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PAPER

Gastrointestinal effects following acupuncture at Pericardium-6 and Stomach-36 in healthy dogs: a pilot study

D. I. Radkey*, V. E. Writt*, L. B. C. Snyder*, B. G. Jones† and R. A. Johnson[1],*

*Department of Surgical Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, Wisconsin, 53706, USA
†Anetech Imaging Services, Fountain Valley, California, 92708, USA

[1]Corresponding author email: rebecca.johnson@wisc.edu

Objectives: **To quantify changes in gastric and intestinal emptying times in the conscious dog following gastrointestinal acupoint stimulation.**

Materials and Methods: **In a randomised, blinded crossover study, six dogs were fed 30×1.5 mm barium-impregnated polyethylene spheres and underwent: (1) no acupuncture (*Control*); (2) stimulation of target points PC6 and ST36 (*Target*) and (3) stimulation of non-target points LU7 and BL55 (*Sham*). Abdominal radiographs were assessed immediately after feeding the spheres and every hour for 12 hours and their number in the stomach and large intestines was counted.**

Results: **The number of barium-impregnated polyethylene spheres found distal to the stomach was less in the *Target* group compared to the *Control* and *Sham* groups between hours 2 and 4, but no differences between groups were seen for the remainder of the treatment period. The number of spheres found within the colon/rectum was less in the *Target* group compared to the *Control* and *Sham* groups between hours 4 and 6, and compared to the *Sham* group only at hour 7 but no differences between groups were seen after hour 8.**

Clinical Significance: **Acupuncture targeted at the gastrointestinal tract of dogs was associated briefly with slowed gastric emptying and gastrointestinal transit time. This foundational study lays the groundwork for additional studies of acupuncture effects associated with altered physiologic states.**

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INTRODUCTION

Visceral motility disorders of the oesophagus, stomach and small and large intestines are commonly associated with critically ill patients and those undergoing sedation, general anaesthesia and operative procedures (Galatos & Raptopoulos 1995a, Galatos & Raptopoulos 1995b, De Miguel Garcia *et al.* 2013, Whitehead *et al.* 2016, Anagnostou *et al.* 2017, Torrente *et al.* 2017). Complications seen in patients with abnormal motility include aspiration pneumonia, oesophagitis and increased risk of bacterial translocation and sepsis, all of which are significant morbidities that can lead to increased length of hospital stay and treatment costs, and may result in patient mortality (Pearson *et al.* 1978, Cotton & Smith 1984, Harai *et al.* 1995, Leib *et al.* 2001, Adamama-Moraitou *et al.* 2002, Adami *et al.* 2011, Whitehead *et al.* 2016). In addition, gastrointestinal (GI) stasis can delay the return to eating and, when encountered in species such as horses and rabbits, can ultimately lead to severe morbidity and, potentially, death (Boscan *et al.* 2006, Bailey *et al.* 2016, Martin-Flores *et al.* 2017). Despite the severity of these consequences, successful preventive treatment options for visceral dysmotility specifically associated with analgesics, sedatives and general anaesthesia are limited, and usually only directed prophylactically at patients with known disease that predisposes them to one or more of these events.

Acupuncture is an ancient form of diagnostic, therapeutic and preventive medicine in which needles, nowadays usually made of stainless steel, are inserted into anatomic points on the body to bring the body back to homeostasis or produce a therapeutic response. In its several thousand-year history, hundreds of individual points have been identified and are linked to specific actions when stimulated. The classic acupuncture point is located just beneath the body surface and is formed by a neurovascular bundle encompassed by a sheath of connective tissue with a high density of free nerve endings (A-delta and C fibres), arterioles, lymphatic vessels and mast cells. These points lie along meridians or channels that follow major nerves, vessels and fascial cleavage planes (Mittleman & Gaynor 2000).

Acupuncture has been studied in various species for its effect on sedation, treatment of acute and chronic pain, inhalant minimum alveolar concentration reduction, ileus and perioperative nausea and vomiting (PONV, Dai *et al.* 1993; Kotani *et al.* 2001, Balestrini *et al.* 2005, Culp *et al.* 2005, Cheong *et al.* 2013, Alizadeh *et al.* 2014, Scallan & Simon 2016, Silva *et al.* 2017, Faramarzi *et al.* 2017), among others. For example, acupuncture at Pericardium-6 (PC6) is considered to be the most effective antiemetic point and has been used in both humans and dogs to prevent nausea and vomiting associated with surgery, chemotherapy, opioids and motion sickness (Dundee *et al.* 1989, Dundee 1990, Dundee *et al.* 1991, Al-Sadi *et al.* 1997, Alkaissi *et al.* 1999, Shen *et al.* 2000, Wang & Kain 2002, Alizadeh *et al.* 2014, Koh *et al.* 2014, Scallan & Simon 2016). In addition, acupuncture at Stomach-36 (ST36) is efficacious in the treatment of GI motility disorders, and improvement of clinical signs has been reported in patients with various disorders of the GI tract (Lin *et al.* 1997, Ouyang *et al.* 2004, Takahashi 2006).

In contrast, points along the lung meridian have been shown to affect the heart rate, body temperature, consciousness, asthma and laryngospasm, but to have no effect on vomiting or nausea (Hosbach 2008). For example, acupuncture at point Lung-7 (LU7) follows the lung meridian and is indicated for use in cough, heart failure, cervical pain, intervertebral disc disease and facial paralysis (Chrisman & Huisheng 2007). The bladder channel has 67 points with varying indications ranging from ocular disease, epilepsy, pancreatic disease, hypertension, bladder disease and many more. Bladder-55 (BL55) is not commonly targeted but is used in thoracolumbar disc disease, pelvic limb paresis and uterine bleeding, but has no effect on GI function (Chrisman & Huisheng 2007).

Thus, the primary objective of this foundational study was to quantify changes in gastric emptying and intestinal transit times in the conscious dog following stimulation of targeted GI acupuncture points PC6 and ST36, with the expectation that our results may then lead to further investigations into dogs with GI motility disorders. We tested the hypothesis that acupuncture at PC6 and ST36 in the dog would hasten gastric emptying and GI transit times when compared to dogs receiving non-target acupuncture at LU7 and BL55 and dogs with no acupuncture.

MATERIALS AND METHODS

Animals

This study was conducted on six, healthy and young adult (<1 year) beagles with a body weight of 13·5 ±1·5 kg (mean ±sd). Dogs were deemed healthy on the basis that no abnormalities were detected during physical examination and packed cell volumes and total protein concentrations were within their respective reference intervals. No dogs showed any clinical signs of GI disease, including diarrhoea or vomiting. All procedures were approved by the Institutional Animal Care and Use Committee.

Experimental procedures

Dogs were allowed to acclimate to their housing environment and new feeding schedule for 7 days before study start. Food was withheld from the dogs 24 hours before each experiment. Similar to previous studies (Allan *et al.* 1996, Johnson *et al.* 2017), on the morning of each study, dogs were fed a kibble meal ration [0·5 resting energy requirement = 0·5×70×(body weight in kg)$^{0.75}$] with 30×1·5-mm (outer diameter) barium-impregnated polyethylene spheres (BIPS; Medical ID Systems Inc) mixed in a small amount of baby food and dispersed throughout the kibble. Within 10 minutes of feeding, ventrodorsal, right lateral and left lateral abdominal radiographs were obtained (baseline), and treatments were started.

In a randomised (www.randomizer.org), blind crossover study, dogs received either: (1) no acupuncture needles inserted (*Control* treatment); (2) sham acupuncture (*Sham*) with needles placed bilaterally at acupoints LU7 (medial aspect of the thoracic limb, proximal to the styloid process of the radius, 1.5 cm proximal to the radiocarpal joint, Fig. 1A) and BL55 (on the caudolateral aspect of the pelvic limb, distal to the stifle, Fig. 1C); or, 3) target acupuncture (*Target*) with needles placed bilaterally at acupoints PC6 (medial side of thoracic limb, 3 cm proximal to the transverse carpal crease, in the groove between the flexor tendons, Fig. 1B) and ST36 (craniolateral aspect of the pelvic limb distal to the stifle, lateral to the cranial aspect of the tibial crest, in the belly of the cranial tibialis muscle, Fig. 1D). At least 7 days elapsed between treatments.

After needles were inserted, they were secured in place with a circumferential strip of porous tape. Needles were left in place for 30 minutes. All treatments involving acupuncture were performed with 0·25×13 mm, 3 gauge, surgical and stainless steel acupuncture needles (Hwa-To Singles, Suzhou Medical Appliance Factory) by the same experienced investigator certified in acupuncture techniques, out of view of the other investigators.

Three-view abdominal radiographs were obtained just after feeding (Time 0) and every hour thereafter during each treatment for 12 hours. Gastric emptying and GI transit times were determined at each time point by assessment of the location and number of BIPS in the stomach, small intestine or large intestine. For the purposes of the study presented here, BIPS that had passed distal to the ileum were counted as in the large intestine, therefore, BIPS in the cecum, colon and rectum were considered within the large intestine. For cases where the location of the

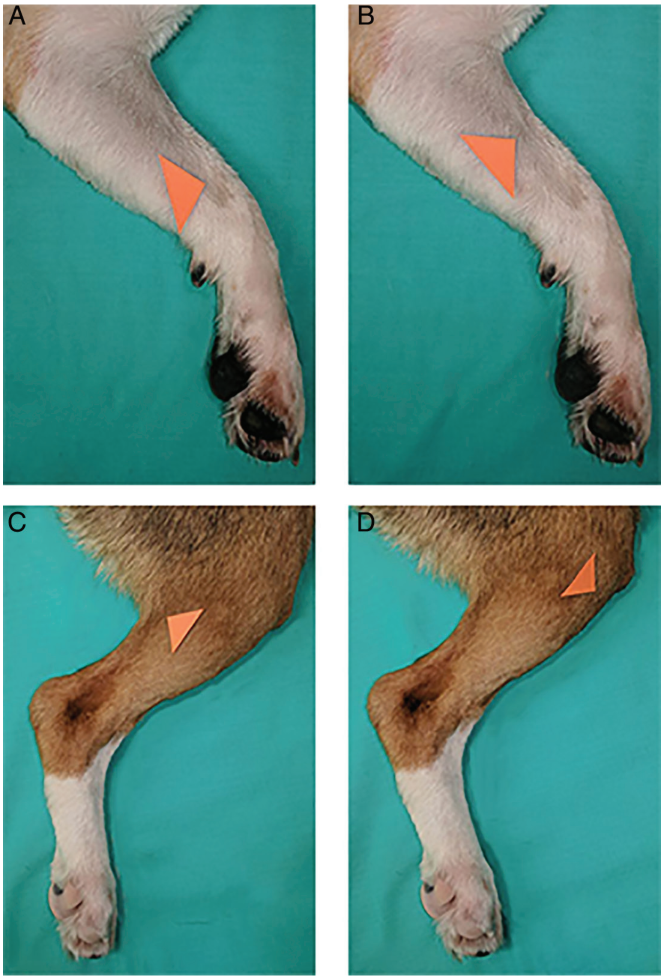

FIG 1. Arrows indicate the specific acupoints used in this study. (A) Lung-7; (B) Pericardium-6; (C) Bladder-55 and (D) Stomach-36

BIPS was uncertain as to whether they were in the stomach or small intestine due to superimposition of the two structures, a conservative selection was carried out so that only BIPS located definitively in the small intestine were counted. The same principle was applied to movement of the BIPS from the small intestine into the large intestine such that only BIPS located definitively in the large intestine were counted as such. Time for each event was recorded to the next hour because of the frequency of radiographic images. The investigator responsible for assessing the location of BIPS was unaware of the treatment and time point for each radiograph.

Statistical analysis

Statistical analyses were performed by use of two-way repeated-measures ANOVAs and Student–Newman–Keuls *post hoc* tests. The Shapiro–Wilk test was used to assess normality of the data. Time, treatment and the time-by-treatment interaction were factors for analyses of BIPS found distal to the stomach and in the large intestine. Data are reported as mean ±SEM. Statistical analyses were performed using commercial software. Results were considered significant if $P<0{\cdot}05$. To determine a 20% difference in the absolute number of BIPS between groups (*e.g.* a difference between 10 out of 30 BIPS in one treatment group compared to 16 out of 30 BIPS in another group located out of the stomach) with an alpha = 0·05 and power = 0·8, a sample size of six dogs was required, similar to other studies (Johnson *et al.* 2017).

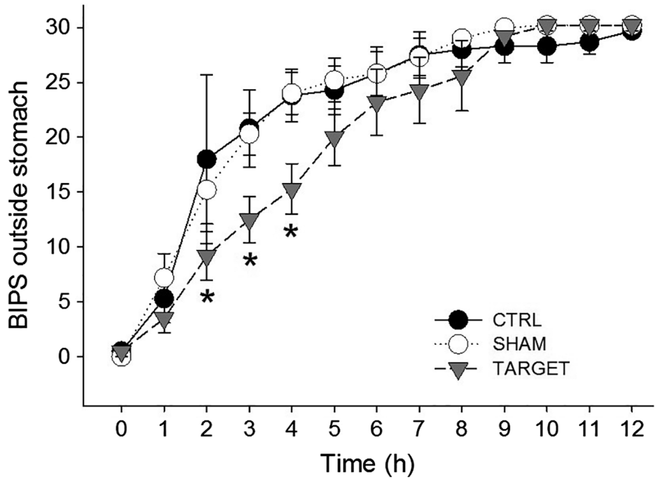

FIG 2. Absolute number of BIPS found distal to the stomach over time. No acupuncture (CTRL, black circles), sham acupuncture (SHAM, white circles) and targeted acupuncture (TARGET, grey triangles). The number of BIPS significantly increased from Time 0 at 1 to 12 hours in the CTRL and SHAM groups and 2 to 12 hours in the TARGET group. *$P<0{\cdot}05$ indicates significantly fewer BIPS distal to the stomach in the TARGET group compared to CTRL and SHAM groups 2 to 4 hours after treatment

RESULTS

Animals

BIPS were easily detected on radiographs obtained from all dogs for all treatments and could be tracked throughout the intestines. No dogs vomited or defaecated during treatments. All dogs were included in final data analysis.

Gastric emptying time

Compared with the baseline value (Time 0), significantly more BIPS were found distal to the stomach from 1 to 12 hours after treatment start in the *Control* and *Sham* groups and from 2 to 12 hours in the *Target* group (Fig. 2; all $P<0{\cdot}001$). Significantly fewer BIPS were found distal to the stomach in the *Target* group compared to the *Control* group at 2 hours (9 ±2 *versus* 17 ±3 BIPS, 48·8% fewer; P=0·013), 3 hours (13 ±2 *versus* 21 ±3 BIPS, 37·5% fewer; P=0·004) and 4 hours (15 ±2 *versus* 24 ±2 BIPS, 35·5% less, P=0·001) and the *Target* group compared to the *Sham* group at 2 hours (9 ±2 *versus* 15 ±3 BIPS, 39·5% fewer; P=0·019), 3 hours (13 ±2 *versus* 20 ±2 BIPS, 38·4% fewer, P=0·003) and 4 hours (15 ±2 *versus* 24 ±2 BIPS, 36·3% fewer, P=0·003). After 4 hours, there were no significant differences in the number of BIPS outside the stomach between groups (all $P>0{\cdot}05$).

GI transit time

Compared with baseline values (Time 0), significantly more BIPS were found in the large intestine from 4 to 12 hours in the *Control* and *Sham* groups and 5 to 12 hours in the *Target* group

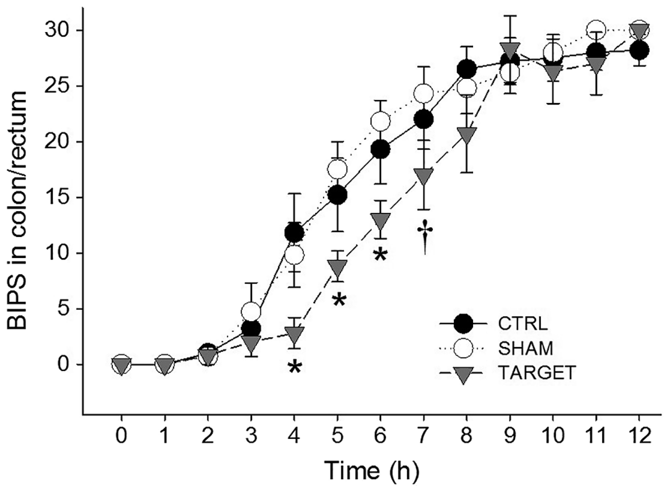

FIG 3. Absolute number of BIPS found in the large intestine over time. No acupuncture (CTRL, black circles), sham acupuncture (SHAM, white circles) and targeted acupuncture (TARGET, grey triangles). The number of BIPS significantly increased from Time 0 at 4 to 12 hours in the CTRL and SHAM groups and 5 to 12 hours in the TARGET group. *P<0·05 indicates significantly fewer BIPS within the large intestine in the TARGET group compared to CTRL and SHAM groups 4 to 6 hours after treatment. †P<0·05 indicates significantly fewer BIPS in the TARGET group compared with that of SHAM only

(Fig. 3; all P<0·001). Significantly fewer BIPS were in the large intestine in the *Target* compared to the *Control* group at 4 hours (3 ±1 *versus* 12 ±3 BIPS, 76·3% fewer; P=0·013), 5 hours (9 ±1 *versus* 15 ±3 BIPS, 42·8% fewer; P=0·017) and 6 hours (13 ±2 *versus* 19 ±3 BIPS, 31·6% fewer, P=0·017) and the *Target* group compared to the *Sham* group at 4 hours (3 ±1 *versus* 10 ±3 BIPS, 71.2% fewer; P=0·009), 5 hours (9 ±1 *versus* 18 ±3 BIPS, 49·7% fewer; P=0·004) and 6 hours (13 ±2 *versus* 22 ±2 BIPS, 42·3% fewer; P=0·003). At hour 7, significantly fewer BIPS were in the large intestine of the *Target* group compared with the *Sham* group only (17 ±3 *versus* 24 ±2 BIPS, 30.0% fewer; P=0·016); the difference between the *Target* and *Control* groups was not significant (17 ±3 *versus* 22 ±3 BIPS, P=0·056). From hour 8 to 12, no differences were present between groups (all P>0.05).

DISCUSSION

Contrary to our initial hypothesis, results of this study suggest that targeted acupuncture at PC6 and ST36 significantly *prolonged* gastric emptying times in dogs, but only up to 4 hours after treatment. GI transit time was also significantly prolonged with targeted acupuncture for 6 hours after treatment. Although we predicted that acupuncture would accelerate visceral transit times, the short-lived effects were not altogether unexpected as acupuncture is predicted to have an autonomic nervous system response in animals with normal visceral activity before returning to normal function (Li *et al.* 2013, Li *et al.* 2015).

Various methods such as conventional and contrast radiography, ultrasonography, nuclear markers, electromyography, radioactive tracers and motility capsules have been used to evaluate small intestinal motility (Caride *et al.* 1984, Dromehl *et al.* 1985, De Ridder *et al.* 1989, Penninck *et al.* 1989, Wiederkehr *et al.* 1992, Choi *et al.* 2001, Balsa *et al.* 2017), in addition to radiopaque markers (Chandler *et al.* 1990, Allan *et al.* 1996, Chandler *et al.* 1997, Sparkes *et al.* 1997, Robertson & Burbidge 2000, Nelson *et al.* 2001, Boscan *et al.* 2006, Johnson *et al.* 2017). Optimal dosing techniques of radiopaque markers in dogs include the use of 1.5-mm spheres fed in canned dog food, baby food or fed in a kibble ration. When small BIPS are ingested, 75% are predicted to exit the stomach of clinically normal dogs by a mean ±sd of 10.8 ±1.4 hours (Nelson *et al.* 2001) to 7.0 ±1·9 hours (Johnson *et al.* 2017). For our control acupuncture treatment, gastric emptying of 75% of BIPS was detected at 3.4 ±1·4 hours, much faster than previously reported values. The reason for this difference is unclear but we speculate that there may be physiologic variations among study populations of dogs (Nelson *et al.* 2001) or our population may have been better acclimated, allowing for more normal gastric emptying times. In addition, although our diet type was similar to that previously reported, differences in kibble or baby food ingredients may also have contributed to normal emptying time differences; these hypotheses were not tested here.

General anaesthesia can predispose patients to varying degrees of GI dysfunction, which may result in delayed return to feeding and increased morbidity, length of hospital stay and mortality (Mythen 2005). More specifically, ileus is especially common after abdominal surgery involving direct manipulation of the GI tract (Tinckler 1965, Harrower 1968, Graves *et al.* 1989, Behm & Stollman 2003, Yanagida *et al.* 2004, Senior *et al.* 2006). In addition, virtually all inhaled and injectable anaesthetics have been implicated in the development of decreased GI motility (Tinckler 1965, Schurizek *et al.* 1989, Durongphongtorn *et al.* 2006, Boscan *et al.* 2014). Although our results do not suggest that targeted acupuncture hastens GI motility in normal, conscious dogs as was predicted, the data confirm that in non-pathologic states, acupuncture has short-lived effects, possibly mediated through the autonomic nervous system, which quickly return function to normal (Li *et al.* 2013, 2015). We speculate, based on the underlying theories of acupuncture, that if visceral dysfunction is present (such as ileus), targeted acupuncture may work to bring the system back to normal homeostasis by enhancing GI motility for more prolonged periods. Alternatively, if GI transit times are abnormally accelerated, acupuncture may slow the dysfunctional motility.

Treatment of GI disturbances associated with anaesthesia is complex. Pharmacologic agents such as cisapride and metoclopramide, used to treat GI motility disorders, are associated with unwanted consequences such as cardiac effects, abdominal cramping, constipation and behavioural changes (Graves *et al.* 1989, Drici *et al.* 1999). In contrast, non-pharmacologic techniques (acupuncture) were superior to placebo and equivalent to antiemetics (metoclopramide, cisapride, *etc.*) in reducing PONV (Lee & Done 1998). Acupuncture may ultimately mediate the release of beta-endorphin in the cerebrospinal fluid, potentiating their endogenous antiemetic actions. The serotonergic and norepinephrinergic fibres may also be activated, and the antiemetic effects of acupuncture may be explained by changes in serotonin transmission (Lee & Done 1998).

In our study, gastric emptying and GI transit time were delayed, comparable to results in healthy human volunteers with no history of GI disease (Haker *et al.* 1999, Li *et al.* 2002, Takamoto *et al.* 2009, Witt *et al.* 2012). We speculate that the primary release of endogenous opioids such as dynorphins and enkephalins, which bind to mu- and delta-opioid receptors, may prolong gastric and GI emptying times (Lin & Wei 2008). Ertha and colleagues (2013) showed ST36 reduces pain-related behaviour in rats, but as many as 84 other points have been shown to have analgesic properties as well (Kotani *et al.* 2001). Serotonin, released during acupuncture, is an important hormone involved in GI homeostasis, although its receptor physiology is complex and its effects are altered in pathologic states such as irritable bowel syndrome, chronic constipation, diarrhoea and functional dyspepsia. Generally, patients with functional ileus benefit from increased levels of serotonin and the opposite is true for patients with rapid GI transit times (Sikander *et al.* 2009). Perhaps, the release of serotonin played a part in the delayed gastric emptying and GI transit times in the current study.

This study had limitations. Similar to other studies involving the use of BIPS, inaccuracies in determining the exact location of each BIP within the GI could have led to underestimation or overestimation of both gastric emptying times and GI transit times. Additonally, each set of radiographs were taken just before the next hour. This means that gastric emptying and GI transit time could have been overestimated because times were rounded up to the next hour. In addition, as mentioned previously, studies in healthy human volunteers have shown that targeted acupuncture results in a variety of outcomes including some similar to those found in our study (*i.e.* bradygastria). Perhaps, if we had chosen dogs with pre-existing GI disease or altered physiology that resulted in pre-existing ileus, we would have seen results consistent with our hypothesis as the ultimate goal of acupuncture therapy is to return homeostasis to unstable systems.

Our study combined both GI acupoints (PC6 and ST36), thus, no conclusions can be made concerning the effects of each individual point on motility. In addition, acupuncture was only performed for 30 minutes. As our results suggest that effects in normal dogs are short-lived, it is possible that the effects could be enhanced or extended if acupuncture was used in dogs with pathology, repeated with more frequency, or performed for a longer duration. We have not yet explored these alternative techniques.

Findings of this study reported here suggest that targeted acupuncture has the potential to alter gastric emptying time and GI transit time in dogs; however, the difference seen between groups was short-lived and is not consistent with our hypothesis of faster transit times. This study lays the groundwork for further investigations of targeted GI acupuncture in dogs with altered physiology or disease states.

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Conflict of interest

No conflicts of interest have been declared.

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