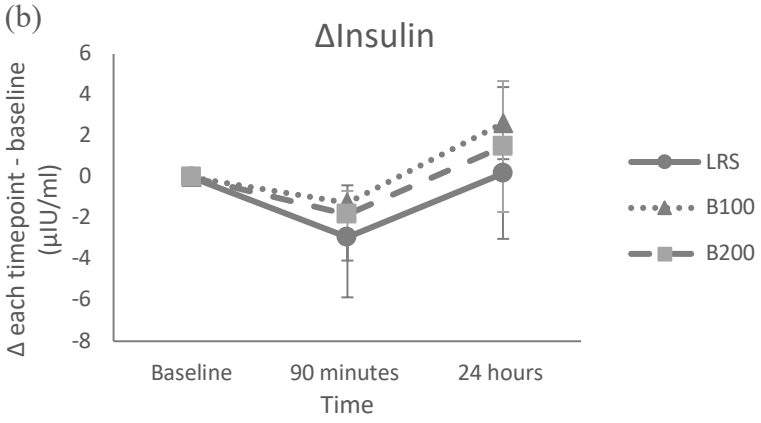


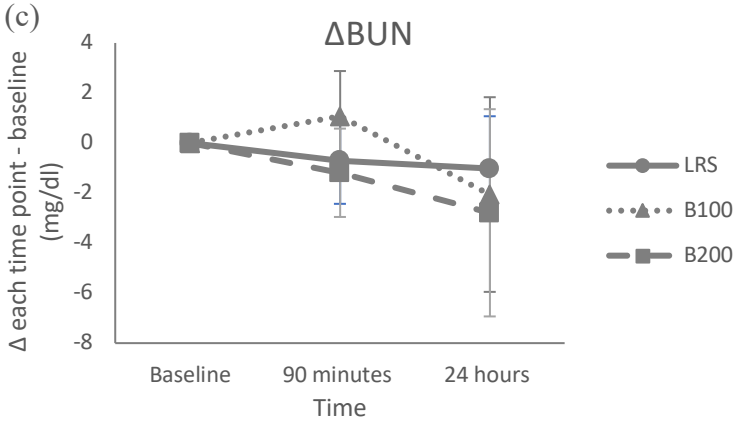
Fig 6. Changes in mean ± SD of differences between each time point – baseline for blood glucose (ΔBG) (a), plasma insulin (Δinsulin) (b), blood urea nitrogen (ΔBUN)(c), and creatinine (ΔCre) (d) for LRS (n = 10, black circles), B100 (n = 10, black triangles), and B200 (n = 10, black squares) during general anesthesia.



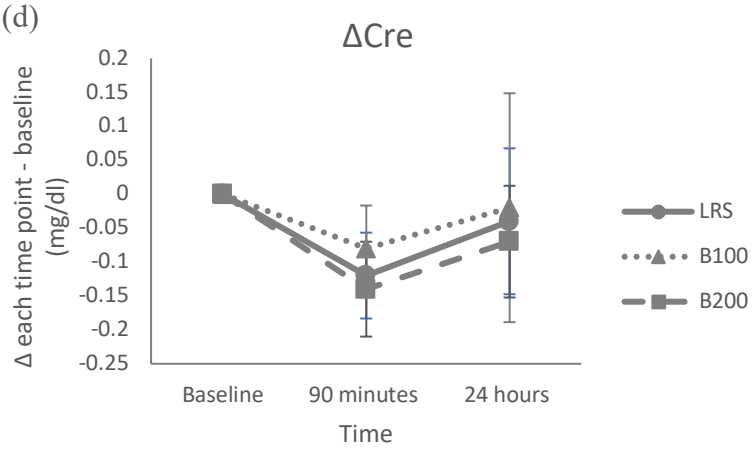
(b)


ΔInsulin
Δ each timepoint - baseline (µIU/ml)
6
4
2
0
-2
-4
-6
-8
Baseline
90 minutes
24 hours
Time
LRS
B100
B200

(c)


ΔBUN
Δ each time point - baseline (mg/dl)
4
2
0
-2
-4
-6
-8
Baseline
90 minutes
24 hours
Time
LRS
B100
B200

(d)


ΔCre
Δ each time point - baseline (mg/dl)
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
-0.25
Baseline
90 minutes
24 hours
Time
LRS
B100
B200

7. Discussion

In this study, the administration of neither low dose nor high dose of BCAAs attenuate heat loss in cats during general anesthesia. Additionally, there was no difference in hemodynamic variables and recovery time in the cats administered BCAAs compared with the control cats. However, the administration of BCAAs appeared to induce hyperinsulinemia, resulting in mild to severe hypoglycemia in cats receiving BCAAs. Any effectiveness of single intravenous BCAAs injection on body temperature in anesthetized cats was absent in this study. The rectal and skin thoracic temperature during the anesthetic period were not significantly different between groups. AAs-NIT has been demonstrated its beneficial effects on heat loss and the recovery quality from general anesthesia in humans and animals (Takashima *et al.* 2016; Aoki *et al.* 2017; Clark-Price *et al.* 2018). In people, a meta-analysis of AAs NIT showed the use of perioperative AAs infusion led to a 0.46℃ increase in body temperature and decrease in the incidence of shivering during recovery (Aoki *et al.* 2017). Furthermore, *Clark-Price* et al. reported that dogs receiving intraoperative AAs infusion had higher temperature by 0.63℃ than control dogs, and this temperature difference led to shorten recovery time and decrease shivering rate (Clark-Price *et al.* 2018). In the thermographic thoracic images, up until 15 minutes after anesthesia induction, cats receiving 200 mg/kg BCAAs had a temporal increase in skin temperature compared to baseline, but the other groups did not. Furthermore, both treatment groups had higher skin temperatures than the control group at 15 minutes after anesthesia induction. Eventually, the level of heat loss from the skin

was equivalent between groups at the end of anesthesia. This might indicated that there was a transient increase in heat production by the single BCAAs administration, but that there was not enough substrate (BCAA) to maintain heat production for the entire anesthetic period in the cats.

There are several postulated causes that contributed to the lack of difference in body temperature between groups that should be considered. First is the constituents of the BCAAs solution as this study used a non-commercially available BCAAs solution. This BCAAs solution was compounded with a BCAAs powder for medical use by licensed pharmacists in our animal hospital. The BCAAs powder was dissolved into sterilized water the day of the experiment. Valine was the highest concentration components, 60% of total BCAAs in our BCAAs solution, however valine may not have strong effects on skeletal muscle anabolism. In fact, leucin is thought to have the strongest anabolic effect on the muscle synthesis of all three BCAAs (Escobar *et al.* 2005; Columbus *et al.* 2014). Escobar et al. showed the magnitude of protein synthesis on skeletal muscle was elevated with leucin dose-dependently rather than valine in pigs (Escobar *et al.* 2005). However, a solution with a higher leucine concentration was not available due to the limitation of the solubility of each BCAA in water. Each BCAA concentration that was compounded was at the highest concentration that would go into solution. A BCAA solution with a higher leucine concentration might induce higher heat production in skeletal muscle and lead to better attenuation of anesthesia related heat loss.

The current study tested 100 and 200 mg/kg BCAA dosages as BCAAs-NIT in cats undergoing general anesthesia. These dosages were based upon the previous dog study as suggested it was likely to be effective (Clark-Price *et al.* 2018). A 10% AA solution was

administered to the dogs at a rate of 8 mL/kg/hr and attenuated heat loss. This infusion rate corresponded to a dosage of BCAA of 196.8 mg/kg delivered over an hour. For this study, we selected two dosages of a single BCAA injection (100 and 200 mg/kg) in cats for evaluation of effects on body temperature during anesthesia. However, dietary protein requirements in cats are approximately twice as those of dogs (Legrand-Defretin 1994). It is unclear if that difference in protein requirement between species directly relate to the magnitude of thermogenesis by BCAAs. Cats may require a higher dosage of BCAAs, more than 200 mg/kg, to achieve an equivalent thermic effect with dogs.

The body surface area to mass ratio is one of the determinants for the intraoperative heat loss (Koop and Tadi 2021). Small animals, such as cats, are more likely to be hypothermic than medium to large size animals during anesthesia. All type of heat loss, radiation evaporation, convection, and conduction, could be enhanced due to the high body surface area to mass ratio. Previous studies in dogs demonstrated the benefit of AA-NIT on temperature enrolled medium to large breed dogs whose median body weight was 19.2 kg and ranged 8.9 – 52.1 kg, while mean body weight of the cats was 3.5 ± 0.7 kg in this study (Clark-Price *et al.* 2018). Thus, in this study, heat loss might have exceeded heat production by BCAAs due to their large body surface area to body mass ration.

Hypoglycemia is recognized as an adverse event for AA-NITs. Indeed, cats receiving BCAA-NITs had a higher incidence of hypoglycemia and more severe decline in BG than control cats at the end of anesthesia. AAs, especially leucin, directly stimulate insulin release from β cell of the islet of Langerhans and increase uptake of glucose into adipose tissue and skeletal muscle from blood, resulting in a decline in BG (Kuhara *et al.* 1991). However, blood insulin concentration at the end of anesthesia was lower than

baseline in all groups. The causes of this discrepancy between BG and insulin concentrations are postulated the interaction of the prolongation of the recovery of BG and dilution of blood. Kuhara et al. studied the changes in blood insulin and BG concentrations for 90 minutes with intravenous leucin administration in awake sheep (Kuhara *et al.* 1991). Blood insulin concentrations peaked 30 minutes after starting the leucine CRI and returned close to baseline values 60 minutes after discontinuation of the leucine CRI while BG reached its lowest value at 30 minutes after the end of leucine CRI and persisted within the timeframe. The cats with BCAAs might have had a similar trend, temporal spiking insulin immediately after the administration of BCAAs and returned it to baseline values at 90 minutes while a persistent low BG continued to 90 minutes. As a result, BG at the end of anesthesia was likely to be lower than baseline in treatment groups. Furthermore, all cats were administered 1.5 – 3.0 ml/kg of treatment solutions (BCAAs or saline) before induction and received 2 ml/kg/hr of an LRS infusion during anesthesia. The increase in blood volume by the fluid administration could cause a dilution of blood and a decrease in measured blood variables. In support, blood creatinine is a common parameter used to assess the hydration of animals in a clinical settings (Smith and Greer 2016). Blood creatinine was lower at the end of anesthesia than baseline in all groups. This suggests that cats were overhydrated after anesthesia. We speculated that the blood volume of the cats increased by fluid administration, resulting in increased dilution of the blood insulin and BG, even in the control group at the end of anesthesia. Thus, the prolonged BG recovery and blood dilution might have caused inconsistency of blood insulin and BG values in all groups.

The BUN value at 90 minutes after anesthesia induction in 100 mg/kg BCAAs group was elevated compared with that of baseline. Similarly, previous studies in dogs and humans demonstrated elevated serum BUN after administration of AAs (Sellden and Lindahl 1998; Takashima *et al.* 2016; Clark-Price *et al.* 2018). AAs are metabolized in the liver through oxidation, and urea is produced as byproduct of the oxidation of AAs (Sellden and Lindahl 1998). We could not clearly explain the reason that serum BUN was not elevated in the 200 mg/kg BCAAs group at the end of anesthesia. However, the elevated BUN values were within clinical reference limits and returned to baseline by 24 hours after induction of anesthesia. Thus, this finding may not be clinically meaningful in healthy animals. Also, creatinine is generated as byproduct of AAs breakdown in liver. A transient decrease of serum creatinine has been reported in dogs after AAs administration (Clark-Price *et al.* 2015, 2018). It was thought that crystalloid fluid administration during anesthesia increased renal excretion of creatinine during the perianethetic period.

The attenuation of perioperative hypothermia shortens recovery time from anesthesia in cats (Redondo *et al.* 2012a; Sakata *et al.* 2020). Hypothermia slows down the anesthetic drug metabolism in the liver due to the reduction of metabolic enzyme activity and hepatic blood flow, which may contribute to a prolonged anesthetic recovery time (Brodeur *et al.* 2017). According to this study, a single injection of BCAAs may not assure an effective serum BCAAs concentration for effective heat production. Changes in rectal temperature were similar between groups after anesthesia and there were no differences in recovery times.

One cat in the LRS (control) group required an additional dose of alfaxalone at 1 mg/kg IV for anesthesia induction because of an inadequate anesthetic depth for endotracheal

intubation. The administration of IV alfaxalone is rapidly metabolized by the liver and eliminated through urine and bile. The elimination half-life of alfaxalone is 45.2 minutes after 5 mg/kg IV, and the recovery time from sedation is 69 minutes in cats (Whittem *et al.* 2008). Moreover, all three recovery times for that cat were similar to the other cats within the group. Thus, the influence of the extra dose of alfaxalone did not appear to affect recovery time.

No hemodynamic improvements were observed with BCAAs-NIT. Elevated insulin concentration, such as those induced by AAs injection, can induce nitric oxide (NO) release from vascular endothelial cells (Muntzel *et al.* 2001). The interaction between insulin and NO causes sympathomimetic responses, resulting in the increase in heart rate and blood pressure (Muntzel *et al.* 2001). However, this positive hemodynamic effect of AAs-NIT was demonstrated by only one study with dogs (Takashima *et al.* 2016). Additional studies are necessary to need to confirm the hemodynamic effect on AAs and BCAAs-NIT.

Finally, another factor as to why there was not a significant amount of heat attenuation may have been due to the difference in the administration method of BCAAs with comparted to previous studies using AAs-NIT. A single injection (our method) could be easily used by any veterinary hospital with minimal effort and no requirement for an electronic pump. However, previous studies in dogs showed the benefits of AAs-NIT using constant rate infusion (CRI) of AAs with a precise syringe pump (Takashima *et al.* 2016; Clark-Price *et al.* 2018). Unlike single injection, CRI can maintain a consistent blood concentration of AAs. A stable serum AA concentration might increase uptake of AAs by skeletal muscle and secretion of insulin, which helps to increase heat production.

While the dosages of BCAAs used was likely not high enough to produce heat for the prevention of heat loss during anesthesia, some cats showed moderate to severe hypoglycemia at the end of anesthesia. The co-administration of dextrose may be needed to prevent perianesthetic hypoglycemia if a higher dosage of BCAAs, over 200 mg/kg, is tested for future studies.

8. Conclusion

In conclusion, a single intravenous injection of BCAAs prior to anesthesia did not attenuate heat loss in healthy cats, whereas some cats receiving BCAAs showed transient mild to moderate hypoglycemia. Further studies are required to evaluate different dosages of BCAAs or methods (e.g. CRI or intermittent injection). However, a simultaneous administration of dextrose may be necessary to prevent hypoglycemia induced by BCAAs-NIT.

9. References

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