Evaluating the Cardiovascular Effects of Lipid Nanoparticle-Encapsulating mRNA Therapeutics in Cynomolgus Monkeys Using Telemetry and Star-Oddi™ Logger Devices

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Introduction

- The use of lipid nanoparticle (LNP)-encapsulated messenger RNA (mRNA) vaccines and therapeutics is a growing modality for various indications and the COVID-19 pandemic highlighted the effectiveness of mRNA vaccines in preventing severe illness caused by viral infections. Currently, there are ongoing or planned clinical trials investigating the use of mRNA-based therapeutics for chronic diseases.
- Since LNPs are comprised of novel lipid excipients and some therapeutic indications may require repeat dosing, it is important to evaluate the cardiovascular (CV) safety of LNP components.
- Traditionally, CV assessments are conducted using telemetry implantation or external jacketed models. However, an alternative approach involves the use of Star-Oddi data loggers, which are subcutaneously implanted to collect heartrate (HR) and body temperature (BT) data. Compared to traditional telemetry models, Star-Oddi data loggers can offer several advantages; they are less invasive while still enabling continuous and uninterrupted data collection of HR and BT. By utilizing Star-Oddi data loggers, it may be possible to investigate LNP-related changes in HR and BT earlier in drug development. This approach aligns with the 3Rs goals (Replacement, Reduction, Refinement) in animal research by providing a minimally invasive option.

Goals of the study

- Compare the sensitivity of subcutaneously implanted Star-Oddi devices to standard implanted telemetry devices at baseline levels within the same animal.
- Determine if Star-Oddi devices can detect changes in HR and BT after the administration of LNP-encapsulated mRNA to nonhuman primates (NHPs) when compared to standard implanted telemetry devices.

Methods

- Two male cynomolgus NHPs (Alice, Tx) were dually implanted within the extra-peritoneal pocket with Data Sciences International™; PhysioTel Digital L21 (DSI) telemetry implants and subcutaneously with Star-Oddi DST Milli-HRT (Star-Oddi) data loggers at the Test Site (Madison, WI) to assess baseline HR and BT levels continuously for 72 hours.
- Four male cynomolgus NHPs (Alice, TX) were implanted intra-abdominally with DSI (TL11M2 or M3) transmitters at the Test Site (Senneville, QC) prior to the test article administration using a dose escalating design. HR and BT were continuously and contemporaneously monitored for 26 hours. Changes in systemic blood pressure and electrocardiographic (ECG) duration/intervals were also evaluated.
- Five male cynomolgus NHPs (Alice, TX) were implanted subcutaneously in the dorsal scapular region with Star-Oddi Data Loggers at the Test Site (Madison, WI) prior to test article administration using a Latin square dosing design. HR and BT were collected continuously for 25 hours.
- After completion of study activities, Star-Oddi data logger implants were removed and the data was downloaded from each device.
- Blood was collected and processed to plasma at multiple timepoints postdose to assess the kinetic timeline for both LNP and translated protein. Samples were collected from separate animals as not to confound HR and BT.
- Animal care and use was conducted in alignment with regulatory requirements at AAALAC accredited animal facilities.
- **Phase 1:** After implantation of both devices, animals were monitored with limited interruption for 72-hours
- **Phase 2:** Animals were administered mRNA-1944 via 60-minute intravenous infusion at either dose levels of 0, 0.3, 1, or 3 mg/kg for animals implanted with DSI transmitters or 0, 1, or 3 mg/kg for animals implanted with Star-Oddi data loggers. mRNA-1944, an LNP-encapsulated mRNA, was utilized due to the encoded protein, a chikugunya monoclonal neutralizing antibody, not being pharmacologically active in normal animals. Results were attributed to the LNPs. This Phase was conducted over two separate studies, one to assess the Star-Oddi data loggers and the other to assess the DSI transmitters.
- **Statistical Analysis:**
  - **Phase 1:** Mean, Standard Deviation (SD) and Observed Range (Range) were assessed for HR and BT from each animal.
  - **Phase 2:** Data were binned into 5 time periods, and HR and BT were analyzed for each time period using analysis of variance (ANOVA), investigating differences due to treatment. Fitted period means for each dose level were calculated using the parameter estimates from the ANOVA model. Comparisons with vehicle control were made using the fitted means. Confidence intervals (CI) for these treatment comparisons were also calculated. If zero is not included within the 95% confidence interval, this indicates statistical significance at the 5% level (i.e., $P \leq 0.05$). The smallest statistically detectable difference (SSDD) was calculated as the smallest Fisher's least significant difference (LSD, i.e., half width of the 95% confidence intervals) and the median value was reported.

Results

Phase 1:

- Both devices were able to detect diurnal variations in HR and BT generated over 72 hours from in each animal. Data generated suggests that sensitivity of each device is comparable (Table 1).

	Star-Oddi				DSI			
	HR (beats/minute)		BT (°C)		HR (beats/minute)		BT (°C)	
Animal	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	78	14.5	35.4	0.9	75	11.25	36.9	0.7
2	120	16.25	35.4	0.7	118	13.5	37.4	0.9

Table 1. Means and SDs of heartrates and body temperatures from Star-Oddi and DSI Telemetry devices implanted in each animal . Values were averaged over the 72 hour collection period for baseline measurements. The mean HR and BT data captured from both implanted devices displayed similar results for each animal, with an average difference of 2-3 beat/minute for HR and 1.5-2°C for BT.

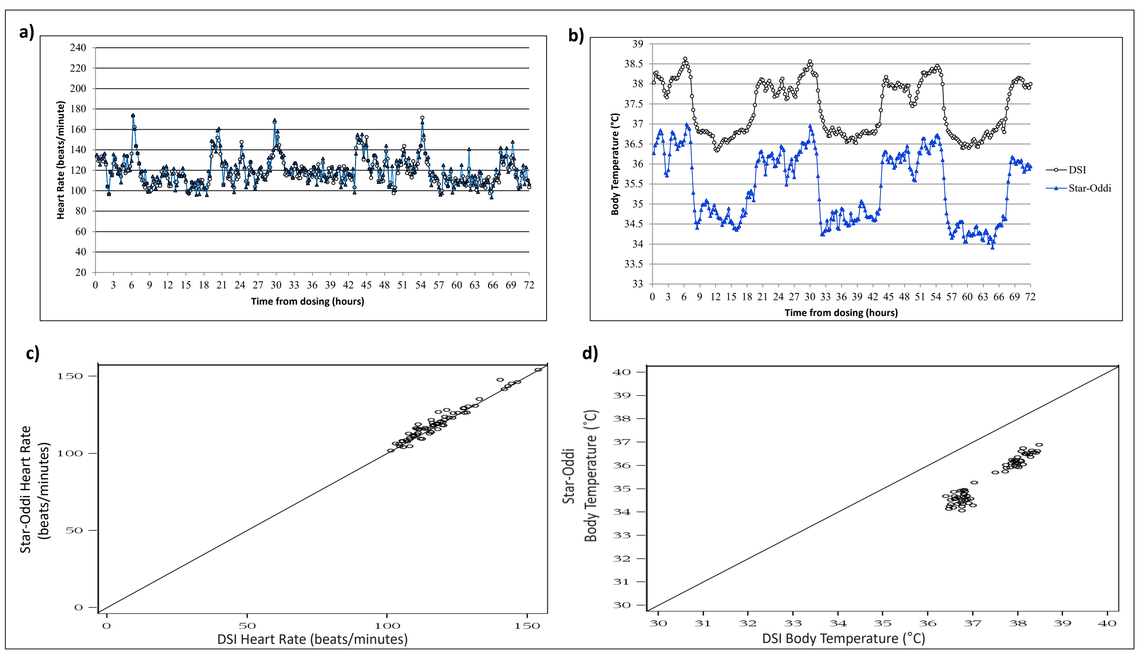
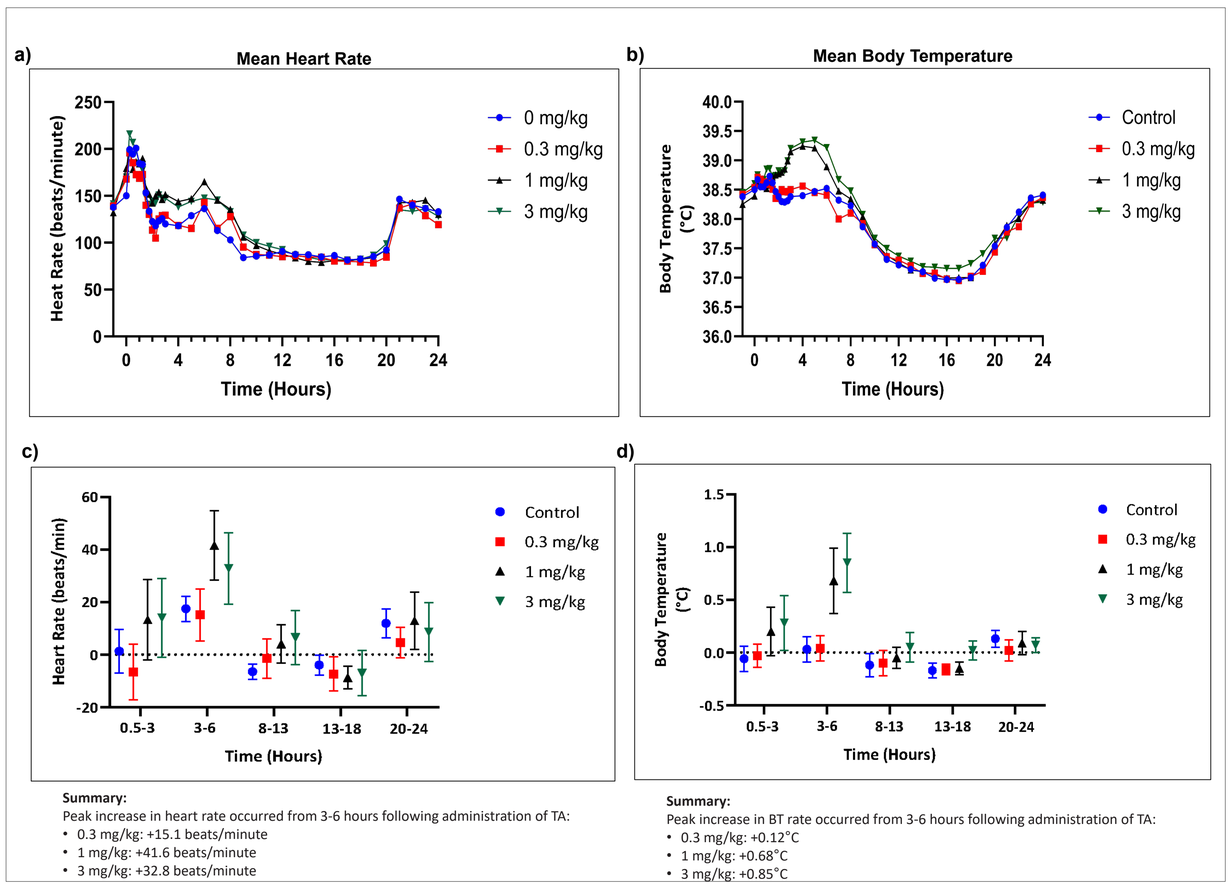


Figure 1. HR and BT values of representative individual animal collected continuously for 72-hours.
60-minute mean time course data for HR (Figure 1a) demonstrate how closely HR data correlate between Star-Oddi and DSI telemetry implants over time. Mean BT time course data (Figure 1b) also demonstrate similarities in temperature changes but a difference between the two devices was consistently evident. 60-minute correlation plots in which the solid line represents the line of unity, show a 1:1 correlation for HR (Figure 1c) and a linear relationship for BT measures (Figure 1d) between both devices with Star-Oddi BT measurements consistently ~2°C lower as would be expected with subcutaneous implantation.

Phase 2:

- Dose dependent mRNA-1944-related increases in HR and BT were detected from both devices (Figures 2 and 3).
- Timing of HR and BT changes were consistent between each device.
- Sensitivity for detection of HR and BT changes were similar in both devices (SSDD values; Table 2).
- Kinetic timeline was determined for LNP and the translated protein (Figure 3).

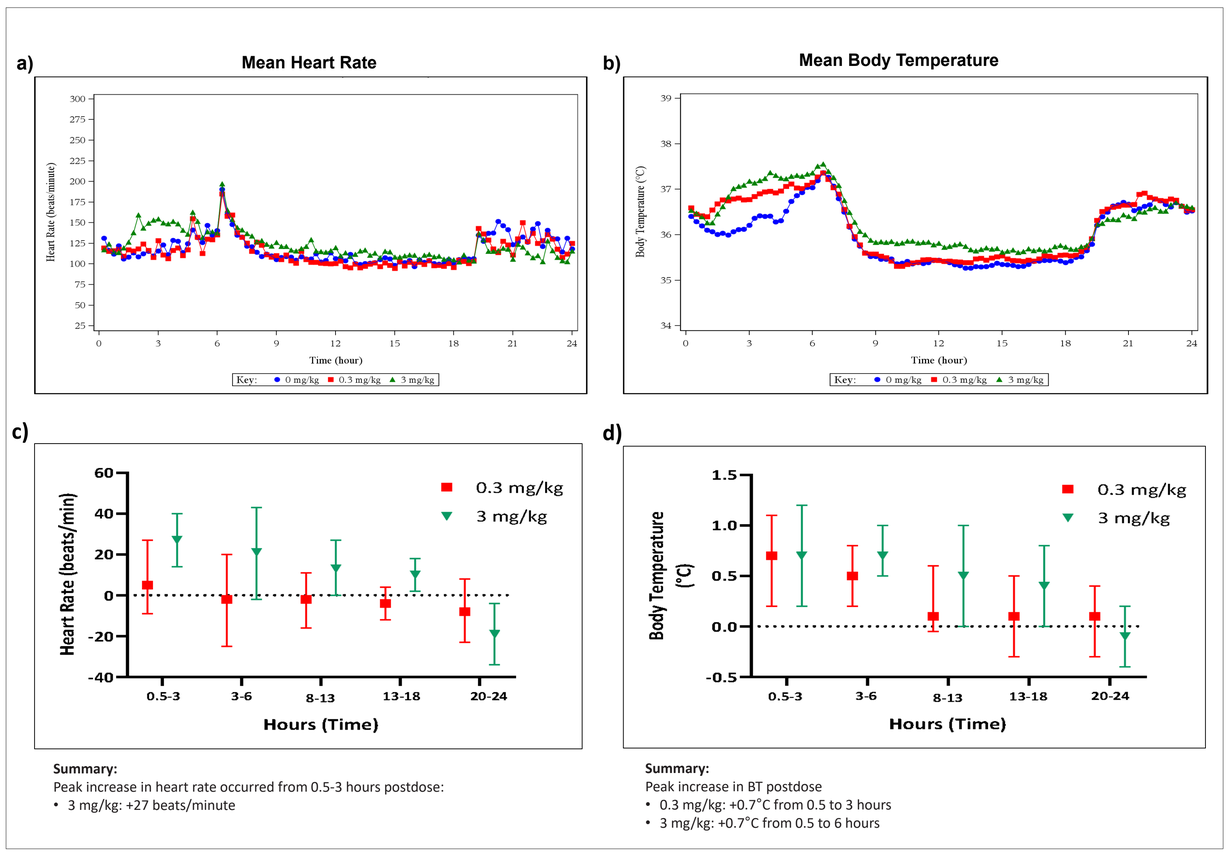
Disclosures
This study was funded by Moderna, Inc. JW, KG, and SG are employees of Moderna, Inc. and may own stock/stock options in the company.
Acknowledgements
Portions of this project were conducted at Charles River Laboratories, Inc.



Summary:
Peak increase in heart rate occurred from 3-6 hours following administration of TA:
- 0.3 mg/kg: +15.1 beats/minute
- 1 mg/kg: +41.6 beats/minute
- 3 mg/kg: +32.8 beats/minute

Summary:
Peak increase in BT rate occurred from 3-6 hours following administration of TA:
- 0.3 mg/kg: +0.12°C
- 1 mg/kg: +0.68°C
- 3 mg/kg: +0.85°C

Figure 2. Mean HR and BT time course data and comparison of test article effects following administration of mRNA-1944 via 60-minute intravenous infusion of animals implanted with DSI Telemetry devices. Mean time course data following the administration of mRNA-1944 at dose levels 1 and 3 mg/kg shows increases in HR (Figure 2a) are detected from 1.25 to 12 hours postdose and increases in BT (Figure 2b) are detected from 1.5 to 8 hours postdose. HR and BT differences from pretreatment are shown as a function of time. Changes in both HR (Figure 2c) and BT (Figure 2d) peaked between 3 and 6 hours from the initiation of dosing. Overall, administration of mRNA-1944 at dose levels of 1 and 3 mg/kg resulted in a transient increase of HR and BT that resolved by 24-hours postdose. In addition to HR and BT, these animals were evaluated for changes in systemic blood pressures and ECG duration/intervals. Similar to HR and BT, changes in arterial blood pressures were observed at dose levels of 1 and 3 mg/kg but resolved within 24 hours. There were no ECG waveform abnormalities noted, however; one animal showed decreases in PR and QT intervals secondary to the increased heart rate observed at the mid and high dose levels.



Summary:
Peak increase in heart rate occurred from 0.5-3 hours postdose:
- 3 mg/kg: +27 beats/minute

Summary:
Peak increase in BT postdose
- 0.3 mg/kg: +0.7°C from 0.5 to 3 hours
- 3 mg/kg: +0.7°C from 0.5 to 6 hours

Figure 2. Mean HR and BT time course data and comparison of test article effects following administration of mRNA-1944 via 60-minute intravenous infusion of animals implanted with Star-Oddi data loggers. Mean time course data following the administration of mRNA-1944 at a dose level of 3 mg/kg shows increases of HR (Figure 3a) from 0.5 to 18 hours postdose and increases in BT (Figure 3b) dose levels of 0.3 and 3 mg/kg from 0.5 to 18 hours postdose. HR and BT differences from control are shown as a function of time. Changes in HR (Figure 2c) peaked between 0.5 and 3 hours postdose changes in BT (Figure 2d) peaked between 0.5 to 6 hours postdose. Overall, administration of mRNA-1944 at 3 mg/kg resulted in a transient increase of HR and administration of 0.3 and 3 mg/kg resulted in a transient increase of BT. Changes in HR and BT resolved by 24 hours postdose.

	SSDD (sensitivity)			
	Star-Oddi HR (beats/minute)	DSI HR (beats/minute)	Star-Oddi BT (˚C)	DSI BT (˚C)
Median	13.75	17	0.40	0.34

Table 2. Comparison of smallest statistically detectable differences (SSDD) for heart rate and body temperature data acquired with Star-Oddi data loggers and DSI telemetry implants.

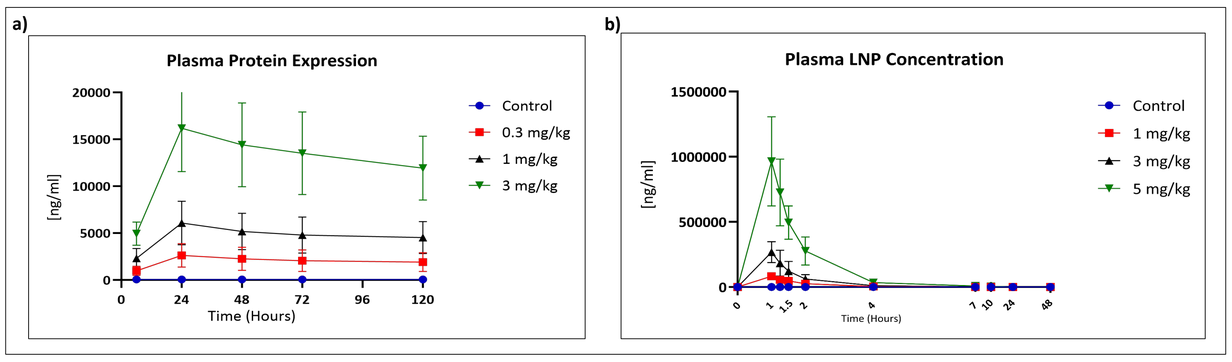


Figure 3. Concentration of expressed protein and LNP levels in plasma following the administration of test article via 60-minute intravenous infusion. Protein levels assessed by analyzing plasma collected at 6, 24, 48,72, 96, and 120 hours postdose (Figure 3a). Data indicates that the highest observed protein concentration in plasma occurs at 24-hours postdose. The level of LNP in plasma was assessed by analyzing plasma collected predose and at 1, 1.25, 1.5, 2, 4, 7, 10, 24, and 48 hours postdose (Figure 3b). Data indicates that the observed highest concentration of LNP in plasma occurs at 1-hour postdose.

Conclusions

- Baseline HR and BT data generated with Star-Oddi data loggers and DSI telemetry implants were highly correlated.
- mRNA-1944-related changes in HR and BT were detected in NHPs and could be assessed using both technologies.
- The occurrence of HR an BT changes are aligned with the observed highest concentration of the LNP which indicates that these changes are related to the LNP and not the expressed protein.
- SSDD values for detection of changes in HR and BT were similar between Star-Oddi data loggers and DSI telemetry implants.
- The CV effects of mRNA-1944 administration were mild and transient in nature.
- Star-Oddi devices offer a less invasive option for early experimental assessment of LNP-induced changes in HR and BT in nonhuman primates, consistent with 3Rs principles.

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