5-2024

Development and Validation of a Noninvasive, Portable, and Low-Cost Device to Detect Peripheral Artery Disease

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Development and Validation of a Noninvasive, Portable, and Low-Cost Device

to Detect Peripheral Artery Disease

By

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A thesis submitted in partial fulfillment of the requirements of the

University of South Alabama Honors College and the

Bachelor of Sciences in Mechanical Engineering degree in the

William B. Burnsed, Jr. Department of Mechanical, Aerospace, and Biomedical Engineering

University of South Alabama

Mobile

May 2024

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Joshua Keller

Committee Member: Dr. Josh Keller

Honors Dean, Douglas Marshall

DEDICATION

This thesis is dedicated to my father, David Nelson, for his unwavering love and encouragement throughout my life. His support means more to me than he knows. I'm honored that I was able to be a part of making his research project come to life.

ACKNOWLEDGEMENTS

The completion of this project would not have been possible without the support of many people. I want to express my appreciation and gratitude to the following:

Dr. Michael Francis, my primary mentor, who guided me through my undergraduate research career. He was always willing to give me suggestions and encouragement, and I truly appreciate the excitement and insight he brought to every meeting. I am very grateful for his mentorship.

Dr. Josh Keller, who always welcomed me into his lab and was willing to help me, whether it was with poster-editing or volunteering for an experiment. I always looked forward to working with him and his lab members.

Dr. David Nelson, my father, who served as a constant source of guidance and support on this project and throughout my life. I am so grateful for his encouragement and willingness to help me, and I am excited to follow in his footsteps by pursuing a Ph.D.

My sister, Paige, for listening to my rants on FaceTime nearly every day. I am lucky to have such a supportive sister who I love spending time with, and who will sit on the phone with me for hours even in complete silence. (And proofread every paper I write. Except for this one.)

Michael Roque, for his love and support over the past several years. He has helped me realize how much I can achieve and has supported me throughout all the hills and valleys of the past few years. He is always a source of joy in my life.

And last, but not least, my mother, Nancy Nelson, who is such an amazing role model. I am so lucky to have a mother who I can always count on. I would not have made it through college without you. Thank you for always thinking of me, whether it's baking me gluten-free brownies or texting me when your plane lands.

ABSTRACT

Peripheral Artery Disease (PAD) is a progressive cardiovascular condition characterized by atherosclerosis in the extremities. It affects up to 10 million adults in the United States and is associated with an elevated risk of heart attack and stroke. Up to 50% of people with PAD are asymptomatic and undiagnosed. This presents the need for a rapid, inexpensive, and noninvasive screening tool that can be easily used to diagnose PAD. We developed a device, REFLO (Rapid Electromagnetic FLOw device), to detect low blood flow and diagnose PAD. The device uses low-power radio frequency energy (35 GHz) to heat the skin and measure the subsequent temperature change. There is a relationship between skin surface temperature and volumetric blood flow in the skin, and we hypothesized that the rate of skin heating in response to millimeter wave irradiation is a function of the underlying volumetric blood flow.

This work presents the results of two cohorts of controlled flow experiments on humans. Each experiment was performed as a series of 3-minute heating periods followed by 3-minute natural cooling periods during baseline, occluded, and post-occluded hyperemic flow. Nonlinear regression analysis was used to fit temperature data and obtain a thermal constant, *k.* In the pilot study, N = 7 and each subject volunteered for 1 visit. During the heating period, significance was observed between the *k*-values in the baseline and post-occlusion periods (p = 0.0039). During the cooling period, significance was observed between the baseline and occlusion periods (p = 0.0014), as well as between the occlusion and post-occlusion periods (p = 0.0469). In the reliability study, N = 5 and each subject returned for 3 visits. During the heating period, significance was observed between the baseline and post-occlusion period (p = 0.0128), as well as between the occlusion and post-occlusion periods (p = 0.0160). During the cooling period, significance was observed between the baseline and occlusion periods (p = 0.0004), as well as between the baseline and post-occlusion periods (p = 0.0044). Two-way ANOVA revealed that flow and subject had a significant effect on the rate of skin heating and cooling, while visit had no effect.

Results suggest that millimeter wave irradiation can be used to distinguish between different volumetric blood flow rates in humans. Utilizing the rate of skin cooling rather than skin heating has proven to be more consistent for distinguishing flow rates. Future clinical testing and device modifications will improve REFLO’s ability to distinguish between flow rates and evaluate the device’s ability to discern PAD patients from individuals without cardiovascular disease.

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LIST OF ABBREVIATIONS

PAD	Peripheral Artery Disease
ABI	Ankle-Brachial Index
REFLO	Rapid Electromagnetic Flow device
NIRS	Near-Infrared Spectroscopy
RF	Radio Frequency

LIST OF FIGURES

INTRODUCTION

Peripheral Artery Disease (PAD) is a progressive condition caused by plaque accumulation on the artery walls of the extremities, most commonly the lower legs. Non-invasively assessing blood flow in the skin could be useful in diagnosing PAD. Persons at high risk for PAD include those with diabetes, those who smoke, and those over the age of 65. They are at an elevated risk of heart attack and stroke [1,2]. At 5 years, approximately 20% of PAD patients will experience a nonfatal cardiovascular event, and 15-20% of PAD patients will die, typically from cardiovascular causes [3,4]. While some PAD cases manifest symptoms such as claudication and limb ischemia, up to 50% of persons with PAD are asymptomatic and undiagnosed [5]. Left untreated, PAD becomes increasingly severe and heightens the risk of serious health complications [1,2]. Early intervention greatly improves outcomes for PAD patients by mitigating disease progression. Treatment options include statins to reduce cholesterol in the blood and prevent plaque buildup on artery walls [6,7]. Lifestyle modifications can be implemented early to slow disease progression [8].

Despite widely available treatment, PAD is severely under-diagnosed. Current clinical recommendations are that anyone over the age of 65, or over 50 with one or more risk factors, be screened for PAD using the Ankle-Brachial Index (ABI) [9]. The ABI is a noninvasive test in which the systolic blood pressure at the ankle is compared to the systolic blood pressure at the arm to identify low blood flow in the legs. An ABI of <0.9 constitutes a diagnosis of PAD. ABI is not reimbursable by insurance when performed on asymptomatic patients. Conducting an ABI test also requires an additional clinic visit for the patient, and insufficient resources exist worldwide to perform a 30-minute ABI test on everyone over the age of 65.

The limitations of the ABI and the under-diagnosis of PAD present the need for a rapid, inexpensive screening tool usable by people of any skill level to accurately screen for PAD. One possible solution is to exploit the relationship between skin surface temperature and blood flow in the underlying tissue during radio frequency (RF) heating [10,11]. This could yield a noninvasive and simple blood flow measurement device. Static thermal images show that reduced blood flow correlates to lower skin temperature under controlled conditions [12], but factors such as skin thickness, ambient temperature, and internal body temperature can affect skin surface temperature readings [13]. This makes static thermographic measurements unreliable for relating body temperature and blood flow. Using similar principles, thermographic measurements can be taken continuously over a period in response to a stimulus to observe subsequent temperature change. The curve-fit time constant can be obtained and used as a normalized metric for blood flow measurement, which removes the effect of limiting factors present in static measurements [12,14]. We developed a device, REFLO (Rapid Electromagnetic FLOw device), that directs low-power radio frequency energy at the skin to provoke shallow heating and simultaneously measures the skin surface temperature change. Radio frequency energy in the K_a band (26.5-40 GHz) is a safe and effective tool for shallow heating of the skin, penetrating to a depth of <1mm below the surface [15]. We hypothesize that the rate of skin heating in response to millimeter wave irradiation is a function of the underlying volumetric blood flow. This work presents the results of two cohorts of controlled flow experiments on humans. The device employs a small microstrip antenna and non-contact infrared (IR) temperature sensor operated under computer control.

METHODS

System Design

The REFLO vascular assessment device was designed to measure blood flow in the skin by measuring the rate of skin temperature change in response to an RF radiation stimulus and correlating this to the amount of blood flow in the underlying tissue. The device emits RF energy using a microstrip antenna ($1\ cm^2$) driven by a transmitter operating at 35 GHz (continuous wave). Skin temperature is measured using a small infrared (IR) temperature sensor (MLX90614, Melexis Technologies NV, Ypres, Belgium). These components were integrated in a compact polylactic acid (PLA) housing that sits atop the skin, created using a three-dimensional printer and designed to maintain a 38-degree angle between the antenna and skin surface. The antenna consists of a rectangular copper patch (2.6 mm x 2.9 mm) separated from the ground plane by a 0.8 mm dielectric substrate layer (RT/Duroid® 5880, Rogers Corp., Chandler, AZ). The substrate and ground plane surfaces are 12 mm x 12 mm. The antenna was driven by a transmitter consisting of an oscillator and an RF power amplifier. The system was driven by an Arduino microcontroller, which also managed data collection. An automatic overheat shutoff feature was implemented in the software to avoid burning the patient's skin. A block diagram of the system is shown in Figure 1. The device transducer is shown in Figure 2.

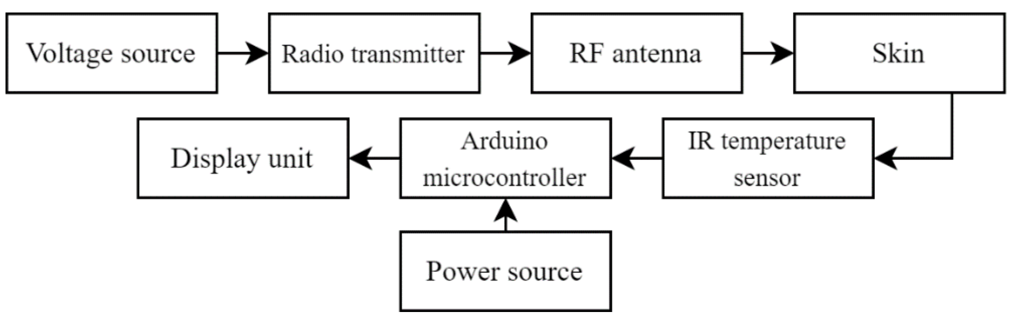

Figure 1. Block diagram of system design. A battery provides voltage to the transmitter, which allows radio frequency energy to be directed at the skin. The IR temperature sensor measures the skin surface temperature, and the temperature readings are stored on an Arduino. A laptop computer provides power to the Arduino and displays data.

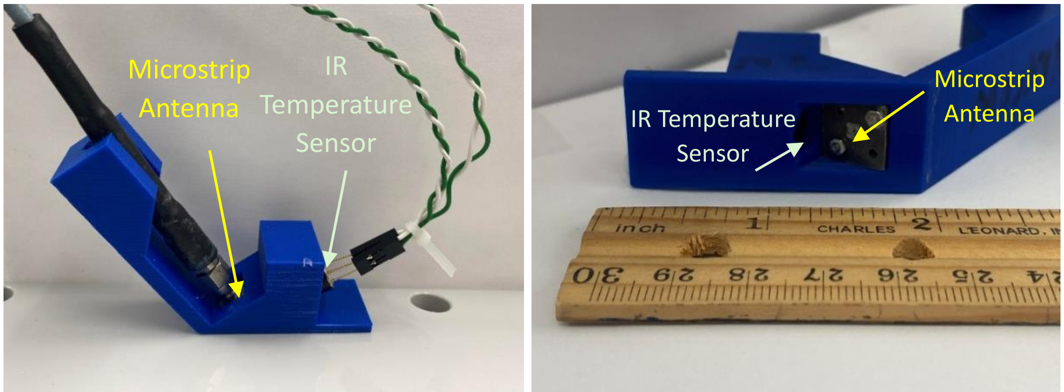

Figure 2a (left) and 2b (right). REFLO transducer. In Figure 2a, the cable attached to the microstrip antenna is shown on the left side of the transducer, while the infrared temperature sensor is shown on the right side of the transducer. Figure 2b shows the bottom of the transducer. This three-dimensionally printed chassis sits atop the skin of the lateral calf and is secured with medical tape. The portion of the transducer in contact with the skin measures about 2 inches. A standard ruler is shown for scale.

Study protocol

Human subject experiments were conducted under protocols approved by the University of South Alabama Institutional Review Board 2088699 and 1876484 (pilot). During the studies, the REFLO vascular assessment device was tested alongside a near-infrared spectroscopy (NIRS) device, an accepted indicator of vascular function [16]. The studies were designed to collect data in healthy, young adults to develop rationale for future studies within clinical populations. Adults of both sexes between the ages of 18-31 with no existing cardiovascular conditions were recruited. In the pilot study, 7 measurements were collected in different subjects. The pilot study assessed the device's ability to differentiate between three blood flow conditions. In the reliability study, measurements were collected in 5 subjects of a separate cohort, with each subject returning for a total of 3 visits. The reliability study assessed the significance of flow, visit, and subject for reliably distinguishing between blood flow conditions. Each experiment was performed in a climate-controlled environment, and subjects were asked to maintain consistency of individual variables, such as caffeine intake and exercise, before each visit.

At the beginning of each visit, the patient acclimated to the room conditions in a prone position for 5-10 minutes. Measurements were performed on the lateral mid-calf region, shown in Figure 3. The skin was dry shaved and cleaned with an alcohol wipe and allowed to dry. The REFLO antenna was cleaned with an alcohol wipe and the transducer was affixed to the lateral calf using medical tape. The NIRS device was affixed to the medial surface of the calf using medical tape and covered with Coban wrap to block light from interfering with the measurements. Data were obtained for each of three flow conditions for each subject: (1) normal, or baseline, (2) occluded, and (3) post-occlusion reperfusion flow. Occlusion was accomplished using rapid-inflation cuffs (E20 Rapid Cuff Inflator, Hokanson Inc., WA, USA). at the thigh and the ankle, as shown in Fig. 3.

Figure 3. Experimental setup. The REFLO and NIRS devices are secured to opposite sides of the lateral calf with clear medical tape. The REFLO transducer is connected to the Arduino microcontroller and REFLO transmitter (out of frame). Cuffs are secured at the ankle and upper thigh for the vascular occlusion protocol.

Each experiment was performed as a series of 3-minute heating periods followed by 3-minute natural cooling periods during baseline, occluded, and post-occluded hyperemic flow. Skin temperature and NIRS measurements were continuously recorded throughout the entire experiment and displayed on a laptop screen. After the baseline heating-cooling period (6 minutes), the cuffs at the ankle and thigh were rapidly inflated (<0.3 seconds) to occlude blood flow. This occlusion protocol is well-accepted in the literature and has been performed for up to 20 minutes with no adverse events reported. A 1-minute rest period was given to allow blood flow to normalize, after which occlusion was maintained for another heating-cooling period (6 minutes). The upper thigh cuff was then deflated to provoke rapid blood re-entry into the leg, while the ankle cuff remained inflated to prevent blood pooling in the foot. Another heating-cooling period followed, after which both cuffs were deflated. Each experiment lasted 19 minutes.

Data processing and analysis

Data was analyzed using GraphPad Prism software. REFLO data was separated into six categories for each subject: baseline heating and cooling, occluded heating and cooling, and post-occluded heating and cooling. REFLO data was separated into baseline, occluded, and post-occluded periods for each subject. Data was fit using nonlinear regression analysis to determine the thermal constant, k, for each heating and cooling period. An exponential plateau model was used for the heating period and a one-phase decay model was used for the cooling period. k is equivalent to the inverse of the time constant τ. Goodness of fit was evaluated via R^2 values. k-values were compared using two-way ANOVA and Tukey's multiple comparisons test. Statistical significance was defined as $p<0.05$.

RESULTS

Skin surface temperature measurements were recorded at 1 s intervals for the duration of each experiment. A thermal model of RF skin heating has been previously established [17,18]. A value k, equivalent to the inverse of the time constant τ for the system, was obtained for the heating and cooling periods during baseline, occlusion, and post-occlusion. Higher τ values indicate slower heating and cooling; accordingly, higher k-values indicate faster heating and cooling.

Pilot Study (Cohort 1)

7 subjects were recruited to participate in the pilot study. The k-values computed from the temperature data are shown in Figure 4.

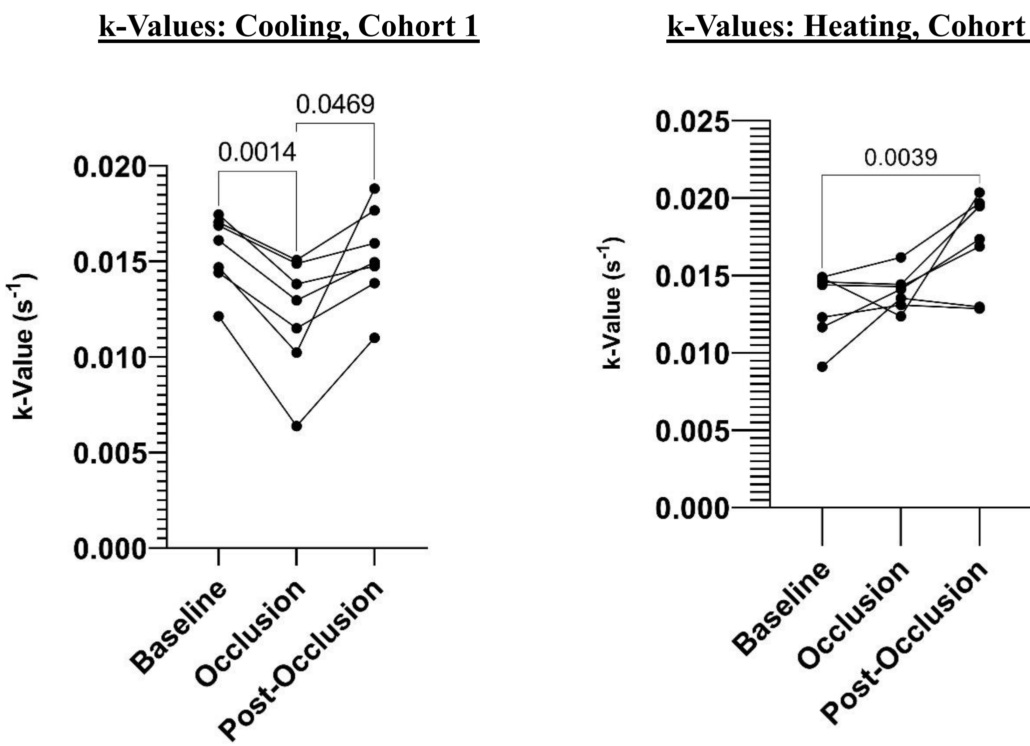

Figure 4. Pilot study thermal constants. Scatter plots show thermal constants during baseline, occluded, and post-occluded cooling (left) and heating (right) periods. Subjects received REFLO measurements during the cooling and heating period during baseline, occluded, and post-occluded conditions. Temperature curves were subjected to nonlinear regression analysis to obtain the rate constant k. Higher k values indicate a faster response. An exponential plateau model was used for the heating period and a one-phase decay model was used for the cooling period. Out of 21 data sets (baseline, occluded, post-occluded heating and cooling are each separate data sets), 16 sets had R^2 values greater than or equal to 0.90, and 3 of the remaining sets had R^2 values greater than or equal to 0.80. One-way ANOVA and Šídák's multiple comparisons test were used to determine significance between k-values during baseline, occluded, and post-occluded periods. During cooling, significance was observed between baseline and occluded periods (p=0.0014) and between occluded and post-occluded periods (p=0.0469). During heating, significance was observed between baseline and post-occluded periods (p=0.0039). N = 7 for all measurements.

The average k-values during heating were found to be $k_{baseline}$ = 0.013111, $k_{occlusion}$ = 0.013996, and $k_{post-occl}$ = 0.017083. The average k-values during cooling were found to be $k_{baseline}$ =

0.01553, $k_{occlusion}$ = 0.012122, and $k_{post-occl}$= 0.015286. Tukey's multiple comparisons test revealed that during cooling, significance was observed between the baseline and occlusion periods (p = 0.0014), as well as between the occlusion and post-occlusion periods (p = 0.0469). During heating, significance was observed between the baseline and post-occlusion periods (p = 0.0039).

Reliability Study (Cohort 2)

The average k-values across subject and visit computed from the reliability study are shown in Figure 5.

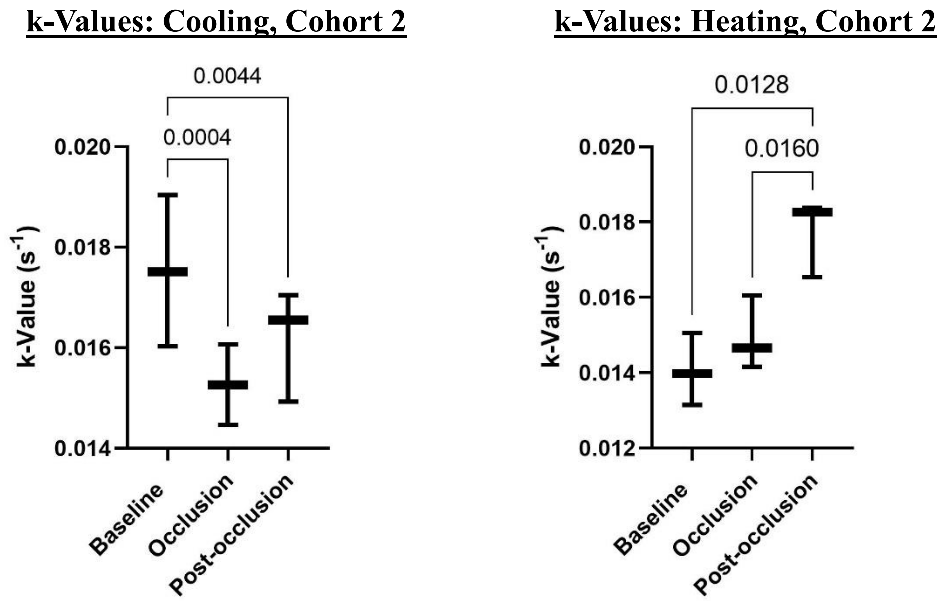

Figure 5. Reliability study thermal constants. Box-and-whiskers plots show average thermal constants across subject and visit during baseline, occluded, and post-occluded cooling (left) and heating (right) periods. Whiskers indicate the interquartile range of the k-values. Temperature data was analyzed using GraphPad Prism software and nonlinear regression analysis was used to obtain the rate constant k. An exponential plateau model was used for the heating period and a one-phase decay model was used for the cooling period. Out of 78 data sets (baseline, occluded, post-occluded heating and cooling are each separate data sets), 63 sets had R^2 values greater than or equal to 0.90, and all sets had R^2 values greater than or equal to 0.80. Two-way ANOVA with Tukey's multiple comparisons testing was used to determine statistical significance between k-values. Significance was defined as p<0.05. During cooling, significance was observed between baseline and occlusion periods (p=0.0004) and between baseline and post-occlusion periods (p=0.0044). During heating, significance was observed between baseline and post-occlusion periods (p=0.0128) and between occlusion and post-occlusion periods (p=0.0160). N = 5 for the cooling and heating period. Each subject returned for a total of 3 visits. Subjects 3 and 5 do not have heating or cooling data for the third visit due to the REFLO device malfunctioning. Subject 2 is missing cooling data for the third visit for the same reason. A total of 25 experiments were successfully completed for this study.

The average k-values during heating were found to be $k_{baseline}$ = 0.014151, $k_{occlusion}$ = 0.014898, and $k_{post-occl}$ = 0.017505. The average k-values during cooling were found to be $k_{baseline}$ = 0.017486, $k_{occlusion}$ = 0.015187, and $k_{post-occl}$ = 0.016327. Tukey's multiple comparisons test revealed that during cooling, significance was observed between the k-values for baseline and occlusion periods (p = 0.0004), as well as between the baseline and post-occlusion periods (p =

0.0044). During heating, significance was observed between the k-values for baseline and post-occlusion periods (p = 0.0128), as well as between the occlusion and post-occlusion periods (p = 0.0160). Two-way ANOVA revealed that during cooling and heating, flow state had a significant effect on k-value (p = 0.0002 cooling, p = 0.0044 heating). Subject also had a significant effect (p = 0.0042 cooling, p = 0.0428 heating) while visit had no significant effect (p = 0.0564 cooling, p = 0.9683 heating). NIRS data for the same cohort of participants is shown in Figure 6.

NIRS, rate of tissue oxygen saturation, cohort 2

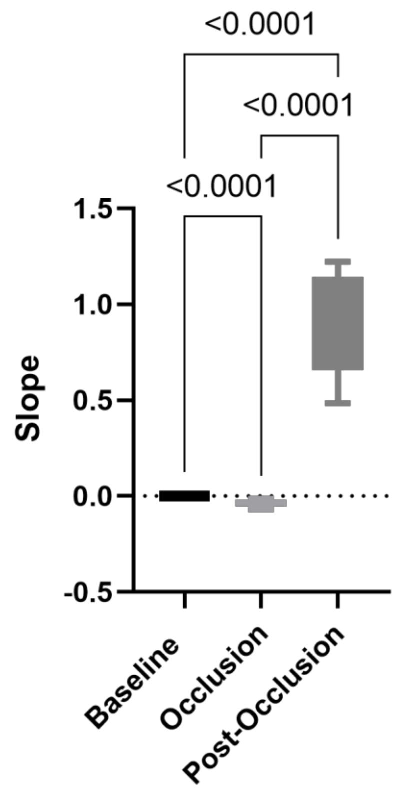

Figure 6. NIRS rates of tissue oxygenation. Box-and-whiskers plot show the average rates of tissue oxygenation across all subjects and visits during baseline, occluded, and post-occluded flow. Subjects received NIRS measurements during baseline, occluded, and post-occluded conditions. Whiskers indicate the interquartile range of the rate of change of oxygenated hemoglobin under each flow condition. Data was analyzed using GraphPad Prism software and linear regression analysis was used to obtain the rate of change (slope) of oxygenated hemoglobin in the blood. One-way ANOVA was used to determine statistical significance between the slope during each flow condition. Significance was defined as $p<0.05$. Significance was observed between baseline and occlusion periods, baseline and post-occlusion periods, and occlusion and post-occlusion periods, with all p values <0.0001. N = 4, and each subject returned for a total of 3 visits.

Statistical significance was found between baseline, occlusion, and post-occlusion periods, with all p-values < 0.0001. The average rates of change of tissue oxygenation were $m_{baseline} =$ 0.001686, $m_{occlusion} =$ -0.03688, $m_{post-occl} =$ 0.0857064, indicating that the post-occlusion period had the most rapid rate of blood reperfusion and that tissue de-oxygenation occurred during the occluded period. Two-way ANOVA revealed that subject and visit had no significant effect on k-value ($p = 0.5750$ and $p = 0.6071$, respectively), while flow had a significant effect ($p < 0.0001$).

DISCUSSION

Decreased blood flow in the skin during the occlusion period was expected to be associated with the lowest k-values because of reduced heat dissipation from the skin and subsequently higher skin heating rates. Hyperemic flow during the post-occlusion period was expected to be associated with the highest k-values because of increased heat dissipation from the skin and subsequently lower heating rates. In both cohorts, the occlusion period was associated with the lowest k-value (and therefore the highest time constant, τ), indicating that the highest rate of skin heating occurs when there is the lowest amount of volumetric blood flow. In both cohorts, the post-occlusion period was not associated with the highest k-value, but it was elevated from the occlusion period. This can be explained by the short time frame during which the post-occlusive hyperemic blood flow response occurs. The NIRS data collected during our experiments suggested that the hyperemic response was about 20 seconds, while REFLO measured the temperature response during hyperemia over a 3-minute heating period. In future studies, we plan to heat and measure the skin over a shorter period during hyperemia to better capture the true response.

Multiple comparisons testing revealed that subject has a significant effect on k-value. This eliminates the possibility of having standardized k-value ranges to distinguish a healthy individual from one with PAD and introduces the need for normalization of values for each patient. Flow also has a significant effect on k-values, so one possibility is to compare the rate of skin surface heating of the leg to that of the arm to create a normalized metric for each person. Analysis also revealed that visit number had no significant effect on k-value, indicating that the device is reliable between uses.

In both the pilot and reliability study, the rate of skin surface temperature change during cooling is more consistent than the rate of change during heating. During the heating periods, inconsistencies in energy emittance from the antenna may contribute to less reliable results. Error is more likely to occur when two factors (skin heating and blood flow response) are present. During cooling, skin heating is not occurring, eliminating this cause for error.

CONCLUSION

These results suggest that millimeter wave irradiation can be utilized to distinguish between different volumetric blood flow rates in humans. This technology creates the opportunity for a low-cost, accessible, and easy-to-use device to screen for cardiovascular disease, a major cause of mortality worldwide. Future protocol revisions and device modifications will aim to improve REFLO's ability to distinguish the post-occluded period of highest blood flow from baseline and occluded periods. Future testing will also involve clinical trials on PAD patients to determine the device's ability to distinguish between healthy patients and those with PAD.

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APPENDIX: DEVICE SOFTWARE

```
#include <Wire.h>
#include <SD.h> //can only support SD cards up to 32GB
#include <SPI.h>
#include <Adafruit_MLX90614.h>

Adafruit_MLX90614 mlx = Adafruit_MLX90614();

const int chipSelect PROGMEM = 8;
const int onButton = 4; //pins that buttons/LEDs/switches are connected to
const int offButton = 5;
const int heatButton = 8;
const int redLED = 3;
const int greenLED = 2;
const int switch1 = 6;
const int switch2 = 7;
const int maxTempC = 40;

int first = 0;
int second = 1;
int third = 2;
int i;

double t0;
double secondsPrecise;
double objTemps[3] = {0,0,0};
double ambTemps[3] = {0,0,0};
float deltaT;

unsigned long initialTime;
unsigned long currentTime;
unsigned long beginTime;
const unsigned long period = 500;

bool fileNamed = false;
bool secondsSet = false;
bool onPress;
bool offPress;
bool heatingPress;
bool finish = true;
bool heating = false;
bool test = true;

String myString = "";
File dataFile;
```

```
void setup() {
  mlx.begin();
  initSDCard();
  Serial.begin(9600);
  Serial.print("\nSetup has begun.");
  initIO();
  switchOff();
  ledOnState();
  delay(1000);
  testConfig();
  openFile();

  Serial.println("\nPress green button to begin recording (no heat), yellow button to begin
recording (heating), or red button to abort test.\n");
  Serial.print("\nAmbient(*C)\tObject(*C)\tDelta(*C)\tTime(s)");
  dataFile.print("\nAmbient(*C)\tObject(*C)\tDelta(*C)\tTime(s)");
}

void loop() {
  onPress = digitalRead(onButton);
  offPress = digitalRead(offButton);
  heatingPress = digitalRead(heatButton);

  if(offPress) {
    Serial.print("\ntestAbort");dataFile.print("\ntestAbort");
    testAbort();
  }
  else if(onPress){
    tempTest();
  }
  else if(heatingPress) {
    heatingTest();
  }
}

void initIO()
{
   pinMode(onButton, INPUT);
   pinMode(offButton, INPUT);
   pinMode(heatButton, INPUT);
   pinMode(switch1, OUTPUT);
   pinMode(switch2, OUTPUT);
   pinMode(redLED, OUTPUT);
   pinMode(greenLED, OUTPUT);
}
```

```
void initSDCard()
{
   while (!Serial){;}
   Serial.print(F("\nInitializing SD card..."));

   if (!SD.begin(chipSelect)) {
      Serial.println(F("Card failed, or not present"));
      return;
   }
   Serial.print(F("card initialized.\n"));
}

void testConfig(){
  Serial.println(F("\nEnter the name of the text file as FILENAME.txt"));
  while (myString == "") {
    while (Serial.available()){
      myString = Serial.readString();
    }
  }

  while(!fileNamed) {
    Serial.print(F("File name selected: "));Serial.print(myString);
    fileNamed = true;
  }
}

void openFile(){
  dataFile = SD.open(myString, FILE_WRITE);
  if(!dataFile){
    Serial.print("Error opening ");Serial.print(myString);Serial.print(" .");
  }
}

void ledOffState(){
  digitalWrite(redLED, HIGH);
  digitalWrite(greenLED, LOW);
}
void ledOnState(){
  digitalWrite(redLED, LOW);
  digitalWrite(greenLED, HIGH);
}
void switchOff(){
   Serial.print(F("\nREFLO is not heating."));dataFile.print(F("\nREFLO is not heating."));
   digitalWrite(switch1, HIGH);
   digitalWrite(switch2, HIGH);
}
```

```
void switchOn(){
   Serial.print(F("\nREFLO is heating."));dataFile.print(F("\nREFLO is heating."));
   digitalWrite(switch1, LOW);
   digitalWrite(switch2, LOW);
}
void testAbort(){
  dataFile.close();
  ledOnState();
  switchOff();
  Serial.println("\nTest aborted.");
  for(;;){}
}

void tempTest(){
  switchOff();
  ledOffState;//wired backwards
  beginTime = millis();
  initialTime = millis();
  t0 = mlx.readObjectTempC();//get initial temperature for delta calc

  heatingPress = digitalRead(heatButton);
  if(heatingPress) {
    heatingTest();
  }

  offPress = digitalRead(offButton);
  if(offPress){
    testAbort();
  }

  while(onPress) {
    heatingPress = digitalRead(heatButton);
    if(heatingPress) {
        heatingTest();
      }

    offPress = digitalRead(offButton);
    if(offPress){
        testAbort();
      }

    currentTime = millis();
    heatingPress = digitalRead(heatButton);
    if(heatingPress) {
        heatingTest();
      }
```

```
    offPress = digitalRead(offButton);
    if(offPress){
       testAbort();
      }

    if((currentTime - initialTime) >= period){
      dataFile.print(F("\n  "));dataFile.print(mlx.readAmbientTempC());Serial.print(F("  "));
      dataFile.print(F("\t     "));dataFile.print(mlx.readObjectTempC());Serial.print(F("  "));
      dataFile.print(F("\t     "));dataFile.print(mlx.readObjectTempC() - t0);Serial.print(F("  "));
      dataFile.print(F("\t     "));
      deltaT =(currentTime-beginTime)/1000;

      Serial.print(F("\n  "));Serial.print(mlx.readAmbientTempC());Serial.print(F(" "));
      Serial.print(F("\t     "));Serial.print(mlx.readObjectTempC());Serial.print(F(" "));
      Serial.print(F("\t     "));Serial.print(mlx.readObjectTempC() - t0);Serial.print(F(" "));
      Serial.print(F("\t     "));Serial.print(deltaT);

      initialTime = currentTime;
    }
  }
}

void heatingTest(){
  i = 0;
  switchOn();
  ledOffState();
  beginTime = millis();
  initialTime = millis();
  t0 = mlx.readObjectTempC();
  while(i <= 360) //this value is dependent upon the length of heating {
     if (objTemps[first] > 40 && objTemps[second] > 40 && objTemps[third] > 40){
       Serial.print("Maximum temperature exceeded. Aborting test.");
       testAbort();
      }
    offPress = digitalRead(offButton);
    if(offPress) {
       testAbort();
      }

    onPress = digitalRead(onButton);
    if(onPress){
       tempTest();
      }

    currentTime = millis();
```

```
    objTemps[third] = mlx.readObjectTempC();
    ambTemps[third] = mlx.readAmbientTempC();

    if((currentTime - initialTime) >= period){
      offPress = digitalRead(offButton);
      if(offPress){
        testAbort();
      }

      onPress = digitalRead(onButton);
      if(onPress){
        tempTest();
      }

    dataFile.print("\n  ");dataFile.print(ambTemps[third]);Serial.print(F("  "));
    dataFile.print(F("\t      "));dataFile.print(objTemps[third]);Serial.print(F("  "));
    dataFile.print(F("\t      "));dataFile.print(objTemps[third] - t0);Serial.print(F("  "));

    Serial.print(F("\n  "));Serial.print(ambTemps[third]);Serial.print(F(" "));
    Serial.print(F("\t     "));Serial.print(objTemps[third]);Serial.print(F(" "));
    Serial.print(F("\t     "));Serial.print(objTemps[third] - t0);Serial.print(F(" "));
    deltaT =(currentTime-beginTime)/1000;
    Serial.print(F("\t     "));Serial.print((deltaT));

    objTemps[first] = objTemps[second];
    ambTemps[first] = ambTemps[second];

    objTemps[second] = objTemps[third];
    ambTemps[second] = ambTemps[third];

    initialTime = currentTime;
    i++;
    }
  }
  tempTest();
}
```