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Sex differences in glycosylated hemoglobin in Mauritian origin long-tailed macaques (Macaca fascicularis)

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SEX DIFFERENCES IN GLYCOSYLATED HEMOGLOBIN IN MAURITIAN ORIGIN LONG-TAILED MACAQUES (*Macaca fascicularis*)

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by

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ABSTRACT

SEX DIFFERENCES IN GLYCOSYLATED HEMOGLOBIN IN MAURITIAN ORIGIN LONG-TAILED MACAQUES (*Macaca fascicularis*)

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Diabetes is a common metabolic condition that affects the body's ability to maintain normal glycemic control. This disease process can occur in primates. Long-tailed macaques (*Macaca fascicularis*) range throughout Southeast Asia and were introduced to Mauritius approximately 400 years ago. This genetically unique population has been the source of a large proportion of captive individuals used in research and macaques are the preferred animal model for diabetic research. Additionally, long-tailed macaques are successful in exploiting habitat overlapping with humans. This urbanization results in changes in the normal diet of these animals which can contribute to poor health outcomes in populations. Similar to humans, spontaneous diabetes is a condition that develops in this species which can have significant health consequences for the affected animals. HbA1C % is a bloodwork parameter that is commonly used to diagnose and monitor diabetic animals. The HbA1C % determines the level of glycosylation occurring on the hemoglobin protein found in the red blood cells. The A1C Now+ System, manufactured by PTS Diagnostics, is a commercially available, portable,

point-of-care device that is used to measure HbA1C%. This device has been validated for use in this species; however, there has been very little research on the applicability of the device for use in the health monitoring of wild nonhuman primates such as, Mauritian origin long-tailed macaques. Furthermore, sex differences in the results of this test have not been reported in this species but have been in such other mammals as chimpanzees, humans, and mice. Sixty-eight long-tailed macaques were sedated for a routine physical exam and HbA1C% was measured using the A1C Now+ device. Blood was also collected for the measurement of blood glucose, hemoglobin, red blood cell count and hematocrit. In our study population, males had significantly higher glycosylated hemoglobin (HbA1C) than females ($p < 0.0001$). Additionally, we found that HbA1C is positively correlated with hemoglobin, red blood cell count, and hematocrit, but sex did not have an impact on the correlations noted between HbA1C and Hb, HCT, and RBC. These results suggest that sex should be considered when assessing the health of a long-tailed macaque using the HbA1C test; for example, what is a normal value in a healthy male (6%) may be indicative of a pre-diabetic or diabetic female. This study confirms the utility of this device for the diagnosis and management of diabetes in captive and free ranging long-tailed macaques. Due to its small size and ease of use in the field, this device can be useful in monitoring the health of free-ranging monkeys that obtain food items that are highly processed, carbohydrate based and sugary. This type of diet is often encountered by macaques living sympatrically with humans. Captive individuals used in research and in zoos may also benefit from this data as the health of these individuals depends on the care provided by the staff.

ACKNOWLEDGMENTS

I would like to thank the operations and veterinary staff employed at Pfizer Inc., Comparative Medicine Department in Groton, CT for their assistance with primate physical examinations, their help with the collection of blood for analysis, and their ongoing dedication to the care and wellbeing of the captive primates at the facility. Additionally, I would like to acknowledge Susan Portugal, Associate Director at Pfizer Worldwide Research and Development, for her help in reviewing the statistics for this study. Lastly, I would like to acknowledge the faculty and staff of the Primate Behavior Program at Central Washington University for their continued support throughout my Master's journey.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Diabetes is a condition that is becoming more prevalent in human populations and diet appears to be a contributing factor. Like humans, nonhuman primates are susceptible to the disease and are frequently used in diabetes research. Glycosylated hemoglobin (HbA1C) is hemoglobin in the red blood cell that has been saturated with glucose molecule. HbA1C levels can be used to diagnose and monitor the progression of diabetes in affected individuals allowing the veterinary staff to care for the monkeys (Sacks, Crainiceanu, Brancati, & Coresh, 2002). Maintaining the health of these captive nonhuman primates is vital to the wellbeing of the animals and crucial to the quality of the research being conducted using them. Long-tailed macaques (*Macaca fascicularis*) are the most frequently used primate model in biomedical research and a large proportion of these monkeys are decedents of the population on the Island of Mauritius. The biology, ecology and behavior of long-tailed macaques as well as the history of the genetically unique population from Mauritius is covered in the subsequent paragraphs.

The genus *Macaca* has the widest geographical distribution of the order primates making it the most successful radiation among nonhuman primates and exploits the widest range of habitats and climates (Yao, Li, Martin, Moreau, & Malhi, 2017). There are 22 species of macaque organized into seven phyletic groups that overlap broadly in their distribution ranges (Yao et al., 2017). There are 10 subspecies of long-tailed macaque, and they are all similar in appearance. Long-tailed macaque habitat stretches to the farthest part of Southeast Asia (Yao et al., 2017). The current study individuals are a subset of animals derived from a population of long-tailed macaques residing on the

island of Mauritius. These primates have been freely ranging on the island for over 400 years and have been studied since the late 1970s (Sussman & Tattersall, 1981; Dunaif & Tattersall, 1987). Tattersall and colleagues note that the animals were brought to the island from Java (Tattersall, Dunaif, Sussman & Jaimieson 1981; Sussman & Tattersall, 1981) and that the animals are genetically similar due to the bottleneck that occurred when the small number of founder animals (~20 monkeys) was brought to the island (Osada, Hettiarachchi, Babarinde, Saitou, & Blancher, 2015; Ebeling et al., 2011). This genetic similarity is thought to contribute to a decrease in the fitness of the population that could cause differences in their physiology and disease processes from other populations of long-tailed macaques (Osada et al., 2015).

Several macaque species live in areas inhabited by humans (Richard, Goldstein & Dewar, 1989). Richard and colleagues (1989) coined the phrase "weed species" when describing four macaque species. Like weed plants, these species do very well in environments where humans are settled and follow humans where they travel. These species often compete with humans for resources and depend on humans to some extent. This 'weed' adaptation strategy is a characteristic of long-tailed macaques as they reach higher populations in regions where humans are abundant. Local human populations contribute to available food sources of this species and this leads to the perception that macaques are a pest due to crop raiding on the Island of Mauritius (Richard et al., 1989). In other areas of the world, this species is considered to have cultural significance and is visited at temples where local people bring gifts of food (Bakar, Amir & Marshal 1981). This exposure to human foods may contribute to negative health outcomes for macaques.

The locomotive strategies of long-tailed macaques are a major part of their daily activity budgets ranging between 18.00- 31.36% (Md-Zain et al., 2010). This species is primarily quadrupedal but leaping has been noted (Cant, 1988) with long leaps occurring when they quickly ascend into the canopy to avoid danger and their long tail allows for stability during these events (Rodman, 1979). Md-Zain et al., 2010 thought that long-tailed macaques move around a lot in order to search for fruit and have an extended range demanding increased locomotive activities due to habitat destruction. In Mauritius, Sussman & Tattersall (1981) found that whole groups did not travel together in search of food.

Long-tailed macaques have an average group size of 20-30 individuals and home ranges of 80-200 ha. In Mauritius, larger groups up to 100 individuals have been documented, and adult sex ratios range from 1M: 2F and 1M: 6F. Overlapping home ranges between groups is common in this species, and individuals commonly move out of their typical home range when resources are limited (Md-Zain et al., 2010; Sussman & Tattersall, 1981). Long-tailed macaque groups may split when the groups become too large (Van Noordwijk & Van Schaik, 1999). Additionally, limited resources may cause individuals to move into human populated areas to search for food.

Long-tailed macaques are mainly frugivorous, but nuts are also a major part of the diet particularly at high altitudes however, they have been noted to destroy seeds making them poor seed dispersers (Lucas & Corlett, 1998). Md-Zain et al. (2010) showed that long-tailed macaques spend 16.29- 24.02% of their time exhibiting feeding behaviors. Sussman & Tatterall (1981) noted that sugar cane made up about 8% of the diet of long-tailed macaques in Mauritius. The sugar cane plantations are a major economic export of

Mauritius and this sometimes leads to primate human conflict. The feeding ecology and diet of animals contribute to their health and wellbeing and negative health outcomes may become more prevalent with ongoing anthropogenic disturbances. Further, these disturbances may alter the social behavior of nonhuman primates and these changes may impact their health.

Long-tailed macaques are sexually dimorphic as males are larger and have bigger canine teeth than females. They live in male dominated, multi-male/multi-female groups where females are the philopatric sex and males disperse from their natal group by adulthood (Gerber, Krutzen, De Ruiter, Van Schaik, & Van Noordwijk, 2016). Long-tailed macaques have a female-bonded social structure that is influenced by a kin based support system (Van Schaik, Netto, Van Amerongen, & Westland, 1989). Although female choice appears to have a greater impact on the paternity of offspring than male rank does in Japanese macaques (*Macaca fuscata*), a positive correlation has been noted between paternity and dominance rank in long-tailed macaques (Soltis, Mitsunaga, Shimizu, Yanagihara, & Nozaki, 1997; Soltis et al., 1997; De Ruiter, Van Hooff, & Scheffrahn, 1994). Further, female dominance rank positively correlates with female birthrate and lifetime reproductive success and when groups become too large and split, there is an increase in average birthrate for each group (Van Noordwijk & Van Schaik, 1999). Since dominance is important for individual fitness the reordering of dominance rank with immigration can occur.

Males obtain their rank by entering a group and falling to the bottom of the hierarchy or by challenging high ranking males and taking their position in the group. Male long-tailed macaques often follow other monkeys from their natal group into

another group. Males often join new groups multiple times throughout their lifetime (Gerber et al., 2016). Males that are born to high ranking mothers tend to become high ranking in the groups that they joing later in life (Van Noordwijk & Van Schaik, 1999). Adult male-immature male social interactions are common in long-tailed macaques and are typically initiated by the immature monkeys. Bardi et al. (2017) found that middle-ranking males had less stress than either high or low ranking males. Additionally, they noted that middle-ranking males contributed to more bouts of affiliative interactions with immature monkeys than high or low ranking males. Dominance rank can be difficult to identify in captive nonhuman primate colonies because often times animals cannot interact in ways they would in their natural environment.

Long-tailed macaques are one of the primary non-human primate species used in biomedical research, contributing to an abundance of literature describing the species' anatomy, physiology, and captive husbandry (Ebeling et al., 2011; Zou et al., 2012). In addition to being the primary non-human primate model for pharmaceutical safety studies, this species is frequently used in diabetes research (Ebeling et al., 2011; Park. et al., 2016).This research not only allows scientists to better understand captive individuals, but it can help us further appreciate the ways in which environmental change and anthropogenic disturbances affect the health and biology of their free-ranging counterparts. It is important to use the data collected from captive animals to better understand free-ranging individuals and contribute to conservation efforts. Wildlife health monitoring is crucial to conservation and anthropogenic disturbances may cause shifts in diet that can lead to metabolic disorders such as diabetes.

Spontaneous diabetes has been reported in a variety of non-human primates spanning New World and Old World monkeys and apes (McTighe, Hansen, Ely, & Lee, 2011; Johnston et al., 2017; Bodkin, 2000; Wagner et al., 2006; Dutton, Parvin, & Gronowski, 2003; Gilardi & Valverde, 1995; Gresl, Baum, & Kemnitz, 2000; Ishizaka et al., 2003; Kavanagh et al., 2007; Stokes, 1986). Diabetes is a condition where the body is in a state of hyperglycemia (increased circulating blood glucose levels) because of body's inability to make or use insulin effectively (Wagner et al., 2006). The condition can be classified into three main types; Type 1 diabetes mellitus, Type 2 diabetes mellitus (T2DM), and gestational diabetes. In Type 1 diabetes, the body's immune system inappropriately destroys the β cells in the pancreas responsible for the production of insulin (Wagner et al., 2006). Given the similarities in the immune systems of both macaques and humans, the disease progression in both species is alike. T2DM is a condition where the body is insulin resistant; therefore, even though insulin is being produced the body is unable to use it to control blood glucose levels. Gestational diabetes is a condition that occurs in the second or third trimester of pregnancy where insulin resistance develops in response to the increased hormonal production by the placental tissue (Wagner et al., 2006; American Diabetes Association, 2018; McTighe et al., 2011). T2DM (Wagner et al., 2006) and gestational diabetes (Wagner, Jayo, Bullock, & Washburn, 1992) have been reported in long-tailed macaques. T2DM in long-tailed macaques has been associated with obesity and age and in its initial stages can be simply treated with caloric restriction alone (Wagner et al., 2006). However, as the condition progresses insulin treatment is necessary to control the hyperglycemia (Wagner et al., 2006). Although different, these types of naturally occurring diabetes have been

documented in nonhuman primates, T2DM similar to that described in humans has been most commonly reported in long-tailed macaques (Wagner et al., 1996; Yue, Ahang, Quintero, Gash, & Zhang, 2017). *Macaca* is the most prolifically studied non-human primate genus regarding spontaneous T2DM (Bodkin, 2000; Bodkin, Metzger, & Hansen, 1989; Hansen, 2003; Hansen, 2010; Hansen & Bodkin, 1986). Evidence of glucose intolerance and diabetes mellitus has been documented for both laboratory-housed long-tailed macaques and their wild counterparts ranging in Mauritius (Honjo, Kondo & Cho, 1976; Jones, 1974; Tattersall et al., 1981; Dunaif & Tattersall, 1987). Additionally, the glucose intolerance was more prevalent in captive long-tailed macaques that were wild-caught than those born in captivity (Honjo et al., 1976). Glucose intolerance alone is not sufficient in diagnosing diabetes.

According to the American Diabetes Association (2018), diagnosis of diabetes can be achieved using the following methodologies: fasting blood glucose level and HbA1C. Fasting blood glucose of ≥ 126 mg/dL in humans (American Diabetes Association, 2018) and ≥ 100mg/dL in rhesus macaques (*Macaca mulatta*) (Hansen, 2010) is indicative of diabetes. An HbA1C % of ≥ 6.5% in humans (American Diabetes Association, 2018; National Institute of Diabetes and Digestive and Kidney Diseases, 2018) and ≥ 7% in long-tailed macaques (Johnston et al., 2017) indicate a diabetes diagnosis. Protein glycosylation is the process by which sugar molecules attach to proteins. Similar to HbA1C, fructosamine is a measurement of protein glycosylation. Contrary to HbA1C, fructosamine measures the glycosylation of serum albumin instead of hemoglobin. Due to the shorter amount of time albumin lasts in the blood, it only

determines the glycemic levels over 2-3 weeks of time (Cefalu, Wagner, & Bell-Farrow, 1993).

Similar to albumen, glucose regularly attaches to the hemoglobin molecule of a red blood cell over the course of the cell's life. In metabolic states in which there is a higher concentration of free glucose molecules in the blood, the hemoglobin becomes more saturated. Since the average lifespan of a human's red blood cell is approximately three months, the glycosylated hemoglobin (HbA1C) measurement identifies the average glucose level for that span of time (Johnston et al., 2017; Higgins, Garlick & Bunn, 1982). The HbA1C measurement is a diagnostic test that is primarily used in the diagnosis and monitoring of humans with T2DM. Previous to this being the standard, daily measurements of blood glucose (BG) were used to monitor patients (Johnston et al., 2017). This latter method has proven to be less effective in the management of diabetic patients than measuring HbA1C (Sacks et al., 2002). Furthermore, the American Diabetes Association's (2018) standards for classification and diagnosis of diabetes indicate that HbA1C levels are a preferred method for diagnosis of the condition because it indicates how well the blood glucose levels are being controlled over a period of ~120 days, neither are short term increases in blood glucose reflected in the results nor is fasting required and the testing is fast and reliable.

HbA1C measurement has been studied in over 20 non-human primate species (Dutton et al., 2003). This diagnostic measurement is helpful in the management of long-tailed macaques that undergo porcine pancreatic islet xenotransplantation as part of diabetic laboratory research (Marigliano, Casu, Bertera, Trucco & Bottino, 2011). In addition to this being the standard of care in human medicine, Dutton et al. (2003),

provided evidence that this is also a useful tool for the diagnosis and management of diabetic haplorrhines. Furthermore, Dutton et al. (2003) stressed that the identification of valid reference ranges for each species is crucial to the utility of this diagnostic test, however, it is also important to identify ranges for each unique population of the same species. This is because many variables in the external environment can have an effect on the biochemical parameters. Additionally, it is important to establish reference ranges for each device or methodology used to analyze blood for certain parameters.

Historically the laboratory methodology used to evaluate HbA1C was only available at specific external facilities that require blood samples to be sent. These tests are expensive, results are not timely, and typically require a large volume of blood (Johnston et al., 2017). Many of the previously available reference ranges for HbA1C% came from work completed at these external facilities. The recent development of new technology has allowed for small portable devices that accurately measure HbA1C. These point-of-care devices can be used by diabetic people in their homes to help manage their condition. The A1C Now^{+} System (PTS Diagnostics, Indianapolis, IN) by Bayer Healthcare is one of these commercially available testing devices. This device has been evaluated in both long-tailed macaques and chimpanzees (*Pan troglodytes*). In two studies (Johnston et al., 2017; McTighe et al., 2011) this device was proven to be effective in the measurement of HbA1C in long-tailed macaques and chimpanzees. Additionally, Johnston et al. (2017) validated the use of the device against the external commercial laboratories in long-tailed macaques and found a good correlation to these commercial facilities which used the best methodology for assessing HbA1C at the time.

The methodology used to evaluate blood parameters is not the only variable that can affect the outcome of results.

A number of variables can have an effect on the measurement of biological parameters. Age, sex, living conditions, social interaction, exercise and diet are some of the variables that can alter biochemical and hematological parameters in long-tailed macaques (Park et al., 2016; Xie et al., 2013; Tattersall et al., 1981; Tigno, Gerzanich & Hansen, 2004; Yue et al., 2017). The effect that these variables have on HbA1C has not been well researched in long-tailed macaques. Furthermore, HbA1C has not been well documented in wild nonhuman primates and the use of this device may provide a conservation tool for health monitoring. Age and sex differences in HbA1C have been identified in healthy Chinese adult humans, in whom the researchers found that HbA1C levels rose with age, and that males have significantly higher levels than females (Ma, Liu, Xiang, Shan & Xing, 2015). Additionally, sex differences have been identified in diabetic and non-diabetic C57BL/6 mice, with males having significantly higher HbA1C levels than females (Dubuc, Scott & Peterson, 1993). No significant sex differences were noted in McTighe et al. (2011) study of HbA1C in chimpanzees; however, differences were noted for chimpanzees in a previous study by Herndon & Tigges (2001). Hemoglobin levels are significantly lower in female long-tailed macaques than males, according to two studies (Xie et al., 2013; Park et al., 2016). This lower hemoglobin level in females could contribute to lower HbA1C in females because HbA1C measures glucose saturated Hb and thus, if there is less Hb then there should be less glucose saturated Hb. Males would likely have higher HbA1C levels because they have a higher amount of Hb that can get saturated by the circulating glucose. Despite the higher levels

of HbA1C in nondiabetic, healthy males, males are not necessarily more susceptible to diabetes because the condition is a result of an inability to control circulating glucose levels causing a higher HbA1C.

The aim of this study is to further evaluate HbA1C using the portable A1C Now+ System in captive Mauritian origin long-tailed macaques. Specifically, the aim is to investigate the effects of sex on HbA1C levels and any correlations with other selected blood work parameters in this population of animals. We hypothesize that HbA1C levels will be lower in female long-tailed macaques than males. Secondly, we believe that HbA1C levels are correlated with Hb, HCT, and RBC values. Lastly, we hypothesize that the quality of the correlation between HbA1C values and Hb, HCT, and RBC is affected by sex.

CHAPTER II

JOURNAL ARTICLE

Sex differences in glycosylated hemoglobin percentage in Mauritian origin long-tailed macaques (*Macaca fascicularis*)

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Running Title: HbA1C in Mauritian long-tailed macaques

ABSTRACT

Long-tailed macaques (*Macaca fascicularis*) live throughout Southeast Asia and were introduced to Mauritius approximately 400 years ago which represents a genetically unique population that has been the source of a large proportion of captive individuals used in research. Additionally, this species has been successful in exploiting habitat sympatrically with humans. Spontaneous diabetes is a condition that develops in this species and can have significant health consequences for the affected animals. The A1C Now+ System is a portable, point-of-care device used to measure HbA1C%. This device has been validated for use in this species; however, there is very little research on the applicability of the device in Mauritian origin long-tailed macaques. Sex differences in the results of this test have not been reported in this species but have been in other mammals. HbA1C% was measured in 68 long-tailed macaques using the A1C Now+ device. Blood was collected to measure hemoglobin, red blood cell count, and hematocrit and blood glucose. In our study population, males had significantly higher glycosylated hemoglobin (HbA1C) than females ($p < 0.0001$). Additionally, we found that HbA1C is positively correlated with hemoglobin, red blood cell count, and hematocrit, but sex did not have an impact on the associations noted between HbA1C and the selected

bloodwork parameters. This study confirms the utility of this device for the diagnosis and management of diabetes in captive long-tailed macaques. Furthermore, it indicates that sex must be taken into account when analyzing the results obtained. Finally, this device's portability can make it a useful tool in monitoring the health of free-ranging monkeys that come into contact with food items that are highly processed, carbohydrate based and sugary (indicative of the "western diet") and captive individuals being used in diabetes research.

Keywords: HbA1C, Mauritius, diabetes, macaque

1 INTRODUCTION

Diabetes is a metabolic condition that can impact the health and wellbeing of long-tailed macaques (*Macaca fascicularis*). This disease state tends to occur as a result of diet changes that consist of a large proportion of highly processed carbohydrates. Diagnosing this disease state in captive animals early on can improve health outcomes for individuals. It is crucial to have accurate reference ranges for each device used in collecting bloodwork parameters. Additionally, each genetically unique population of animals should be evaluated to determine specific reference ranges for the desired population. This is important because the ecology and genetics of a population can have an effect on physiology and health.

The genus *Macaca* has the widest geographical distribution making it the most successful radiation among nonhuman primates and exploits the widest range of habitats and climates (Yao, Li, Martin, Moreau, & Malhi, 2017). There are 22 species of macaque organized into seven phyletic groups that overlap broadly in their distribution ranges (Yao et al., 2017). There are ten subspecies of long-tailed macaque and they are all similar in appearance. Long-tailed macaque habitat stretches to the farthest part of

Southeast Asia (Yao et al., 2017). The current study individuals are a subset of animals derived from a population of long-tailed macaques residing on the island of Mauritius. These primates have been freely ranging on the island for over 400 years and have been studied since the late 1970s (Sussman & Tattersall, 1981; Dunaif & Tattersall, 1987). Tattersall and colleagues note that the animals were brought to the island from Java (Tattersall, Dunaif, Sussman & Jaimieson 1981; Sussman & Tattersall, 1981) and that the animals are genetically similar due to the bottleneck that occurred when the small number of founder animals (~20 monkeys) was brought to the island (Osada, Hettiarachchi, Babarinde, Saitou, & Blancher, 2015; Ebeling et al., 2011). This genetic similarity can have an impact on health outcomes and physiology.

Several macaque species sympatrically occupy areas inhabited by humans (Richard, Goldstein & Dewar, 1989). Richard and colleagues coined the phrase "weed species" when describing four macaque species in their 1989 paper. Like weed plants, these species do very well in environments where humans are settled and follow humans where they travel. Macaques often compete with humans for resources and often depend on humans in fragmented and urban environments. For example long-tailed macaques reach higher populations in regions where humans are abundant. This behavioral adaptation contributes to the success of the species. Local human populations contribute to the food sources of this species and this leads to the perception that macaques are a pest due to crop raiding on the Island of Mauritius (Richard et al., 1989). In other areas of the world, this species is culturally significant and visited at temples where local people bring gifts of food (Bakar, Amir & Marshal 1981).

Long-tailed macaques are one of the primary non-human primate species used in biomedical research, contributing to an abundance of literature describing the species' anatomy, physiology, and captive husbandry of the species (Ebeling et al., 2011; Zou et al., 2012). In addition to being the primary non-human primate model for pharmaceutical safety studies, this species is frequently used in diabetes research (Ebeling et al., 2011; Park. et al., 2016).This research not only allows scientists to better understand captive individuals, but it can help us further appreciate the ways in which environmental change and anthropogenic disturbances affect the health and biology of their free-ranging counterparts.

Spontaneous diabetes has been reported in a variety of non-human primates spanning New World and Old World monkeys and apes (McTighe, Hansen, Ely, & Lee, 2011; Johnston et al., 2017; Bodkin, 2000; Wagner et al., 2006; Dutton, Parvin, & Gronowski, 2003; Gilardi & Valverde, 1995; Gresl, Baum, & Kemnitz, 2000; Ishizaka et al., 2003; Kavanagh et al., 2007; Stokes, 1986). Diabetes is a condition where the body is in a state of hyperglycemia (increased circulating blood glucose levels) because of body's inability to make or use insulin effectively (Wagner et al., 2006). The condition can be classified into three main types; Type 1 diabetes mellitus, Type 2 diabetes mellitus (T2DM), and gestational diabetes. In Type 1 diabetes, the body's immune system inappropriately destroys the β cells in the pancreas responsible for the production of insulin. T2DM is a condition where the body is insulin resistant; therefore, even though insulin is being produced the body is unable to use it to control blood glucose levels. Gestational diabetes is a condition that occurs in the second or third trimester of pregnancy where insulin resistance develops in response to the increased hormonal

production by the placental tissue. (Wagner et al., 2006; American Diabetes Association, 2018; McTighe et al., 2011). T2DM (Wagner et al., 2006) and gestational diabetes (Wagner, Jayo, Bullock, & Washburn, 1992) have been reported in long-tailed macaques. T2DM in long-tailed macaques has been associated with obesity and age and in its initial stages can be simply treated with caloric restriction alone (Wagner et al., 2006). However, as the condition progresses insulin treatment is necessary to control the hyperglycemia (Wagner et al., 2006). Although all of these different types of naturally occurring diabetes have been documented in nonhuman primates, T2DM similar to that described in humans has been most commonly reported in long-tailed macaques (Wagner et al., 1996; Yue, Ahang, Quintero, Gash, & Zhang, 2017). *Macaca* is the most prolifically studied non-human primate genus regarding spontaneous T2DM (Bodkin, 2000; Bodkin, Metzger, & Hansen, 1989; Hansen, 2003; Hansen, 2010; Hansen & Bodkin, 1986). Evidence of glucose intolerance and diabetes mellitus has been documented for both laboratory-housed long-tailed macaques and their wild counterparts ranging in Mauritius (Honjo, Kondo & Cho, 1976; Jones, 1974; Tattersall et al., 1981; Dunaif & Tattersall, 1987). Additionally, the glucose intolerance was more prevalent in captive long-tailed macaques that were wild-caught than those born in captivity (Honjo et al., 1976).

According to the American Diabetes Association (2018), diagnosis of diabetes can be achieved using the following methodologies: fasting blood glucose level and HbA1C. Fasting blood glucose of ≥ 126 mg/dL in humans (American Diabetes Association, 2018) and ≥ 100mg/dL in rhesus macaques (*Macaca mulatta*) (Hansen, 2010) is indicative of diabetes. An HbA1C % of ≥ 6.5% in humans (American Diabetes

Association, 2018; National Institute of Diabetes and Digestive and Kidney Diseases, 2018) and ≥ 7% in long-tailed macaques (Johnston et al., 2017) indicate a diabetes diagnosis. Similar to HbA1C, fructosamine is a measurement of protein glycosylation. Contrary to HbA1C, fructosamine measures the glycosylation of serum albumin instead of hemoglobin. Due to the shorter amount of time albumin lasts in the blood, it only determines the glycemic levels over 2-3 weeks of time (Cefalu, Wagner, & Bell-Farrow, 1993).

Similar to albumen, glucose regularly attaches to the hemoglobin molecule of a red blood cell over the course of the cell's life. This process is termed glycosylation. In metabolic states in which there is a higher concentration of free glucose molecules in the blood, the hemoglobin becomes more saturated. Since the average lifespan of a human's red blood cell is approximately three months, the glycosylated hemoglobin (HbA1C) measurement identifies the average glucose level for that span of time (Johnston et al., 2017; Higgins, Garlick & Bunn, 1982). The HbA1C measurement is a diagnostic test that is primarily used in the diagnosis and monitoring of humans with T2DM. Previous to this being the standard, daily measurements of blood glucose (BG) were used to monitor patients (Johnston et al., 2017). This latter method has proven to be less effective in the management of diabetic patients than measuring HbA1C (Sacks, Crainiceanu, Brancati, & Coresh, 2002). Furthermore, the American Diabetes Association's (2018) standards for classification and diagnosis of diabetes indicate that HbA1C levels are a preferred method for diagnosis of the condition because it indicates how well the blood glucose levels are being controlled over a period of ~120 days. This is a more reliable method

because, neither short term increases in blood glucose are reflected in the results nor is fasting required and the test is fast.

HbA1C measurement has been studied in over 20 non-human primate species (Dutton et al., 2003). This diagnostic measurement is helpful in the management of long-tailed macaques that undergo porcine pancreatic islet xenotransplantation as part of diabetic laboratory research (Marigliano, Casu, Bertera, Trucco & Bottino, 2011). In addition to this being the standard of care in human medicine, Dutton et al. (2003), provided evidence that this is also a useful tool for the diagnosis and management of diabetic haplorrhines. Furthermore, Dutton et al. (2003), stressed that the identification of valid reference ranges for each species is crucial to the utility of this diagnostic test.

Historically the laboratory methodology used to evaluate HbA1C was only available at specific external facilities to which the blood samples were sent. These tests are expensive, results are not timely, and typically required a large volume of blood (Johnston et al., 2017). The recent development of new technology has allowed for small portable devices that accurately measure HbA1C. These point-of-care devices can be used by diabetic people in their homes to help manage their condition. The A1C Now^{+} System (PTS Diagnostics, Indianapolis, IN) by Bayer Healthcare is one of these commercially available testing devices. This device has been evaluated in both long-tailed macaques and chimpanzees (*Pan troglodytes*). In two studies (Johnston et al., 2017; McTighe et al., 2011) this device was shown to be effective in the measurement of HbA1C in long-tailed macaques and chimpanzees. Additionally, Johnston et al. (2017) validated the use of the device against the external commercial laboratories in long-tailed

macaques and found a good correlation to these commercial facilities which used the best methodology for assessing HbA1C at the time.

A number of variables can have an effect on the measurement of biological parameters. Age, sex, living conditions, social interaction, exercise and diet can alter biochemical and hematological parameters in long-tailed macaques (Park et al., 2016; Xie et al., 2013; Tattersall et al., 1981; Tigno, Gerzanich & Hansen, 2004; Yue et al., 2017). The effect these variables have on HbA1C has not been well researched in long-tailed macaques. Furthermore, HbA1C has not been well documented in wild nonhuman primates and the use of this device may provide a conservation tool for health monitoring. However, for humans age and sex differences in HbA1C have been identified in healthy Chinese adult humans, in which the researchers found that HbA1C levels rose with age, and that males have significantly higher HbA1C levels than females (Ma, Liu, Xiang, Shan & Xing, 2015). Additionally, sex differences have been identified in diabetic and non-diabetic C57BL/6 mice, with males having significantly higher HbA1C levels than females in both groups (Dubuc, Scott & Peterson, 1993). No significant sex differences were noted in McTighe et al. (2011) study of HbA1C in chimpanzees; however, differences were noted for this species in a previous study by Herndon and Tigges (2001). Hemoglobin levels are significantly lower in female long-tailed macaques than males, according to two studies (Xie et al., 2013; Park et al., 2016). This lower hemoglobin level in females could contribute to lower HbA1C in females because HbA1C measures glucose saturated Hb and thus, if there is less Hb, then there should be less glucose saturated Hb. This lower HbA1C range in females may indicate that the same reference ranges cannot be used for both sexes.

The aim of this study is to further evaluate HbA1C in captive Mauritian origin long-tailed macaques. Specifically, to investigate the effects of sex on HbA1C levels and any correlations with other selected blood work parameters in this population of individuals. We hypothesize that HbA1C levels will be lower in female long-tailed macaques than males based on previous research in chimpanzees, humans, and mice. Secondly, we believe that HbA1C levels are correlated with Hb, HCT, and RBC values. Lastly, we hypothesize that the quality of the correlation between HbA1C values and Hb, HCT, and RBC is affected by sex.

2 METHODS

2.1 Animals

The current study population was comprised of 68 (31 male and 37 females) adult long-tailed macaques (*Macaca fascicularis*). All animals are of Mauritian origin and free of *Macacine herpesvirus* 1, SIV, STLV, measles, SRV (1-5), and tuberculosis on arrival to the facility. Additionally, all macaques receive an M-Vac Measles vaccine (Serum Institute of India) at the breeder site in Mauritius and HAVRIX Hepatitis A vaccine while housed at a U.S. vendor site. All study animals were within normal limits on recent physical exam and bloodwork parameters at the time of study. The study population ranged from 3.8 to 11.6 years of age (3.8 to 8.5 years of age for females and 4.7 to 11.6 years of age for males). The distribution of age for both the female (M = 5.2 yr) and male (M = 6.3 yr) groups is presented in Figure 1.

These macaques are dam reared in an outdoor AAALAC-accredited facility in Mauritius and weaned > 6 months in age. They are socially or pair housed with conspecifics when possible unless aggression is noted. At the time of sample collection,

animals were fed twice daily (Labdiet Certified Hi Fiber Primate Diet 5K91; Purina, St Louis, MO) and had ad libitum access to reverse osmosis water. They were given environmental enrichment, food foraging opportunities and fruits and/or vegetables daily. The animals were tested for tuberculosis biannually by intradermal palpebral tuberculin. They were screened for *Macacine herpesvirus* 1, SIV, STLV, measles, and SRV annually.

Housing and care for all animals are provided in accordance with the standards set forth in the *Guide for the Care and Use of Laboratory Animals* and the Animal Welfare Act. At the facility the animals are maintained on a 12 hour light/ 12 hour dark cycle. Temperature is set at 21.1 degrees Celcius and 50% relative humidity for all macaques. All study procedures described were approved by the Pfizer Inc. Groton, CT Institutional Animal Care and Use Committee.

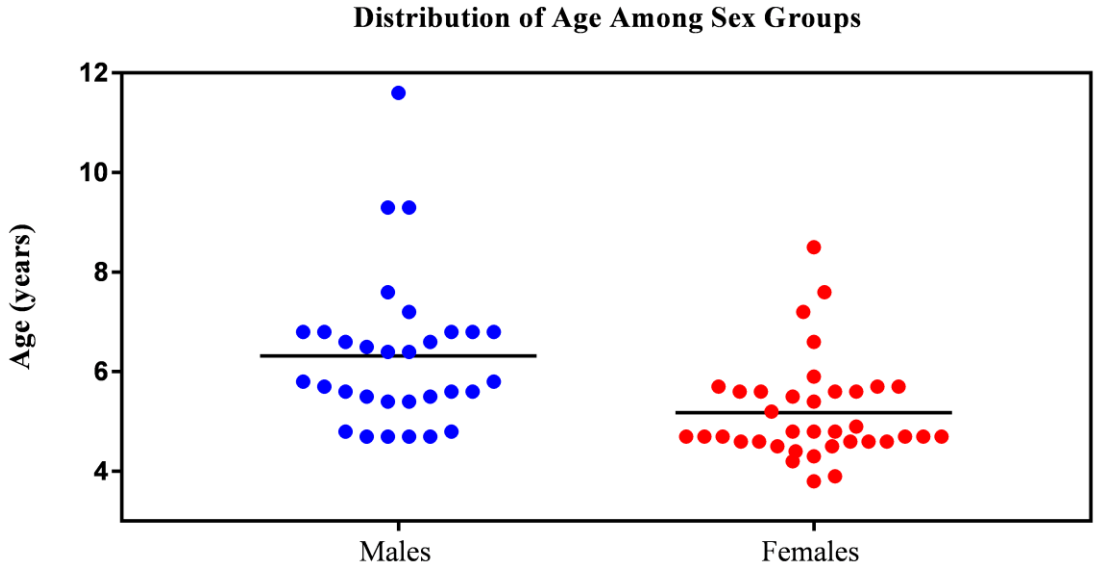

Figure 1. Distribution of age among sex groups. Data are presented as individuals within each group and the mean age per group. Data were collected for all 68 individuals on study.

2.2 Experimental design

The animals were sedated with 10mg/kg of ketamine (Zoetis, Kalamazoo, MI) intramuscularly for their routine bi-annual physical exam and blood collection. The inguinal triangle was disinfected with a dilute chlorhexidine solution prior to blood collection. Blood was collected from the femoral vein using a 22 gauge needle and vacutainer (Figure. 2). A total of 6.5 mL of blood were collected from each monkey. Three vacuettes of blood were collected, 2 mL of blood were collected into a sterile vacuette containing EDTA, 2.5 mL of blood were collected into a sterile vacuette containing a serum separator and clot activator, and 2 mL of blood were collected into a sterile vacuette containing lithium heparin. A complete blood count and blood chemistry were run as part of the bi-annual physical exam for each animal and select data from these tests were used in our analysis. The lithium heparin vacuette was placed on ice and used for HbA1C testing.

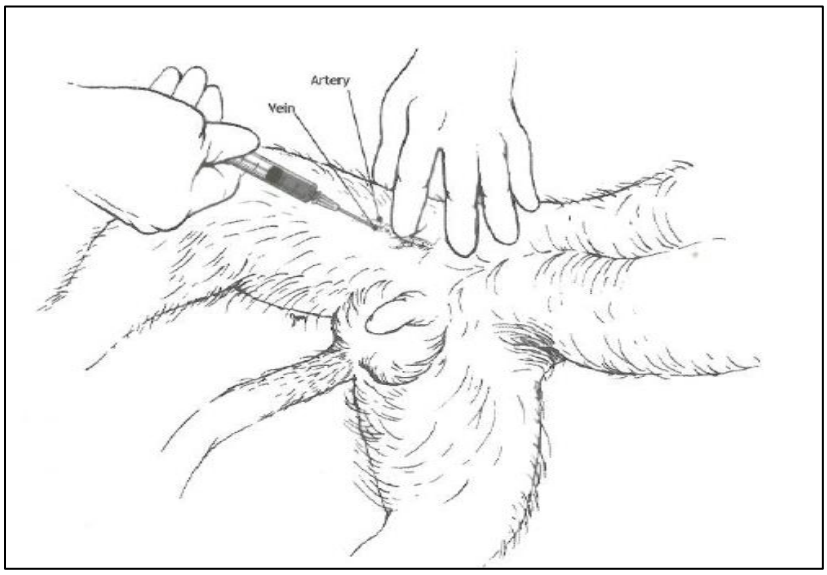

Figure 2. Blood collection from the femoral vein in a primate. The needle is inserted into the femoral triangle at approximately a 45 degree angle medial to the palpated pulse. Adapted from *The Laboratory Nonhuman Primate* (p. 163) by J.D. Fortman, T.A. Hewett and B.T. Bennett, (2002).

HbA1C percentage was measured for each animal using the A1C Now^{+} System (PTS Diagnostics, Indianapolis, IN). This device has been shown to be accurate in measuring HbA1C percentage in the management of diabetes in humans. This point of care device allowed for a result in five minutes and requires a small blood sample to obtain an accurate result. This device measured both A1C and hemoglobin (Hb) in the blood sample introduced via immunoassay and chemical technology (McTighe et al., 2011). The result was expressed as A1C% ((total hemoglobin/ A1C) x 100). The test results were immediately recorded. In addition to the HbA1C values, the animal's BG, Hb, hematocrit (HCT), and red blood cell count (RBC), at the time of testing, were obtained from the complete blood count and clinical chemistry data. Sex and age were also recorded for each animal. Due to equipment error, HbA1C % and BG could only be obtained for 67 and 64 animals, respectfully. Additionally, Hb, HCT, and RBC could only be obtained for 63 animals (Table 1).

2.3 Analysis

We aim to better refine the normal reference ranges for A1C% in these animals. Additionally, we propose to identify the differences in A1C% between male and female Mauritian origin adult long-tailed macaques. To this end, we determined the mean A1C% for the study population and then identified the mean A1C% for both males and females. The statistical mean for BG, Hb, HCT, and RBC data obtained from the biannual bloodwork results were calculated for both groups. Data were analyzed using Prism 7 for windows (Version 7.04 GraphPad Software, Inc.). An unpaired two-tailed *t* test with a 95% confidence interval was run between male and female data for each parameter to test for sex differences. Pearson's correlation coefficient (*r*) was calculated between HbA1C

and the other parameters to investigate correlations between HbA1C and other potential diagnostic blood parameters. A linear regression was calculated for each parameter between the sex groups and overall HbA1C with each parameter in order to assess any correlations between the parameters and whether those correlations change with respect to sex. Statistical significance was set to 0.05 and mean values were reported with the standard error of the mean (SEM).

3 RESULTS

3.1 Age and HbA1C%

The F-test indicated unequal variances for the age ($F = 2.364$, $p = 0.0144$) of the monkeys between both sex groups. Therefore, all assumptions of the *t* test would not be met and a *t* test was not completed. Instead the equivalent nonparametric Kolmogorov-Smirnov test was run, due to unequal variances, and there was a significant difference ($D = 0.4281$, $p = 0.0041$) in the ages of the males and the females in the study population. The results of the Pearson's correlation (r) showed no significant correlation between HbA1C% and age ($r(67) = 0.02997$, $p = 0.1612$).

3.2 Sex differences between hematological and biochemical parameters

The results analyzing the effect of sex on hematological and biochemical parameters in Mauritian origin long-tailed macaques are presented in Table 1. Values for HbA1C and BG were successfully measured in 31 males and 36 females, 27 males and 37 females, respectfully. Values for Hb, HCT, and RBC were measured in 28 males and 35 females. The F-test indicated equal variances for HbA1c ($F = 1.33$, $p = 0.4151$), BG ($F = 2.074$, $p = 0.0560$), Hb ($F = 1.446$, $p = 0.3273$), HCT ($F = 1.131$, $p = 0.7275$), and RBC ($F = 1.288$, $p = 0.5021$) which satisfies the equal variances assumption of the *t* tests.

The population mean for HbA1C was 5.7% (M = 5.7, SEM = 0.05167). There was evidence for significant differences of HbA1C% of sex with males (M = 5.897, SEM = 0.06902) having a higher HbA1C% than females (M = 5.439, SEM = 0.05553), $t(65) = 5.225$, $p < 0.0001$ (Figure 3 and Figure 4). However, males (M = 62.3, SEM = 1.747) and females (M = 66.11, SEM = 2.058) did not differ significantly in their BG results ($t(62) = 1.57$, $p = 0.056$). For Hb there was evidence for a significance between sexes, males (M = 13.66, SEM = 0.191) had higher values than females (M = 12.8, SEM = 0.2055), $t(61) = 3.016$, $p < 0.0037$. In addition, evidence for significant differences occurred for HCT with males (M = 47, SEM = 0.746) higher than females (M = 44.01, SEM = 0.6276), $t(61) = 3.083$, $p < 0.0031$. Finally, RBC count was significantly higher in males (M = 6.62, SEM = 0.1199) than females (M = 6.207, SEM = 0.1217) group, $t(61) = 2.378$, $p < 0.0205$ (Figure 5).

TABLE 1

Bloodwork Values in Mauritian Origin Long-tailed Macaques for HbA1C, BG, Hb, HCT, and RBC in Males and Females

	Males					Females				
	n	Mean	SEM	Min	Max	*n*	Mean	SEM	Min	Max
HbA1C	31	5.9	± 0.069	4.8	6.6	36	5.4	± 0.056	4.6	6.0
BG	27	62	± 1.8	30	81	37	67	± 2.2	44	101
Hb	28	13.7	± 0.19	10.1	15.1	35	12.8	± 0.21	7.2	14.3
HCT	28	47	± 0.75	35.6	57.5	35	44	± 0.63	26.4	48.5
RBC	28	6.6	± 0.12	4.73	8.3	35	6.2	± 0.12	3	7.11

Note. Data are presented as means ± SEMs, range of results, and number of individuals tested per sex group (*n*).

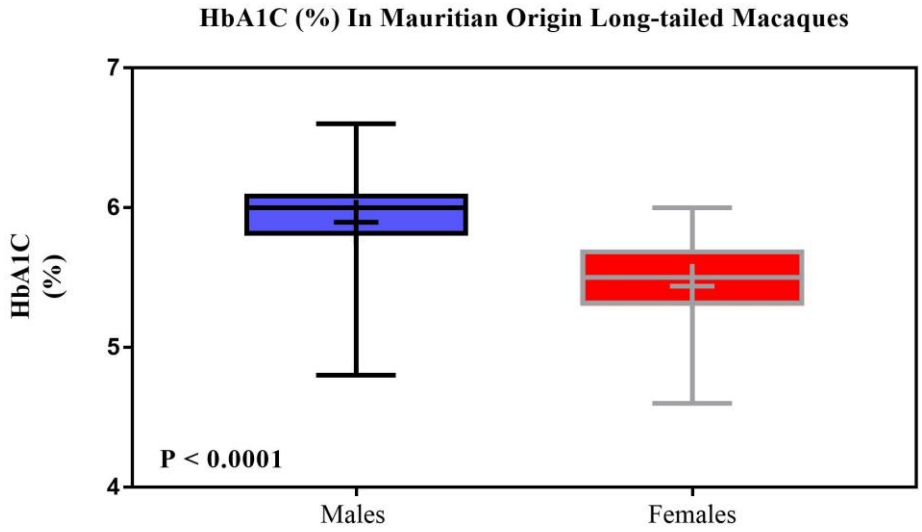

Figure 3. HbA1C values for Mauritian origin long-tailed macaques between males and females. Data are presented as the spread of data points, the shaded box extends from the 25th to 75th percentiles. The longer line in the box is the median and the dash represents the mean for the group. Significant difference between sex groups ($P < 0.05$).

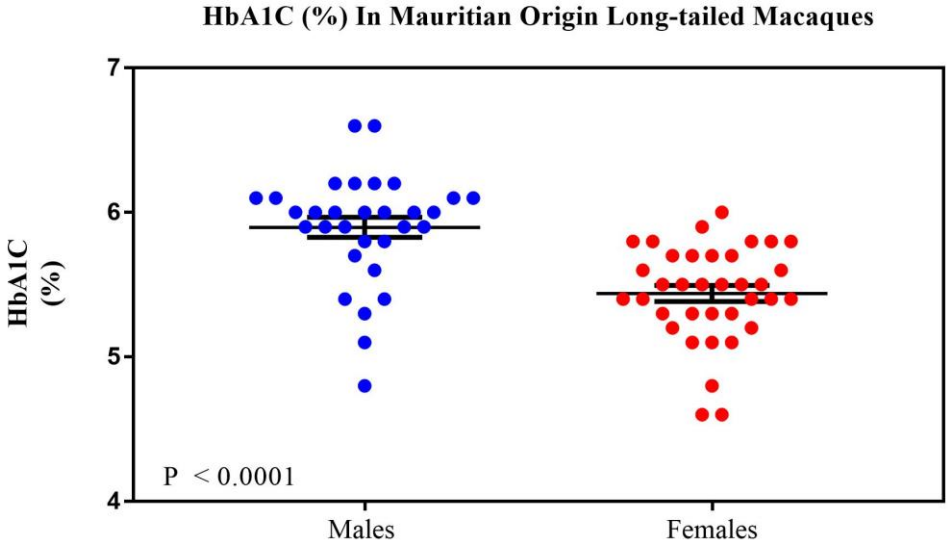

Figure 4. HbA1C values for Mauritian origin long-tailed macaques between male and female individuals. Data are presented as individuals and means ± SEMs separated by sex. Significant difference between sex groups as reflected by ($P < 0.05$).

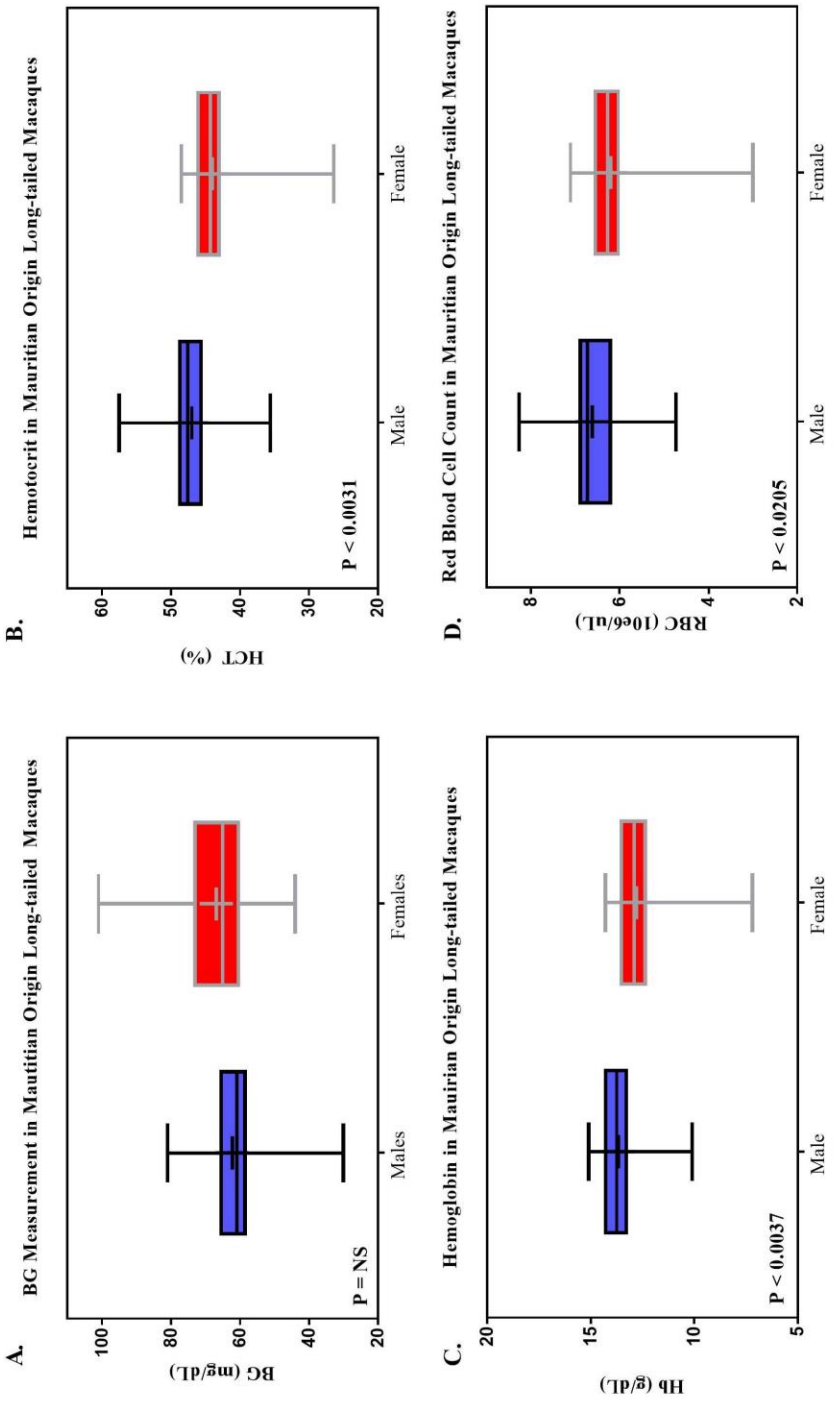

Figure 5. Blood work values in Mauritian origin long-tailed macaques (A), BG (B), HCT (C), Hb (D), RBC in males and females. Data are presented as the spread of data points, the shaded box extends from the 25th to 75th percentiles. The longer line in the box is the median and the dash represents the mean for the group. Significant difference between sex groups ($P < 0.05$). NS, not significant.

3.3 Correlation of HbA1C% and BG, Hb, HCT and RBC

Values for HbA1C and BG were successfully measured in 31 males and 36 females, 27 males and 37 females, respectfully. Values for Hb, HCT, and RBC were successfully measured in only 28 males and 35 females. The results of the Pearson's correlation (r) showed positive associations between HbA1C% and Hb ($r(62) = 0.460$, $p = 0.0002$), HCT ($r(62) = 0.414$, $p = 0.0008$), and RBC ($r(62) = 0.401$, $p = 0.0012$). Additionally, Hb and HCT ($r(62) = 0.876$, $p < 0.001$), Hb and RBC ($r(62) = 0.752$, $p < 0.001$), and HCT and RBC ($r(62) = 0.859$, $p = < 0.001$) were positively correlated. BG ($r(63) = -0.174$, $p = 0.172$) was not significantly correlated with HbA1C%.

3.3 Regression analysis of HbA1C% and Hb, HCT and RBC

Values for HbA1C and BG were measured in 31 males and 36 females, 27 males and 37 females, respectfully. Values for Hb, HCT, and RBC were successfully measured in only 28 males and 35 females. A linear regression analysis further showed that Hb ($R^2 = 0.212$, $F(1,60) = 16.14$, $p = 0.0002$), HCT ($R^2 = 0.171$, $F(1,60) = 12.38$, $p = 0.0008$), and RBC ($R^2 = 0.1606$, $F(1,60) = 11.48$, $p = 0.0012$) when compared to HbA1C%, were positively correlated in our study population (Figure 6). A linear regression analysis was run separately between the sexes for all three hematologic parameters compared to HbA1C% (Figure 7). The Hb regression indicates that overall the slopes are identical ($F(1,58) = 0.7802$, $p = 0.7810$) between males and females, however, there was a significant difference between intercepts ($F(1,59) = 14.52$, $p = 0.0003$). The HCT regression shows that overall the slopes are identical ($F(1,58) = 0.1486$, $p = 0.7013$) between males and females, however, there was a significant difference between intercepts ($F(1,59) = 15.25$, $p = 0.0002$). The RBC regression indicates that overall the

slopes are identical ($F(1,58) = 2.265$, $p = 0.1377$) between males and females, however, there was a significant difference between intercepts ($F(1,59) = 19.67$, $p < 0.0001$).

4 DISCUSSION

In this study, serum biochemical and hematological data were compared to the HbA1C% in long-tailed macaques and the effect of sex was analyzed for significant difference. Since long-tailed macaques are frequently used research, there is abundant literature regarding the care of captive individuals; however, literature specifically on the captive care, behavior and ecology of Mauritian origin long-tailed macaques is scarce. The results of this study can help to improve the standard of veterinary care for captive Mauritian origin long-tailed macaques, as well as provide information vital to the health monitoring of the wild population living on the island. The establishment of better defined reference ranges obtained from a large sample of individuals will provide researchers with normal HbA1C ranges that can be utilized in assessing an animal for diabetes.

This study further validates the use of the A1C Now+ point-of-care device for the diagnosis and monitoring of diabetes in long-tailed macaques. This device is useful in the medical care of captive individuals because it is easy to use, results are obtained immediately, and the device is accurate. Additionally, these point-of-care devices are invaluable in the field due to their portability and clinical significance. As expected, our study subjects were relatively within the reference range (4.8-7%) identified for this device by Johnston et al., 2017 for healthy long-tailed macaques. McTighe et al. (2011), established that normal, healthy chimpanzees and rhesus macaques have an HbA1C % < 5.6 and < 4.7, respectively. Our population's results (4.6-6.6%) indicate that a proportion

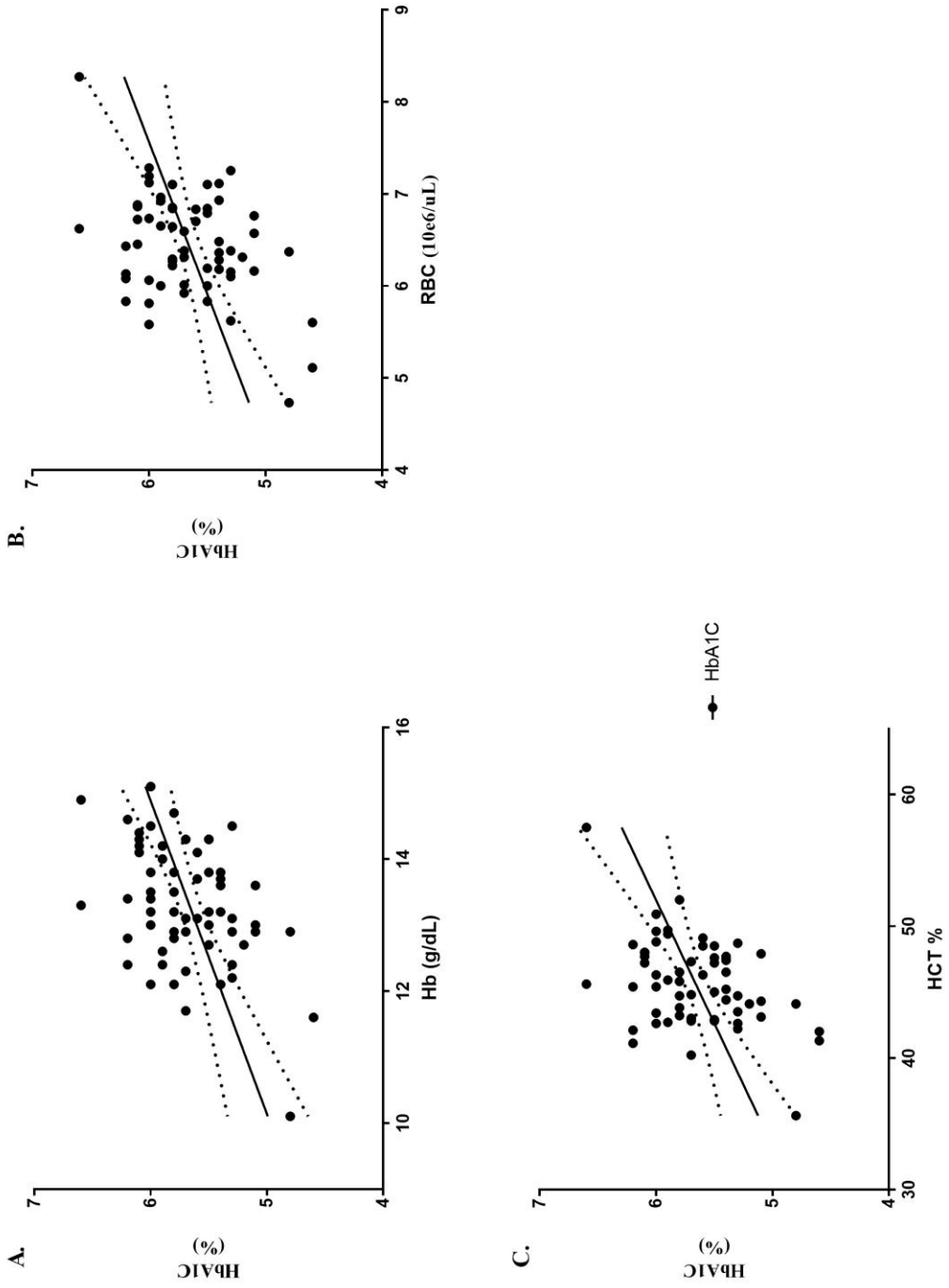

Figure 6. Linear regression plot of HbA1C and (A), Hb (B), RBC (C), HCT for all individuals. The data are presented as a dot for each individual where the intercept occurs for the two parameters. One regression line is present for all data regardless of sex.

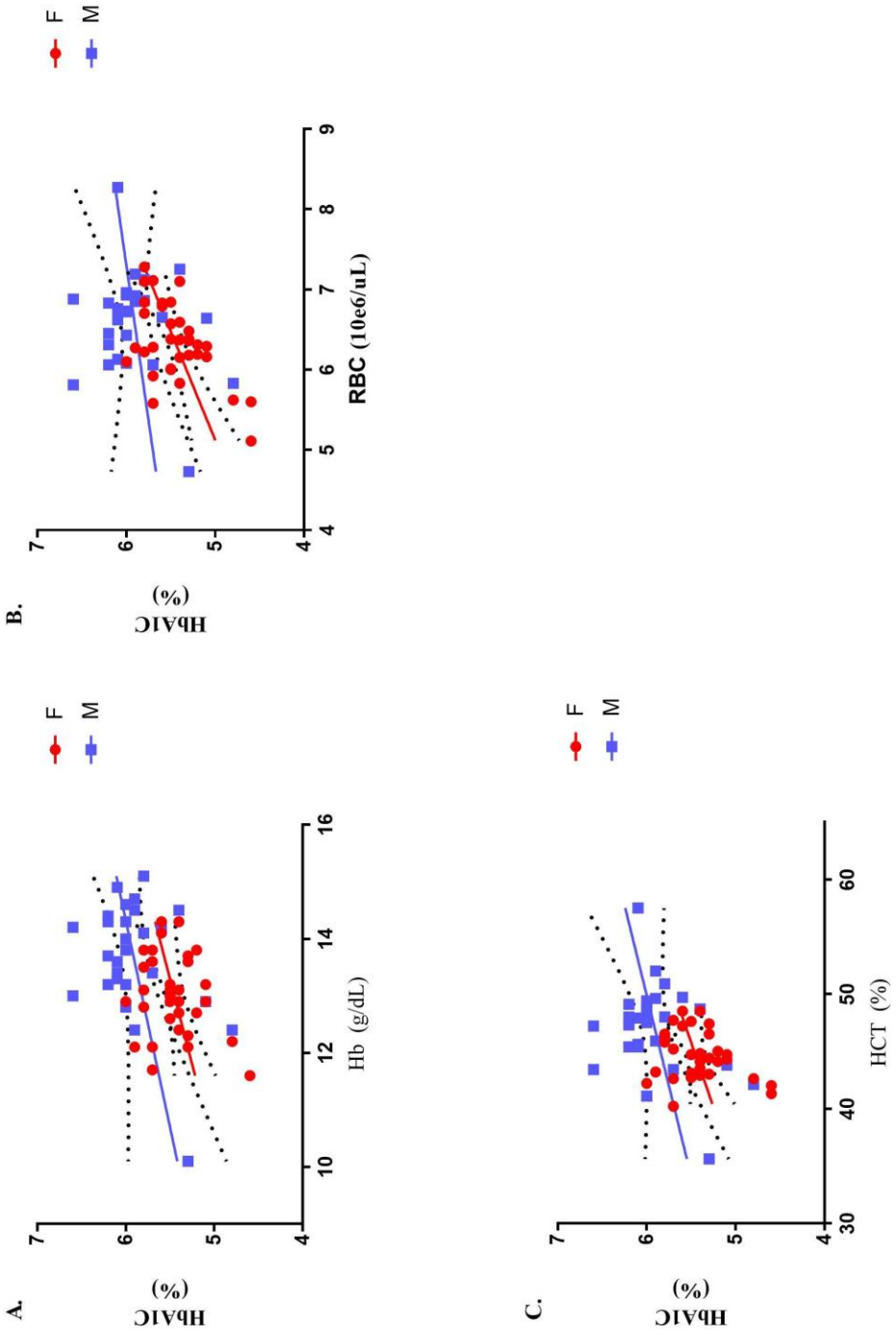

Figure 7. Linear regression plot of HbA1C and (A), Hb (B), RBC (C), HCT for males and females separately. The data are presented as a dot for each individual where the intercept occurs for the two parameters. Two regression lines are present for data per sex group.

of the animals are higher than the upper limit stated by McTighe et al. (2011) for rhesus macaques. Given that all animals on this study were deemed healthy by all other measures at the time of blood collection, this data is valuable to the development of well-established reference ranges in this species. It further suggests that HbA1C% for normal, healthy long-tailed macaques can be higher than the normal values identified for other primate species.

Furthermore, the uniqueness of our study population indicates that the genetic bottleneck that occurred on the island due to a small number of founder animals does not impact the reference range for this parameter. Our larger sample size also does not impact the results found by Johnston et al. (2017). Our results indicate that the A1C Now+ device is appropriate for monitoring HbA1C values in both captive and free-ranging Mauritian origin long-tailed macaques.

Sex and age are variables that can impact hematological and serum biochemical values in this species (Xie et al., 2013; Park et al., 2016). The effect of sex on the HbA1C values in the current study aligns with those observations noted by previous studies on chimpanzees, humans and C57BL/6 mice. Males tend to have higher HbA1C values than do females (Herndon & Tigges, 2001; Ma et al., 2016; Dubuc et al., 1993) as in the current study the mean HbA1C% is significantly higher in males than in females. This indicates that close attention should be paid to an individual's sex when evaluating HbA1C values for health monitoring purposes because the recent published reference ranges for the species, in general, do not account for differences than occur in males versus females. Some researchers have suggested that blood pressure, menstrual/estrous cycles, and blood lipid levels may be contributing to the differences seen in these groups

(Ma et al., 2016). Others indicate that RBC life span and rate at which hemoglobin becomes glycosylated may affect the values obtained by the test (Cefalu et al., 1993; McTighe et al., 2011). In order to validate our findings, we analyzed values for Hb, HCT, and RBC in our study population to show similar trends for sex differences that have been previously noted in the literature for this species. The significant differences we noted between males and females for Hb, HCT, and RBC were also noted in previous research (Xie et al., 2013). Similar to our findings, Ma et al. (2013), noted that BG was not significantly different in males versus females.

The mean age in our male group was significantly higher than the mean age of our female group; however, age did not have a significant correlation with HbA1C% in our study population. This makes it less likely that the differences in mean age between our two groups contributed to the differences in sex noted between the two groups. Since there is no correlation between age and HbA1C%, sex appears to be the variable that contributes to the differences noted between the male and female groups.

In the current study, we assessed if there were measureable and significant correlations between HbA1C% and Hb, HCT, and RBC. As expected, HBA1C% significantly correlated with Hb, HCT, and RBC. This indicates that anemia, hypo/hyperhemoglobinemia, and polycythemia can all impact whether the HbA1C results obtained are accurate. BG did not significantly correlate with HbA1C% in our study, however, Dubuc et al. (1993), noted that there was a significant correlation between BG and HbA1C% in mice. This could be explained by the routine overnight fasting that occurs at our facility prior to collection of bloodwork. The mean BG value for our study population (62.3 for males and 66.9 for females) was lower than the published mean

established by Park et al. (2016; 76.1 for males and 72.9 for females). The lower BG in our study population may have caused our lack of correlation between HbA1C and BG in this study.

Overall, the linear regressions analyzing the correlations between sex groups showed that there was no significant difference between the sex groups. The slopes of the regression lines are similar for both males and females. This indicates that Hb, HCT, and RBC are equally correlated with HbA1C% for both males and females. The intercepts for the regression lines were different between the sex groups and this is due to males having significantly higher mean HbA1C%, Hb, HCT, and RBC values than females. The difference in intercepts indicates an upward shift in the regression line for males and this can be seen in Figure 7. Although, R values for the regression analysis appear to be low, this could be due to the amount of variability in the data points around the trend line. The graphs clearly show a positive trend indicating that as Hb, HCT, and RBC increase so does HbA1C% even though there is not tight data spread around the trend line.

Ultimately, these results indicate that the exact same HbA1C reference ranges cannot be used for both males and females. Additionally, a level of 6% in a male along with no other abnormalities may indicate that the animal is normal; however, the same result in a female may indicate that she is suffering from a diabetic condition.

Urbanization of primate home ranges is contributing to human-primate conflict (Riley, 2017). As humans encroach on nonhuman primate habitat and nonhuman primates become more accustomed to human food, the health of these nonhuman primate populations may begin to decline. This is especially true of the long-tailed macaques in Mauritius, as well as, other parts of Southeast Asia. Understanding the physiological

parameters of each genetically diverse population will provide researchers and veterinary staff with the best references for evaluating population health. This is vital as further urbanization may be a health detriment to these animals, specifically, the change in diet may be contributing to a diabetic state in some of these populations. Since it is evident that spontaneous diabetes occurs in this species and diet is a contributing factor to its development, monitoring these populations for diabetes may help to inform wildlife management organizations. Specifically, the data could be used to educate local people about the health impacts of human food on the nonhuman primates, however, future studies on the differences in HbA1C for urban versus nonurban macaques would aid in this discussion.

Future studies will be important for identifying the biological mechanism(s) that causes the difference in average HbA1C % noted in this study between male and female long-tailed macaques originating from Mauritius. Since changes in social environment have been associated with significant changes in bloodwork parameters for this species, it would be interesting to investigate the effect that pair/group housing and single housing has on HbA1C %. Future research studies should also include an ample number of monkeys of the same age representing all life stages i.e. infant, juvenile, young adult, mid adult, and old adult as defined in a previous study that investigated age related changes in bloodwork (Hood et al., 2013). It is imperative that the information gained by captive nonhuman primates be used to improve conservation and wildlife management efforts for free ranging counterparts. It would be interesting to evaluate any differences in the HbA1C% of Mauritian long-tailed macaques that frequently raid human settlements or sugar cane plantations and those that live far away from humans and eat what is typical of

their natural diet. This could identify if the modification of diet in urban macaques is contributing to a health problems, such as, diabetes.

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APPENDIX

Complete data set including blood work values, sex, and age in Mauritian origin long-tailed macaques.

Study ID	Age (yrs)	Sex	HbA1C (%)	BG	Hb	HCT	RBC
1	4.2	Female	6.0	76	12.9	42.2	6.10
2	5.4	Female	5.3	73	13.7	46.5	6.36
3	5.6	Female	5.4	47	12.4	43.5	6.15
4	5.9	Female	5.3	75	12.1	44.4	6.18
5	7.6	Female	5.4	64	12.7	42.9	5.83
6	4.5	Female	5.5	94	13.1	44.7	6.38
7	4.7	Female	5.3	65	13.6	47.4	6.48
8	4.6	Female	5.4	78	12.9	44.1	6.37
9	5.7	Male	6.0	66	13.2	47.5	6.93
10	4.9	Female	4.8	56	12.2	42.6	5.62
11	4.7	Female	5.3	72	12.3	43.0	6.38
12	4.4	Female	5.7	53	13.8	47.7	7.11
13	4.7	Female	5.4	62	13.1	44.8	6.59
14	5.7	Female	5.7	81	13.6	45.2	6.28
15	5.6	Female	5.4	62	14.3	48.5	7.10
16	3.9	Female	5.5	64	12.6	42.7	6.00
17	4.7	Female	5.9	44	12.1	43.2	6.27
18	5.6	Female	5.8	70	13.1	46.3	6.70
19	3.8	Female	5.6	63	14.3	47.2	6.79
20	5.6	Male	5.4	55	14.5	48.7	7.25
21	8.5	Male	5.3	74	10.1	35.6	4.73
22	4.6	Female	5.5	65	13.0	43.1	6.57
23	5.7	Male	4.8	68	12.4	42.1	5.83
24	4.3	Female	5.1	74	13.2	44.7	6.29
25	4.5	Female	5.8	65	13.5	46.3	7.28
26	6.6	Male	6.2	57	14.4	48.0	6.45
27	4.6	Male	6.1	64	13.6	47.9	6.76
28	4.6	Male	5.1	64	12.9	43.8	6.64
29	4.8	Male	5.8	76	15.1	50.9	7.12
30	4.7	Male	6.0	56	14.0	49.4	6.96
31	4.7	Male	5.9	61	12.4	45.9	6.92
32	4.8	Male	5.9	81	14.5	49.6	7.19
33	5.2	Male	6.0	62	13.8	48.8	6.73
34	4.6	Male	6.0	60	12.8	41.1	6.08
35	4.8	Male	6.2	57	13.7	49.1	6.83
36	5.5	Male	5.6	65	14.2	49.7	6.65
37	5.4	Male	5.9	61	14.7	52.0	6.85
38	5.5	Male	5.8	68	14.1	48.0	6.86

39	5.4	Male	6.1	58	13.3	45.6	6.62
40	6.8	Female	4.6	62	11.6	42.0	5.60
41	6.8	Female	5.8	101	12.8	45.8	7.10
42	6.8	Female	5.6	73	14.1	48.5	6.83
43	5.6	Female	5.5	67	13.2	47.6	6.84
44	4.7	Female	5.7	66	11.7	40.2	5.92
45	5.5	Female	5.8	51	13.5	45.8	6.22
46	4.7	Male	6.6	65	13	43.4	5.81
47	4.7	Male	6.0	60	14.3	47.7	6.72
48	5.8	Female	5.5	70	13	42.8	6.00
49	4.8	Female	5.8	59	13.8	46.5	6.84
50	5.6	Male	6.1	71	14.9	57.5	8.27
51	7.2	Female	x	95	7.2	26.4	3.00
52	9.3	Female	4.6	79	11.6	41.3	5.11
53	4.7	Male	6.6	59	14.2	47.2	6.88
54	4.8	Female	5.1	72	12.9	44.3	6.16
55	6.5	Male	6.1	61	13.4	45.4	6.13
56	6.8	Female	5.2	44	12.7	44.1	6.31
57	6.8	Female	5.1	61	X	X	X
58	5.6	Female	5.2	55	13.8	45.0	6.19
59	6.6	Male	6.2	61	14.3	47.3	6.31
60	9.3	Female	5.5	67	12.9	42.8	6.01
61	5.7	Male	5.7	56	13.4	43.4	6.06
62	11.6	Female	5.7	50	12.1	42.6	5.58
63	6.4	Male	6.0	66	14.6	48.6	6.43
64	6.4	Male	6.2	30	13.2	45.4	6.06
65	7.6	Male	6.0	x	X	X	X
66	5.8	Male	5.9	x	X	X	X
67	6.6	Male	5.9	x	X	X	X
68	6.7	Male	5.4	x	X	X	X

Note. HbA1C = glycosylated hemoglobin, BG = blood glucose, Hb = hemoglobin, HCT = hematocrit, RBC = red blood cell count