Journal of the American Association for Laboratory Animal Science
Vol 56, No 1
January 2017
Pages 84–89

Evaluation of Infrared Thermometry in Cynomolgus Macaques (*Macaca fascicularis*)

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Recording an accurate body temperature is important to assess an animal's health status. We compared temperature data from sedated cynomolgus macaques (*Macaca fascicularis*) to evaluate differences between rectal, infrared (inguinal and chest), and implanted telemetry techniques with the objective of demonstrating the diagnostic equivalence of the infrared device with other approaches. Infrared thermometer readings are instantaneous and require no contact with the animal. Body temperature data were obtained from 205 (137 male, 68 female) cynomolgus macaques under ketamine (10 mg/kg IM) sedation over a 3-mo period during scheduled physical examinations. Infrared measurements were taken 5 cm from the chest and inguinal areas. We evaluated 10 (9 functional devices) sedated cynomolgus macaques (5 male, 5 female) implanted with telemetry units in a muscular pouch between the internal and external abdominal oblique muscles. We determined that the mean body temperature acquired by using telemetry did not differ from either the mean of inguinal and chest infrared measurements but did differ from the mean of temperature obtained rectally. In addition, the mean rectal temperature differed from the mean of the inguinal reading but not the mean of the chest temperature. The results confirm our hypothesis that the infrared thermometer can be used to replace standard rectal thermometry.

Temperature regulation is a core component in maintaining homeostasis and appropriate physiologic processes in animals. The normal temperature range in cynomolgus macaques (*Macaca fascicularis*) is 98.6 to 103.1 °F (37.0 to 39.5 °C).[5] To obtain a rectal temperature in an alert macaque, it must be hand caught and held for examination, whereas the infrared device can be used without hand capture (that is, cageside), thus reducing stress and potential injury for both the animal and handler. In addition, the infrared thermometer allows us to do cageside evaluation, which helps to maintain an animal's normal physiology due to decreased sympathetic override of the potential underlying pharmacology or toxicology. For example, restrained squirrel monkeys normally have a temperature of 100.4 to 103.1 °F (38,0 to 39.5 °C), and the body temperatures in healthy active, struggling animals may rapidly increase to as high 105.8 °F (41.0 °C).[1] A study assessing the behavioral and physiologic responses to repeated physical restraint in rhesus macaques showed a decrease in behavioral agitation and cortisol concentrations during 7 straight days of physical restraint.[9] When the macaques were restrained for a single session 6 mo later, they maintained the decrease in behavioral agitation, but the majority of changes in adrenocortical responsiveness was no longer present suggesting that habituation to physical restraint is not lasting.[9]

Working with laboratory cynomolgus macaques is challenging due to the difficulty and danger in handling them. The distribution of the scientific data collected might be skewed as discussed earlier, due to their fear response and the resulting physiologic changes. Under ideal circumstances, implanted telemetry devices are used to collect temperature and other data continuously and without human interaction. However, the drawbacks of telemetry are the need for an invasive surgical procedure, associated healing time, and possible surgical complications such as delayed wound healing and infection. A viable alternative is using the noncontact thermometer for body temperature collection.

Infrared thermometers capture invisible infrared energy or electromagnetic radiation emitted from all objects, including the skin. Infrared radiation is, quite literally, heat and lies in the electromagnetic spectrum between microwave and UV radiation. The sensor in an infrared thermometer collects a small amount of energy (usually 0.0001 W) that is radiated from the target, generates an electrical signal that is amplified by a precision amplifier, and converts it into voltage output. The signal is digitized by an analog-to-digital converter, and an arithmetic unit solves a temperature equation based on the Planck Radiation Law and compensates for the ambient temperature and emissivity (emitted energy), resulting in a temperature reading within a fraction of a second after the 'read' button on an infrared thermometer is pushed.[12]

The Centers for Disease Control has a guidance document[4] for the use of noncontact infrared thermometers for screening subjects for febrile illness (most recently for Ebola monitoring in 2014) at ports of entry. The overall performance characteristics reported were: sensitivity, 80% to 99%; specificity, 75% to 99%; and positive predictive value, 31% to 98%. This document confirms the utility of infrared thermometers to detect fever and presents advantages and disadvantages of the noncontact thermometer approach. Stated advantages of these devices included their noncontact feature, accuracy, low cost, small size, and ease of training and use. Some models are FDA-approved and can be used in medical facilities. Disadvantages include slower for screening large numbers of people compared with mounted, camera-style scanners.[4] A further review of the human literature showed 3 studies[3] with conclusions in favor of infrared skin thermometry, whereas 3 studies[3] stated that this type of device lacks accuracy. These studies compared infrared skin thermometers with rectal, oral, axillary, pulmonary artery

Received: 21 Apr 2016. Revision requested: 03 Jun 2016. Accepted: 01 Jul 2016.
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catheter, and nasopharyngeal probes. The studies[3] comparing infrared with rectal temperatures showed weak correlation (correlation coefficient, 0.48) between the 2 devices, with a mean difference of 0.1° F.[3]

Our goal was to establish a method of assessing body temperature in cynomolgus macaques that is a refinement of traditional methods, which are invasive and require hand capture of alert animals.

Materials and Methods

Animals, housing and feeding. All animals used for the project were Chinese-origin, captive-bred cynomolgus monkeys (*Macaca fascicularis*) from Charles River Laboratories Safety Assessment (Reno, NV) and were received by the test site from Charles River (Houston, TX). Macaques weighed 2.0 to 5.5 kg, were 2 to 6 y old, and were of both sexes. The study site (Charles River Laboratories, Reno, NV) is fully AAALAC-accredited, is periodically audited (including site visits), and is in full compliance with all applicable guidelines of the US Department of Agriculture. The study protocol was reviewed and approved by the site's IACUC. Prior to study assignment, macaques were placed in pairs and evaluated by behavior staff for compatibility. Animals were housed in stainless steel cages, and the room environment maintained to meet the specifications of the US Animal Welfare Act (9 CFR, Parts 1, 2, and 3) and as described in the *Guide for the Care and Use of Laboratory Animals*.[7] Purina Primate Diet (certified; Richmond, IN) was provided daily in amounts appropriate for the size and age of the animals and was supplemented with fruits, vegetables, and other treats as part of the facility's environmental enrichment program.

Surgical procedure and telemeterized cohort. The telemeterized group (5 male and 5 female) were previously implanted with radiotransmitters (D-70 PCT, Data Sciences International, St Paul, MN) for other projects. Animals were anesthetized with ketamine hydrochloride (Putney, Portland, ME) cageside and taken to the anesthesia prep area, where an intravenous catheter was placed in peripheral vein and anesthesia was induced with ketamine (10 mg/kg) and valium (0.50 mg/kg; Hospira, Lake Forest, IL). Perioperative opioids were administered, an endotracheal tube was inserted, and animals were placed on 2 L/min oxygen and 1% to 2% isoflurane for anesthesia. The right flank, right and left thorax, and right inguinal region were prepared for surgery. Macaques were placed in dorsal recumbency on the surgical table and prepped with chlorhexadine and alcohol prior to procedure. Telemetry units were placed in muscular pockets between the internal and external abdominal oblique muscles, and the pressure catheters were tunneled to the inguinal incisions, where they were placed in femoral arteries and advanced to the abdominal aorta. ECG leads were tunneled to the thorax incisions and sutured to muscle. All incisions were closed with absorbable monofilament suture and animals were recovered from anesthesia. Postprocedural opioids and NSAID were provided for 3 d.

The radiotransmitter devices record core body temperature, due to their placement in a muscular pocket in the flank of the animal adjacent to the abdominal body wall. Other data available from these devices were beyond the scope of the current study. For the current study, these 10 animals were anesthetized cageside with ketamine hydrochloride (10 mg/kg IM) several months after surgical recovery. They were placed on a telemetry receiver, and body temperatures were recorded on 9 of them; one animal had a nonfunctioning telemetry device. After recordings were obtained, the animals were returned to their home cages for recovery and monitoring.

Healthy animal cohort, rectal thermometry, and infrared thermometry. A total of 205 body temperature recordings were gathered from cynomolgus macaques (137 males and 68 females) over a period of approximately 6 mo. All animals were clinically healthy, and the temperatures were collected between 0900 to 1200. All macaques were sedated with ketamine hydrochloride (10 mg/kg IM; Putney) prior to recordings and exams. In addition to recording of body temperature, all macaques underwent physical examination, tuberculosis testing, weighing, and tattooing. Body temperatures were measured by using a digital rectal thermometer (Filac 1500, Coviden, Dublin, Ireland) and the infrared thermometer at both the inguinal and chest locations.

The rectal thermometer was advanced 2 to 3 cm, and data were recorded by hand from the digital display. The infrared device (TempIR, Den of Goods, Gibraltar, Gibraltar) was placed approximately 5 cm from the skin of the inguinal or chest regions of the animals, and the temperature reading recorded by hand. These thinly haired sites were chosen to minimize hair interference. The noncontact infrared thermometer is capable of measuring body temperature, surface temperature, and ambient temperature, and has a high-temperature alarm.

Rectal and IR thermometers were calibrated after purchase and used according to the manufacturers' recommendations. Measurements from the infrared thermometer can be in degrees centigrade or Fahrenheit. Telemeterized macaques were removed from their cages and placed on a telemetry receiver, and the infrared, core, and rectal body temperatures were recorded consecutively. The telemetry body temperatures were captured by using Data Quest software, version 4.3 (Data Sciences International).

Statistical analysis (SAS/STAT 9.2, SAS Institute, Cary, NC) of temperature data for all animals used linear regression to determine whether a sex-associated effect was present. A P value of less than 0.05 was defined as being statistically significant. Given the absence of a sex-associated effect, data for both sexes were pooled for correlation analysis and pairwise comparison of mean values. No pain or distress was noted in any of the monkeys used in this project.

Results

The plot graph (Figure 1) was created by using the dataset of 205 macaques and compared rectal and IR thermometry. We found that readings from the chest location were most closely aligned with rectal measurements. The temperature data corresponding to the 2 groups of macaques, one comprising 9 animals (which yielded data by telemetry) and the other including all 205 animals, were submitted separately to the following inferential statistical analysis.

To assess the relationships between body temperatures obtained by telemetry and those obtained by infrared inguinal (Figure 2), infrared chest (Figure 3), and digital rectal (Figure 4) techniques ($n = 9$) and between rectal temperature and infrared inguinal and chest temperatures ($n = 205$ each technique; Figures 5 and 6, respectively), a test of regression lines equality was performed for each pairwise comparison of interest to determine whether a sex-associated effect was present during the correlation analysis. The results of these comparisons (Table 1) revealed no significance at the 5% level ($P > 0.05$). Consequently, the data from both sexes were pooled for the following corresponding regression analysis, correlation analysis, and means pairwise comparisons were conducted for pooled sexes.

For the regression analysis, the response variable 'telemetry' was regressed separately on each of the independent variables

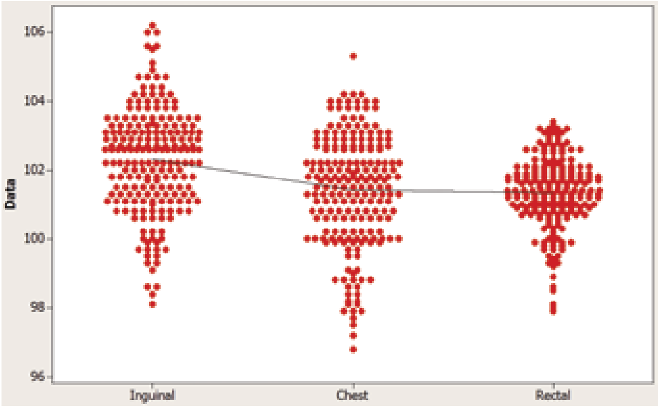

Figure 1. Inguinal, chest, and rectal temperatures of 205 macaques. This plot compares rectal and infrared thermometry and helped us to determine that readings from the chest location were most closely aligned with rectal measurements.

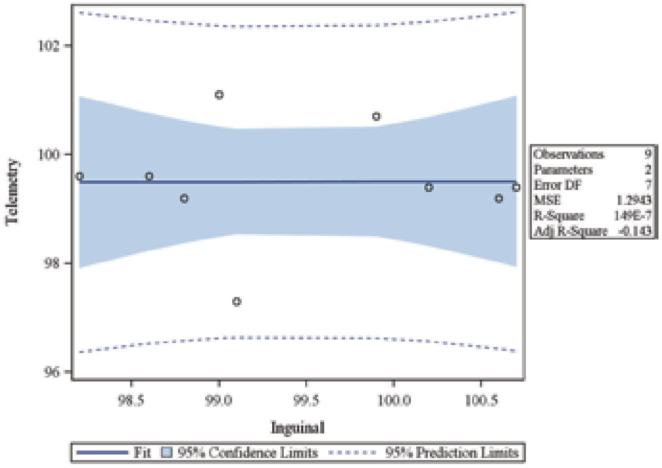

Figure 2. Regression of telemetry on inguinal: dataset containing 9 macaques.

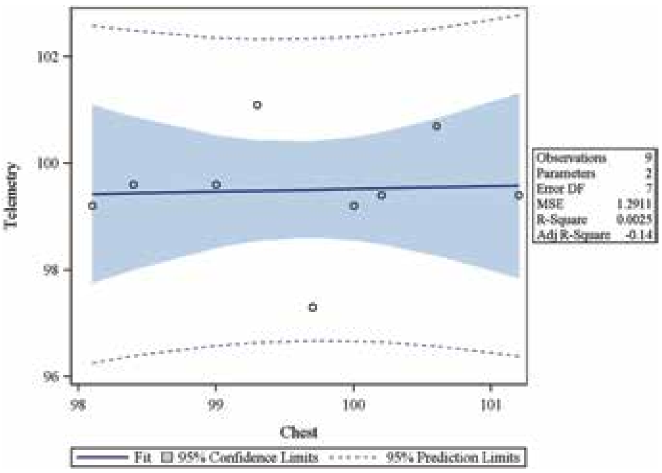

Figure 3. Regression of telemetry on chest: dataset containing 9 macaques.

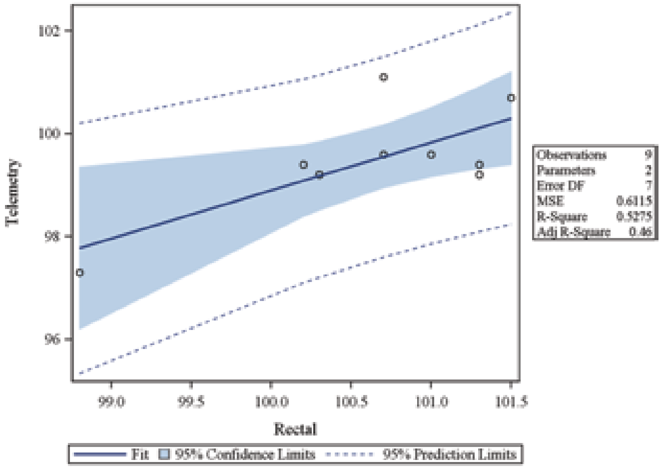

Figure 4. Regression of telemetry on rectal: dataset containing 9 macaques.

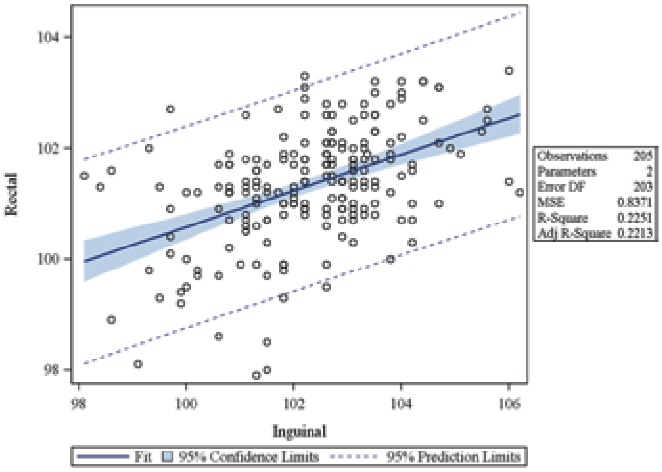

Figure 5. Regression of rectal on inguinal: dataset containing 205 macaques.

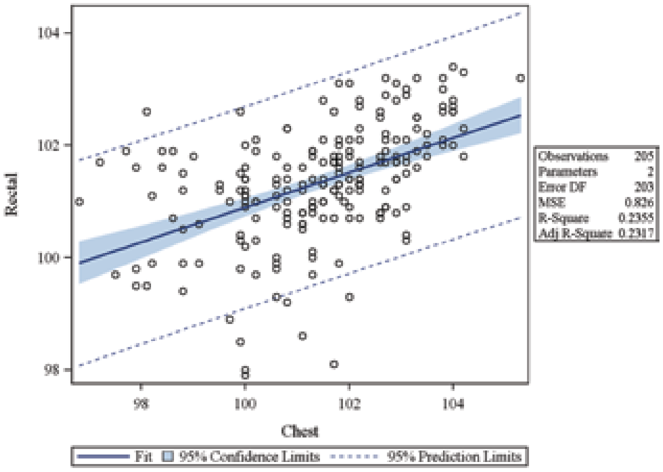

Figure 6. Regression of rectal on chest: dataset containing 205 macaques.

'inguinal,' 'chest,' and 'rectal' in the dataset containing 9 macaques, and the response variable 'rectal' was regressed on each of the independent variables 'inguinal' and 'chest' in the dataset containing 205 macaques. For accuracy assessments, the null hypotheses that the corresponding true regression line intercept and slope are respectively 0 and 1 were tested separately. In addition, for precision assessments, the null hypothesis that the corresponding true Pearson correlation coefficient is 0 was tested. The results of these tests are summarized in Table 2.

Table 1. Results of analysis for potential sex-associated effect

Dataset	Pairwise comparison	*P*
9 macaques	Telemetry compared with inguinal	0.8764
	Telemetry compared with chest	0.8378
	Telemetry compared with rectal	0.9159
205 macaques	Rectal compared with inguinal	0.1449
	Rectal compared with chest	0.7878

For both pairwise comparisons in the dataset with 205 animals, the *P* values (Table 2) indicate that the correlation coefficient was highly significantly different from 0 and that the regression line intercept and slope were highly significantly different from 0 and 1, respectively. However, for the dataset with 9 animals, the *P* values (Table 2) show that the 2 correlation coefficient were not significantly different from 0 for the pairwise comparisons between 'telemetry' and 'inguinal' and between 'telemetry' and 'chest' but did differ between 'telemetry' and 'rectal' (Figure 8). The regression line intercept and slope were barely significantly different from 0 and 1, respectively, for the pairwise comparison between 'telemetry' and 'chest' but did not differ from 0 and 1, respectively, for the pairwise comparisons between 'telemetry' and 'inguinal' and between 'telemetry' and 'rectal.'

Furthermore, paired *t* tests were conducted on both datasets of macaques to compare the temperature means obtained by using telemetry with those from infrared inguinal, infrared chest, and digital rectal thermography and to compare rectal thermography with inguinal and chest infrared thermography (Table 3). For the dataset with 9 macaques, the mean temperature acquired by telemetry was not significantly different from either of those acquired by infrared thermometry (Figures 7 and 8) but did differ the mean of rectal thermography (Figure 9). However, for the dataset containing 205 animals, the results show that the mean temperature for rectal thermography differed from the mean of inguinal (Figure 10) but not chest (Figure 11) infrared thermography.

Discussion

The basis for comparing cynomolgus macaque temperature data was to evaluate the differences between rectal, infrared (inguinal and chest), and implanted telemetry readings (core body temperature) in sedated healthy animals. The rectal temperature technique is somewhat invasive, given the slight risk of rectal trauma, and it takes 30 to 60 s to record the temperature. The infrared thermometer readings are instantaneous and require no contact with the animal. There are animal-wellbeing benefits to a noncontact approach to body temperature collection, such as the infrared and implanted telemetry devices we used. A positive correlation between data from these noncontact methods with digital rectal temperatures (standard method) would support the use of a noncontact method and thus decreased handling of and stress to our cynomolgus monkey population.

Our primary goal with this project was to develop a new standard procedure for collecting body temperatures in macaques to avoid the requirement of physical restraint or sedation, the potential for rectal trauma, and the time needed to collect rectal temperatures. In addition, using the infrared thermometer, we evaluated the macaques cageside, without removal, by gently squeezing them to the front of the cage and taking a reading approximately 5 cm from the chest or inguinal region. Compared with other methods, the no-touch infrared method is potentially less stressful and traumatic, especially for alert animals being hand captured for an examination. Overall, the results confirmed our hypothesis that infrared thermometry can be used in place of the rectal method, given that all readings were within the normal temperature range for cynomolgus macaques (98.6 to 103.1 °F; 37.0 to 39.5 °C).

The implanted telemetry device is placed into a muscular pocket between the internal and external abdominal oblique muscles in the flank of cynomolgus monkeys; therefore temperature readings likely closely reflect the core body temperature for comparison with data from the other methods we evaluated. Implanted telemetry is beneficial not only because of accurate core body temperature analysis but also because it reduces animal handling and the stress induced by hand capture. The disadvantages of telemetry include the needs for invasive surgery, pain management, and associated recovery time; the need for a receiver and computer software to capture the data; and limitations due to transmitter battery lifespan. Infrared thermometry is the next best method, compared with having to manually restrain cynomolgus monkeys and place a rectal temperature probe. Interestingly, the infrared measurements correlated more closely with those from implanted telemetry than with those from rectal thermometry in the current study, in which animals were sedated to remove the variable of manual restraint. In our experience, manual restraint of ill macaques leads to slightly higher temperatures than does cageside infrared thermography, which is consistent with the increased animal stress and movement associated with hand capture.

Data from the telemeterized cohort show that the mean of telemetry-acquired temperatures did not differ from either of the means of inguinal and chest infrared thermography but did differ from the mean of rectal digital thermography at the 1% significance level. In addition, for the nontelemeterized cohort, the mean of body temperatures collected through rectal thermography differed from that of inguinal infrared thermography at the 0.01% significance level but was similar to that from chest infrared thermography. Therefore, we are confident in using chest infrared thermography as our standard method for monitoring body temperature in our cynomolgus macaques.

We believe the inguinal temperature measurements were lower than those from rectal and infrared chest thermography possibly due to hair interference and the concave anatomy of the femoral groove compared with the flat surface of the chest. The vast majority of the macaques from which we collect temperature data are healthy, sedated, and removed from their primary enclosure, such that the 5-cm required distance can be accurately and consistently achieved.

We were successful in determining that sedated cynomolgus macaques can be accurately evaluated for body temperature by using the infrared thermometer. The statistical results revealed a correlation at the 5% significance level between infrared measurements (both inguinal and chest) with rectal temperatures in more than 200 macaques and between telemetry-acquired core body temperature and rectal thermography in 9 animals with functioning telemetry devices.

In the laboratory animal context, the infrared alternative to rectal thermometry is probably best used on sedated NHP, as in our study, but could be explored for alert temperature readings for animals in or outside of their primary enclosure. It is a practical alternative to rectal thermometry in our facility, where we consistently handle large numbers of sedated animals and can easily obtain temperatures with the infrared thermometer is placed 5 cm from the chest. However, 3 studies describing the use of IR thermometers in NHP had markedly different outcomes from our findings. For example, one study[13]

Table 2. Results of regression analysis

Dataset	Pairwise comparison	Pearson correlation coefficient	*P*		
			Correlation	Intercept	Slope
9 macaques	Telemetry compared with inguinal	0.00386	0.9925	0.0581	0.0582
	Telemetry compared with chest	0.04980	0.9028	0.0482	0.0480
	Telemetry compared with rectal	0.72630	0.0241	0.8822	0.8565
205 macaques	Rectal compared with inguinal	0.47450	<0.0001	<0.0001	<0.0001
	Rectal compared with chest	0.48524	<0.0001	<0.0001	<0.0001

Table 3. Results of paired *t* tests

Dataset	Means evaluated	Difference between means	*P*	95% confidence interval
9 macaques	Telemetry – inguinal	0.0444	0.9265	1.0318 to 1.1207
	Telemetry – chest	–0.1111	0.8219	–1.2126 to 0.9904
	Telemetry – rectal	–1.1444	0.0016	–1.7081 to –0.5808
205 macaques	Rectal – inguinal	–0.9681	<0.0001	–1.1558 to –0.7804
	Rectal – chest	–0.0795	0.4309	–0.2782 to 0.1191

evaluated a temporal artery thermometer in rhesus and cynomolgus macaques under sedation. Agreement between rectal and temporal artery temperature measurements indicated that values from arterial thermometry were lower than those from rectal thermometry by 1.57 °C. These differences were attributed to a decreased body surface temperature after the administration of an anesthetic agent. The authors determined that the arterial thermometer is satisfactory for clinical use.[13] The second study[2] compared noncontact IR thermometry and 3 subcutaneous temperature transponding microchips with rectal

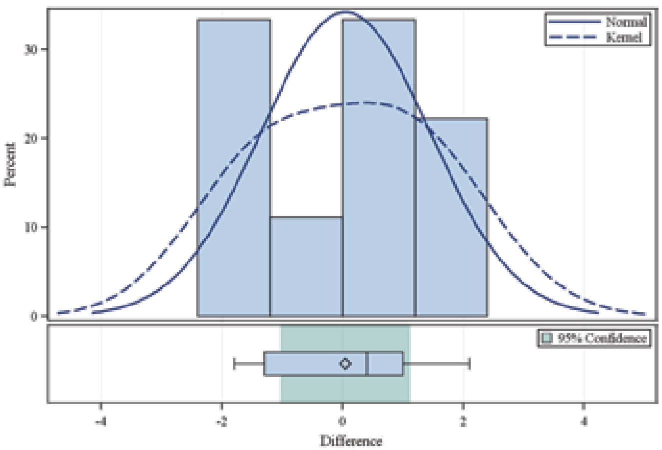

Figure 7. Comparison of mean temperatures from telemetry and inguinal infrared thermography: dataset containing 9 macaques.

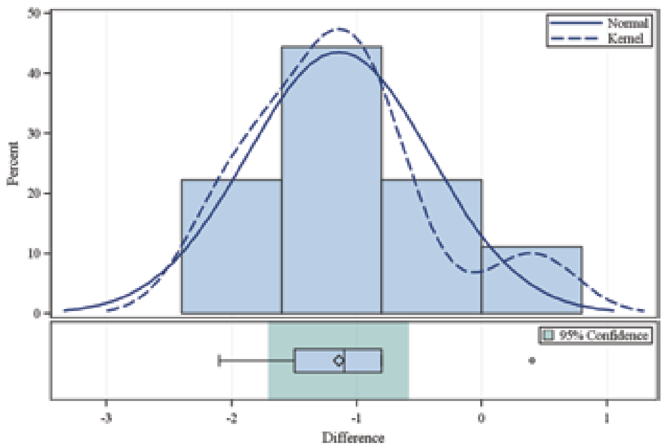

Figure 9. Comparison of mean temperatures from telemetry and rectal digital thermography: dataset containing 9 macaques.

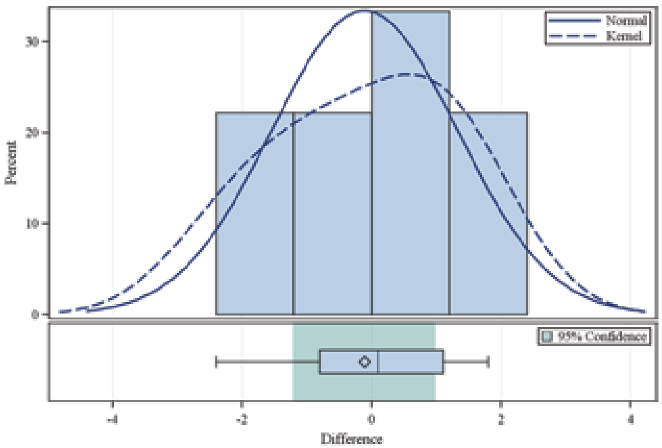

Figure 8. Comparison of mean temperatures from telemetry and chest infrared thermography: dataset containing 9 macaques.

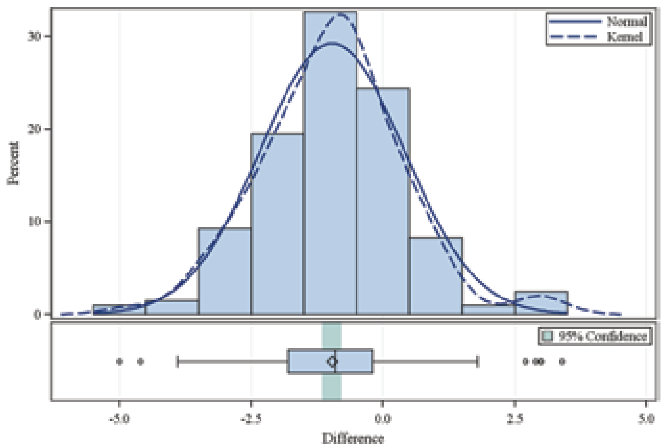

Figure 10. Comparison of mean temperatures from rectal digital thermography and inguinal infrared thermography: dataset containing 205 macaques.

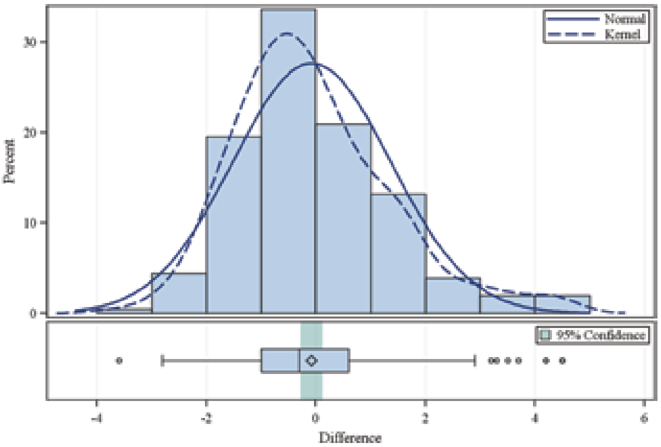

Figure 11. Comparison of mean temperatures from rectal digital thermography and chest infrared thermography: dataset containing 205 macaques.

thermometry in rhesus macaques. Temperatures obtained with microchips agreed more closely with rectal temperatures than did those obtained by using the infrared method. The infrared temperatures were both higher and lower than were rectal readings at different time points. The chest and abdomen were the areas targeted for infrared temperature readings, and the IR thermometer was held 15 cm from those areas.[2] In comparison, in our study, we clipped the hair from the chest and inguinal regions before taking the temperature, because hair interference will prevent accurate temperature readings. In addition, we followed the recommendation of the manufacturer of the IR thermometer in that we used and obtained temperatures at a focal distance of 5 cm from skin surface.

The third study[11] that yielded data that differed from ours compared rectal and infrared thermometry in cynomolgus macaques. The authors concluded that temperatures obtained by using the infrared technique did not correlate with rectal temperatures and was not a valid alternative. The study targeted the axilla, abdomen, shoulder, and face of the monkey, and resulting temperatures from the infrared device were lower than rectal measurements. The authors noted that using a shaved area of skin improved accuracy but did not feel that was a practical solution without sedation or restraint.[11] In our study, all macaques were clipped and sedated, to allow for accurate temperature recordings by infrared thermography, thus perhaps explaining the differences between our current report and the previous studies we presented.

Like previous groups,[11,13] we used the chest as a target area and found it to yield the data most closely correlated with rectal measurements in cynomolgus macaques. In comparison, data from infrared and digital thermometers in cats were weakly correlated (correlation coefficient, 0.62); the mean difference was 0.13 °F (0.07 °C); and the limits of agreement were 2.6 °F (1.43 °C) and –2.5 °F (–1.36 °C), which the authors deemed unacceptable for clinical purposes.[8] Another study found that tympanic infrared thermometry correlated well with traditional rectal thermometry in goats and sheep, but rectal temperatures were higher than tympanic and subcutaneous (transponder chip) temperatures in horses and sheep.[6] We similarly noted that rectal temperatures were higher than telemetry-acquired measurements in cynomolgus macaques. A comparison of rectal temperatures with infrared thermometry at different skin locations including the ear, back, tail, and sole in mice revealed that ear and back skin temperatures were closely correlated with the rectal temperatures in various situations. Conversely, temperatures from sole skin and tail skin were much lower and much more variable than were rectal temperatures.[10]

In conclusion, we have developed a simplified and more efficient alternative to digital rectal thermography for cynomolgus macaques and will exclusively use noncontact infrared thermography for future evaluations. We demonstrated that the temperature data obtained from in healthy macaques correlated closely among all 3 methods evaluated, with a trend toward lower temperatures from infrared thermography and telemetry. Infrared thermography should be evaluated for use in other preclinical species such as dogs and rabbits, to simplify examination and body temperature evaluation in those animals. Although the current cohorts (naïve and implanted) were considered definitive for the current investigation, we will evaluate the correlation between these methods in macaques that have an altered health status.

Acknowledgments

Thanks to Charles River Laboratories for providing us the funding and ability to perform a method analysis and essentially improve the wellbeing of our animals.

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