

**THE DEVELOPMENT OF A NEW REFERENCE RANGE FOR FRUCTOSAMINE FOR THE  
PATHCARE PATHOLOGY GROUP, SOMERSET WEST, SOUTH AFRICA**

**By**

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**Thesis submitted in fulfilment of the requirements for the degree**

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**In the Faculty of Health Sciences**

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**October 2010**

## DECLARATION

I, Francois Christiaan Smit, declare that the contents of this thesis represents my own unaided work, and that this thesis has not previously been submitted for academic examination towards any qualification. Furthermore, this thesis represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

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## ABSTRACT

Fructosamine is a generic name given to a compound known as plasma protein ketoamines. It is formed by a non-irreversible enzymatic reaction between glucose and serum proteins, mainly albumin. Fructosamine together with glycated haemoglobin (HbA1c) are used to monitor the state of hyperglycaemia in diabetics. It provides the clinician with an index of glycaemia over a shorter period of time than HbA1c, about 4 weeks due to the high turnover of human serum albumin in blood and the degree of glycation in serum proteins. The evolution of automation in Clinical Chemistry necessitates that each pathology laboratory provides relevant sets of reliable reference values that are population and analyzer or method specific. Currently, the reference range for fructosamine at PathCare ranges between 200 to 285  $\mu\text{mol/L}$ .

Four hundred and forty six (120 white females, 117 white males, 114 mixed ancestry females, 95 mixed ancestry males) apparently healthy participants visiting the PathCare, Somerset West practice, were recruited for this study. Fructosamine, random blood glucose, HbA1c, total protein, albumin, and lipid profile was performed on all individuals. Nonparametric methods, whereby the sample 2.5 and 97.5 percentiles are used to form the 95% reference interval, were used to determine the reference values for fructosamine.

Though no significant differences ( $p = 0.086$ ) were observed between males and females in the total population group the mixed ancestry males had significantly higher fructosamine levels ( $p = 0.01$ ) compared to their female counterparts. The reference range of the entire sample was 223 – 295  $\mu\text{mol/L}$ , however differed in the different population groups (white females = 228 - 291  $\mu\text{mol/L}$ , white males = 223 – 296  $\mu\text{mol/L}$ , mixed ancestry females = 217 - 293  $\mu\text{mol/L}$  and mixed ancestry males = 222 – 304  $\mu\text{mol/L}$ ).

The new fructosamine reference range obtained in this study showed a significant difference than the one used at Pathcare, which is 200 – 285  $\mu\text{mol/L}$ . Our results further strengthen the recommendations by pathology bodies that laboratories must establish reference values that are representative of local populations. The single reference range (226 - 293  $\mu\text{mol/L}$ ) for the Caucasian males and females is recommended, however due

to the significant differences observed between the mixed ancestry males and females it is recommended that gender specific references ranges be used for this population group, which are: 1) 222 - 304  $\mu\text{mol/L}$  for the mixed ancestry males and 2) 217 - 293  $\mu\text{mol/L}$  for the mixed ancestry females respectively.

## AKNOWLEDGEMENTS

I hereby wish to give thanks to:

- My Lord and Saviour, Jesus Christ, for giving me the strength, courage, and health during the good and bad times to complete this thesis. To Him all the Glory and Praise.
- My supervisor, Professor Thandi Matsha, for your continued support, encouragement and mentorship. It truly was a honour to have you as my supervisor.
- My co-supervisors, Dr Cor Aalbers (PathCare), Dr Oluwafemi Oguntibeju (CPUT) and collaborator Dr Esmè Hitchcock (PathCare). Thank you for the advice and continued support.
- Dr John Douglas, Pathcare Chief Executive Officer, and the PathCare Executive Committee for grating me permission to do this project.
- Professor Johan Esterhuyse, Dean, CPUT Biomedical Department, and Mrs. Corlie Grovè (PathCare Somerset West Area Manager) for all the meetings and time spent cutting through the red tape to get the project off the ground.
- All the PathCare, Somerset West, staff who, in their individual capacity, contributed towards this study.
- Mr. Thys Mouton, CPUT Biomedical Department for the processing of the HbA1c samples at the CPUT Campus, Bellville.
- Professor Rajiv Erasmus and Professor Martin Kidd from the University of Stellenbosch for your suggestions, advice and contributions.
- Mr. Pieter Conradie for the regular transportation of the blood samples from Somerset West to the CPUT Bellville Campus for sample processing.
- My family and friends for your encouragement, love and support which carried me through.
- The financial assistance of the Cape Peninsula University of Technology towards this research project is acknowledged. The opinions expressed in this thesis and the conclusions arrived at, are those of the author, and are not necessarily to be attributed to the Cape Peninsula University of Technology.

## **DEDICATION**

To my parents, Fasia and Brenda Smit, for your love, support and encouragement all through my school- and academic years. Without you, I would not be the person that I am today.

## LIST OF ABBREVIATIONS, SYMBOLS, TERMS AND UNITS

>	Greater than
<	Less than
≥	Greater and equal than
≤	Less and equal than
A	Alpha
B	Beta
Y	Gamma
° C	Degrees Celsius
μmol	Micromol per Liter
mmol/L	Millimol per Liter
Nm	Nanometers
kD	Kilo Dalton
g/L	Grams per Liter
g/mL	Grams per Milliliter
μL	Microliter
ADA	American Diabetes Association
Adrenache	Term used for the increase in the function of the adrenal cortex, which occurs at the age of approximately 8 years
AIDS	Acquired Immunodeficiency Syndrome
ALB	Albumin
Apo	Apolipoprotein
Caucasian	White ethnic group
CPUT	Cape Peninsula University of Technology
CVD	Cardiovascular Disease
Depranocytosis	Alternative name for Sickle Cell Anaemia
Dehydration	Excessive water loss
DIC	Disseminated Intravascular Coagulation
Dysalbuminaemia	Abnormal structure of albumin in circulation resulting in a decrease in circulating albumin
DKA	Diabetic Ketoacidosis
DM	Diabetes Mellitus
DNA	Deoxyribonucleic Acid

Dyslipidaemia	Abnormal blood lipid profile
EAG	Estimated Average Glucose
Erythrocytes	Red blood cells
Ethics	A system of moral principles
FRUC	Fructosamine
GDM	Gestational Diabetes Mellitus
GSP	Glycated Serum Protein
HbA1c	Glycated Haemoglobin
HDL	High – Density Lipoprotein
HPLC	High Performance Liquid Chromatography
Haemolysis	Intra – or Extravascular breakdown of erythrocytes
Hepatocyte	Liver Cell
Hyperalbuminaemia	Plasma albumin concentration above the reference value
Hypoalbuminaemia	Plasma albumin concentration below the reference value
Hyperglycaemia	Plasma glucose concentration above the reference value
Hypoglycaemia	Plasma glucose concentration below the reference value
Hyperproteinaemia	Plasma total protein concentration above the reference value
Hypoproteinaemia	Plasma total protein concentration below the normal reference value
Hypertension	Increased Blood Pressure
IDF	The International Diabetes Federation
IFCC	The International Federation of Clinical Chemistry
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
Immunoglobulins	Antibodies
IVD	Ischaemic Vascular Disease
LDL	Low – Density Lipoproteins
Lipolysis	Breakdown of fat in storage for energy
Mean	Average obtained from a group of observations
MODY	Maturity Onset Diabetes of Young
Mortality	Rate of measurement of the number of deaths
Mixed Ancestry	Coloured ethnic group
NCCLS	National Committee for Clinical Laboratory Standards
Nephropathy	Damage or disease of the kidney
Neuropathy	Nerve damage



Obesity	Excess body fat accumulation to such an extent that it has negative effect on health
Polydipsia	Consume large volumes of fluids
Polyphagia	Constant hunger
Polyuria	Excess production and excretion of urine
QC	Quality Control
RBG	Random Blood Glucose
Retinopathy	Non – inflammatory damage to the retina of the eye
SANAS	South African National Accreditation System
SD	Standard Deviation
Tachycardia	Above normal heart rhythm
TC	Total Cholesterol
TG	Total Triglycerides
Tinnitus Aurium	Ringling sound in the ears
Venipuncture	Procedure whereby blood is obtained from a patient's vein or artery
VLDL	Very Low – Density Lipoprotein
WHO	World Health Organisation

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# **CHAPTER ONE: LITERATURE REVIEW**



## **1.1 Introduction**

Fructosamine is a generic name given to a compound known as plasma protein ketoamines. It is formed by a spontaneous non-enzymatic reaction between a carbonyl group of a glucose molecule and an amino group of a protein (Burton, Ashwood, & Burns, 2006). Fructosamine and glycated haemoglobin (HbA1c) are used to monitor the state of glycaemia in diabetics. HbA1c indicates the average glucose concentration over the preceding 6 weeks, as the average life span of a red blood cell is 120 days. However, this test is inaccurate in patients with haemoglobinopathies, such as sickle-cell disease, recent change in diet over a 6 week period, blood loss accompanied afterward by blood transfusions and haemolytic anaemia (Bishop, Fody, & Schoeff, 2005). In such cases, serum fructosamine plays an important role in glycaemic control and diabetes monitoring. Furthermore, fructosamine provides the clinician with an index of glycaemia over a shorter period of time than HbA1c, which is about 4 weeks. This is due to the high turnover of human serum albumin in blood and the degree of glycation of serum proteins

Diabetes mellitus is a group of metabolic diseases, characterized by an overall increase in plasma glucose concentration, otherwise known as hyperglycaemia. Hyperglycaemia in diabetics is mainly caused by: 1) a decrease in insulin production by the  $\beta$ -cells of the Islet of Langerhans in the pancreas, 2) a defect in insulin action or 3) a combination of both. If left untreated or poorly managed, uncontrolled hyperglycaemia in diabetes would result in dysfunction and failure of various organs including: the eyes, kidneys, nervous system, heart and blood vessels (American Diabetes Association, 2008). These symptoms usually appear gradually as the disease progresses until irreversible damage has occurred. Complications that may develop due to uncontrolled hyperglycaemia may include: 1) retinopathy, which may lead to blindness, 2) nephropathy, leading to renal failure, 3) neuropathy, causing an increased risk of developing foot ulcers and 4) cardiovascular disease, which may lead to heart disease and coronary artery disease, stroke and poor peripheral blood circulation (World Health Organization, 2006).

## **1.2 Diabetes Mellitus and Plasma Glucose**

Plasma glucose concentration is maintained within a narrow range by means of the endocrine system and other metabolic processes. This is quite important, because some tissues in the body (nervous system, erythrocytes or red blood cells and the retinal cells) are dependent on glucose for a steady energy supply, since these organs are incapable of storing glycogen and converting it to glucose for energy. When the plasma glucose

concentration falls below a certain concentration, these tissues lose their primary energy source and are thus incapable of maintaining their normal function.

Hypoglycaemia, or a low blood glucose concentration, can have many causes. Some can be transient, while others can have life-threatening consequences. The signs and symptoms of hypoglycaemia are related to the central nervous system and symptoms include: headache, perspiration, tachycardia (increased heart rate), hunger, tinnitus aurium (ringing sound in the ear), and blurred vision, to name a few (Davis, 2009).

Hyperglycaemia or a blood glucose concentration above the normal reference range is seen more often than hypoglycaemia in diabetes. Diabetes mellitus is the most well known hyperglycaemic condition.

There are two distinct states that precede diabetes mellitus: Impaired Fasting Glucose (IFG) and Impaired Glucose Tolerance (IGT) (Rasmussen *et al.*, 2008). Although IFG and IGT are the precursors to diabetes mellitus, the time of progression to overt disease is not known (Santaguida, *et al.*, August 2005). The difference between IFG and IGT are shown in Table 1.3. Diabetes mellitus is classified into 2 major types, insulin dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM). The other less common types include: gestational diabetes mellitus (GDM) and maturity onset diabetes of youth (MODY).

### **1.2.1 Insulin Dependent Diabetes Mellitus (IDDM)**

This is also known as type 1 diabetes mellitus or juvenile onset diabetes mellitus. The disease occurs mostly in individuals under the age of 30 years. The pathophysiology of this disease involves the  $\beta$ -cells of the pancreas that stop producing insulin due to their destruction as a result of an autoimmune disease (Harrison *et al.*, 1996). This results in abnormalities of carbohydrate and lipid metabolism. Type 1 diabetes mellitus remains the most common form of diabetes mellitus diagnosed in children (Cooke & Plotnick, 2008). One of the main complications type 1 diabetic patients that can develop is diabetic ketoacidosis or DKA, which is uncommon in non-insulin dependent diabetes mellitus. The reason for this is the lack of insulin that exists in type 1 diabetes mellitus. This leads to increased glucagon levels in circulation, leading to excess gluconeogenesis (glucose formation occurring in the liver from stored glycogen) and lipolysis (fat breakdown). Subsequently, plasma ketones are produced which are: 1) acetone, 2)  $\beta$ -hydroxybutyric acid and 3) acetoacetic acid. The formation of these ketones will lead to DKA if left untreated. Diabetic ketoacidosis remains one of the leading causes of death in children with type 1

diabetes mellitus (Plotnick & Cooke, 2008). Onset of type 1 diabetes mellitus is usually fast and dramatic, and patients are treated by insulin injections, together with a balanced diet (Diabetes South Africa, 2010).

### **1.2.2 Non-Insulin Dependent Diabetes Mellitus (NIDDM)**

Non-insulin dependent diabetes mellitus, otherwise known as type 2 diabetes mellitus, occurs mostly in individuals over the age of 40. There has been a worldwide increase of type 2 diabetes mellitus in children and adolescents, which can be linked to an increase in childhood obesity. This also parallels the increase in type 2 diabetes mellitus seen in adults. The cause can be attributed to an increase in insulin resistance syndrome in children with complications for example polycystic ovarian syndrome and a normal physiological developmental process known as adrenarche. Adrenarche is a term used for the increase in the function of the adrenal cortex, which occurs at the age of approximately 8 years (Dorland, 1981). Other complications such as: hypertension (high blood pressure), dyslipidaemia and other cardiovascular risk factors may occur (Silverstein & Rosenbloom, 2001). A longitudinal study performed on a Native North American tribe, called the Pima Indians, showed that this tribe has the highest incidence of NIDDM in the world accounting for about 70 % in the 55 – 64 year age group. Obesity is prevalent in this population group, but was not the sole cause for the emergence of type 2 diabetes. One main factor was found to be the profound changes in lifestyle that aided in the emergence of diabetes in this native tribe (Charles, Eschwège, & Bennet, 1997). The pathogenesis of NIDDM may be due to two factors namely: 1) the insufficient insulin production by the pancreas or 2) improper insulin function. The onset of the disease is slow and gradual. Patients will usually be able to control it with the correct diet and exercise (Diabetes South Africa, 2010).

### **1.2.3 Gestational Diabetes Mellitus (GDM)**

Gestational diabetes mellitus is a temporary condition which occurs during pregnancy. Both mother and child have an increased risk of developing diabetes later in life (Diabetes South Africa, 2010). Over the years, there has been a marked increase of diabetes and obesity worldwide. Obesity, particularly in the youth, has been identified as being one of the factors for the increase of GDM worldwide. Other contributing factors include: increased insulin resistance (as discussed earlier with type 2 diabetes mellitus) and a lack of physical exercise. Gestational diabetes mellitus is caused by an insufficient insulin secretion to meet the demands, which will lead to insulin resistance. Two causes of this insulin resistance in

GDM have been identified namely: 1) the physiological insulin resistance in late pregnancy and 2) the chronic form of insulin resistance in GDM that exists long before pregnancy which is exacerbated by the physiological changes that occur during pregnancy. Thus, women with GDM, as a group, have a higher degree of insulin resistance during pregnancy than normal women because they have a combination of acquired chronic insulin resistance and insufficient insulin secretion to meet the demands during pregnancy (Metzger *et al.*, 2007).

#### **1.2.4 Maturity Onset Diabetes of Young (MODY)**

This is a very rare form of type 2 diabetes mellitus. It is known to be a disease found in families due to an autosomal dominant inheritance of the diabetes susceptibility gene (Cox *et al.*, 1992). Onset is typically before the age of 25. Often, it is misdiagnosed as either type 1 or type 2 diabetes mellitus (Giuffrida & Reis, 2005; Frueguel & Velho, 1999).

### **1.3 Prevalence of Diabetes Mellitus in South Africa**

According to the World Health Organization (WHO), the current estimate of patients suffering from diabetes worldwide is 180 million. This figure is estimated to increase to 366 million by the year 2030 (World Health Organization, 2008).

In a publication released by the International Diabetes Federation (IDF) in 2009, it was estimated that in 2010 the African continent's total estimated population for the age range 20 - 79 will be 378.55 million, while the estimated figure for the South African population for the same age range will be 28.55 million. This is 7.5 % of the African continent as depicted in the IDF's Diabetes Atlas, Fourth Edition.

Table 1.1 shows data compiled by the International Diabetes Federation (IDF) of the estimated number of South Africans suffering from diabetes mellitus and IGT aged 20 to 79 years in 2010. All comparative data were compiled by the IDF using the comparative prevalence which was adjusted to the world population. The national prevalence indicates the percentage of the country's population that has diabetes mellitus. It is an ideal tool to use to try and establish the burden diabetes mellitus has on the country. (International Diabetes Federation, 2009).

**Table 1.1:** The estimated prevalence of diabetes mellitus and impaired glucose tolerance for the year 2010 – South African and African Region. (International Diabetes Federation, 2009).

	DIABETES	IGT
<b>Prevalence (%)</b> <b>South Africa</b>	4.5 %	7.6 %
<b>Prevalence (%)</b> <b>Comparative</b>	4.5 %	8.7 %
<b>Estimated total of male patients aged 20 – 79 years in South Africa</b>	5 012 000	13 453 000
<b>Estimated total of female patients aged 20 – 79 years in South Africa</b>	7 822 000	8 185 000
<b>Estimated total of patients in South Africa aged 20 – 79 years of age (male and female)</b>	12.83 million	21.64 million
<b>Estimated total of patients aged 20 – 39 in South Africa (male and female)</b>	2 282 000	6 681 000
<b>Estimated total of patients aged 40 – 59 in South Africa (male and female)</b>	6 496 000	7 432 000
<b>Estimated total of patients aged 60 – 79 in South Africa (male and female)</b>	4 056 000	7 525 000
<b>Estimated total of South African population aged 20 – 79 years (male and female)</b>	28.55 million	28.55 million

Table 1.2 below indicates the IDF's projected totals and percentage of patients suffering from IGT and diabetes mellitus in 2030.

**Table 1.2:** The estimated projected prevalence of diabetes mellitus and impaired glucose tolerance for the year 2030 - South African and African Region (International Diabetes Federation, 2009).

	DIABETES	IGT
<b>Prevalence (%)</b> <b>South Africa</b>	4.9 %	8.4 %
<b>Prevalence (%)</b> <b>Comparative</b>	5.6 %	10 %
<b>Estimated total of male patients aged 20 – 79 years in South Africa</b>	6 554 000	17 430 000
<b>Estimated total of female patients aged 20 – 79 years in South Africa</b>	9 888 000	10 650 000
<b>Estimated total of patients in South Africa aged 20 – 79 years of age (male and female)</b>	16,44 million	28,08 million
<b>Estimated total of patients aged 20 – 39 in South Africa (male and female)</b>	2 529 000	7 464 000
<b>Estimated total of patients aged 40 – 59 in South Africa (male and female)</b>	7 373 000	8 860 000
<b>Estimated total of patients aged 60 – 79 in South Africa (male and female)</b>	6 540 000	11 573 000
<b>Estimated total of South African population aged 20 – 79 years (male and female)</b>	33,46 million	33,46 million

#### 1.4 The Signs and Symptoms of Diabetes Mellitus

Using the signs and symptoms found in diabetes mellitus are not recommended for the diagnosis of the disease, as these symptoms are not specific to diabetes mellitus alone. For the diagnosis of diabetes mellitus, patients with the following characteristic symptoms should be subjected to further testing and investigation to rule out any other conditions that might cause similar symptoms. These symptoms are: polyuria (the urge to urinate frequently), polydipsia (the desire to drink large amounts of water to quench the constant thirst), polyphagia (the frequent urge to eat or binge), weight loss over a short period of time, dehydration, vomiting, mental state changes and weakness (Kitabchi *et al.*, 2009).

Under normal physiological conditions, the liver, pancreas and other endocrine glands in the body are responsible for maintaining normal glucose concentrations within a narrow range. Normal glucose concentration in blood is primarily under the control of 2 hormones synthesized by the pancreas namely: insulin and glucagon. Other neuroendocrine substances and hormones also exert some control over blood glucose concentrations but to a lesser extent (Bowen, 2010).

Insulin is produced by the  $\beta$ -cells in the pancreas and released in response to an increase in blood glucose concentration. It is responsible for glucose entering from the circulation into body cells where it is either converted to glycogen or immediately used as a source of energy, thereby increasing its metabolism and breakdown. The ultimate effect of insulin is to decrease the blood glucose concentration. (Bowen, 2010). In diabetes mellitus, insulin is either absent (as seen in IDDM) or dysfunctional (as seen in NIDDM).

Glucagon is produced by the  $\alpha$ -cells in the pancreas and responsible for the increase in blood glucose concentration. Therefore, it is secreted in response to a decreased blood glucose concentration. It is released during fasting periods and under stress conditions. Glucagon causes glycogenolysis (breakdown of glycogen) in the liver and promotes gluconeogenesis. The ultimate goal of glucagon is to increase the blood concentration of glucose to normal physiological concentration (Alberti & Zimmet, 1998).

Polyuria and polydipsia in diabetes mellitus are caused by hyperglycaemia. There will be an increase in the serum osmolality due to the hyperglycaemia. The hyperglycaemia results in large amounts of glucose being filtered by the kidneys into the urine causing an osmotic diuresis with concomitant loss of water and large amounts of electrolytes, but specifically sodium and potassium, causing further dehydration. Due to this water loss patients are constantly thirsty (World Health Organization, 2006).

Weight loss in diabetes mellitus is due to the relatively low levels of circulating insulin, which is incapable of inhibiting fat breakdown (lipolysis). If lipolysis takes place, the implication is that, despite hyperglycaemia, the amount of insulin secreted is insufficient to inhibit lipolysis. Research has shown that overweight diabetics who exhibited significant weight loss showed a ~ 25% decrease in mortality rate (Williamson *et al.*, 2000).

### **1.5 The Diagnosis of Diabetes Mellitus**

Patients who are asymptomatic on physical examination but are suspected of either being diabetic or suffering from possible IGT or IFG should be subjected to an additional random or fasting glucose test. If the test results comply with the predetermined criteria for the diagnosis of diabetes mellitus, the diagnosis is made. The patient can also be subjected to a 75g oral glucose tolerance test (OGTT) after an overnight fast of 8-12 hours. Again, depending on the glucose levels, the diagnosis of diabetes or impaired glucose tolerance can be made. The diagnosis of diabetes mellitus is made or excluded using the cut-off values depicted in table 1.3. Because the development of diabetes is not a sudden physiological occurrence the diagnosis of diabetes is expanded to include pre-diabetic conditions like IGT and IFG.

The diagnosis of diabetes mellitus is made by measuring blood glucose levels. The World Health Organization (WHO) and the American Diabetes Association (ADA) have set out criteria based on the blood glucose concentrations with cut-off values for the diagnosis of diabetes mellitus (World Health Organization, 2006). However, the WHO states that the diagnosis of diabetes mellitus should not be made by measuring a single glucose concentration (WHO, 1999). Table 1.3 below summarises the WHO and ADA criteria for the diagnosis of diabetes mellitus, IFG and IGT by measuring blood glucose concentrations.



**Table 1.3:** The American Diabetes Association versus the World Health Organization’s criteria for the diagnosis of diabetes mellitus, impaired fasting glucose and impaired glucose tolerance. \* indicates the plasma blood glucose concentration 2 hours after the ingestion of 75 grams of glucose.

	ADA (2008)	WHO (2006)
<b>DIABETES</b>		
<b>Fasting glucose</b>	≥ 7.0 mmol/L	≥ 7.0 mmol/L
<b>2 hour glucose*</b>	<b>OR</b> ≥ 11.1 mmol/L	<b>OR</b> ≥ 11.1 mmol/L
<b>IFG</b>		
<b>Fasting glucose</b>	5.6 to 6.9 mmol/L	6.1 to 6.9 mmol/L
<b>2 hour glucose*</b>	If measured; < 11.1 mmol/L	<b>AND / OR (if measured)</b> < 7.8 mmol/L
<b>IGT</b>		
<b>Fasting glucose</b>	Not required	< 7.0 mmol/L
<b>2 hour glucose*</b>	≥ 7.8 and / or < 11.1 mmol/L	<b>AND</b> ≥ 7.8 and / or < 11.1 mmol/L

When a patient has been diagnosed as a diabetic, treatment and continuous monitoring of blood glucose levels at regular intervals during the day, especially before and after meals, is initiated. Patients then need a complete and intense change in lifestyle and their dietary requirements regarding the amount of glucose, carbohydrates and starches they ingest have to be strictly monitored (Li *et al.*, 2008).

Patients suffering from IDDM need lifelong insulin injections, as the pancreas in these individuals are not capable of producing insulin. Usually, they require a certain number of units before each meal, with regular blood glucose checks done by themselves via a fingerpick test several times during the day, as too much insulin can cause hypoglycaemia which can be life threatening if not well managed (Takahashi, Xiao, & Hu, 2008).

Patients diagnosed with NIDDM on the other hand are initially treated by managing their diet and changing their lifestyle by initiating a more regular exercise program and possible weight loss if required. In some patients this will keep their diabetes under control, while other patients are required to take oral antidiabetic medication like Metformin in addition to their altered lifestyle and diet to manage their diabetes. If all of these treatment options fail,

NIDDM patients, in the most severe cases, are also placed on insulin injections to make their diabetes more manageable.

## **1.6 The Short- and Long- term Monitoring of Diabetes Mellitus**

The management of blood glucose concentrations in diabetic patients is very important as mentioned before. Methods of monitoring glucose concentration include: 1) self monitoring by means of a hand held device using a droplet of blood, obtained from a fingerprick and 2) blood taken by a trained medical professional via venipuncture from the patient's vein in the arm. The latter sample is sent to the laboratory for glucose analysis on an automated analyser.

This glucose measurement is a reflection of the glycaemic state of the patient at that given moment. Over the years other tests have been developed to give the healthcare professional a better clinical picture of how the glycaemic control has been in the diabetic patient over a period of time and are thus more advantageous than looking just at the blood glucose concentration alone. These tests are glycated haemoglobin (HbA<sub>1c</sub>) and fructosamine.

### **1.6.1 HbA<sub>1c</sub>**

Glycated haemoglobin is the term used to describe a spontaneous reaction between glucose, which is a reducing agent, and haemoglobin found in the erythrocyte. This reaction occurs non-enzymatically (a spontaneous reaction) to form a ketoamine. The rate of HbA<sub>1c</sub> formation is directly proportional to the concentration of plasma glucose present. The glucose control is usually assessed in diabetics by HbA<sub>1c</sub> measurement.

The WHO recognises HbA<sub>1c</sub> as the gold standard in assessing glycaemic control of diabetics, as well as assessing glucose control in patients without diabetes. It is a reflection of the average glucose concentration over a period of 4 to 6 weeks and its gives an equal or almost equal sensitivity and specificity of a glucose measurement in most cases (World Health Organization, 2006).

Haemoglobinopathies and other conditions may also interfere with HbA<sub>1c</sub> assessment and these are discussed in the following sections. HbA<sub>1c</sub> determination is based on the assumption that the analysis of blood is performed on blood containing normal haemoglobin (Smaldone, 2008). Hence in patients who do not have normal haemoglobin, the assay cannot be interpreted accurately. Abnormalities that will alter the HbA<sub>1c</sub> assay are as follows:

### 1.6.1.1 Haemolytic Anaemia

Haemolytic anaemia is a condition that develops due to excessive breakdown of erythrocytes. A normal erythrocyte has an average lifespan of 120 days. When a haemolytic event or haemolysis occurs and the bone marrow cannot compensate with adequate erythrocyte production due to the increase in demand, haemolytic anaemia develops. This can be caused by two mechanisms namely: 1) intravascular haemolysis and 2) extravascular haemolysis (Tabara, 1992).

Intravascular haemolysis may occur due to complement fixation, trauma or extrinsic factors. Examples of such conditions would be disseminated intravascular coagulation or DIC, thrombotic thrombocytopenic purpura, glucose-6-phosphate deficiency or trauma to name a few (Tabara, 1992).

Extravascular haemolysis occurs more commonly when the red blood cells are removed from the circulation by the mononuclear phagocytic system either because they are intrinsically abnormal, or because of bound immunoglobulins (antibodies) on their outer membrane surfaces (Tabara, 1992).

The causes of intra- and extravascular haemolysis can be either genetic or acquired.

Genetic abnormalities that may cause intra- or extravascular haemolysis may be as a result of erythrocyte membrane abnormalities as seen in sickle cell anaemia, haemoglobin abnormalities as seen in thalassaemia or enzyme defects as seen in glucose-6-phosphate deficiency.

Acquired abnormalities leading to intra- or extravascular haemolysis may be due to immune reaction as seen in a blood transfusion reaction, autoimmune as seen in many leukaemias or non-immune as seen in major traumatic events (Tabara, 1992).

Haemoglobinopathies interfere with HbA<sub>1c</sub> measurements. HbA<sub>1c</sub> determination is based on the analysis of blood containing normal haemoglobin. Thus, haemoglobinopathies could affect this test in three ways: 1) it could alter the normal process of glycation of HbA to HbA<sub>1c</sub>; 2) create an abnormal peak in the chromatography analysis, making the estimation of HbA<sub>1c</sub> unreliable and 3) making the red blood cells more prone to haemolysis, thus decreasing the time for the glycosylation process to occur and producing false low HbA<sub>1c</sub> results. The use of fructosamine as an alternative test is recommended to assess glycaemic control in such cases (Smaldone, 2008).

Abnormalities in glycated serum proteins, such as fructosamine, are not inherited, thus the measurement of such analytes are better indicators of glycaemic control in patients with

haemoglobinopathies, since haemoglobinopathies are inherited and will affect the HbA<sub>1c</sub> measurement (Cohen, 2007).

#### **1.6.1.2. Sickle Cell Anaemia**

Sickle cell anaemia, also known as sickle cell disease or drepanocytosis, is a life long disorder and is classified as a haemolytic anaemia. It occurs when an erythrocyte assumes a rigid sickle shape, instead of its normal round biconcave shape. This condition occurs due to an abnormality found on the haemoglobin gene causing a homozygosity for a mutation that causes haemoglobin S (HbS). This happens when a point mutation occurs in the  $\beta$ -globin chain of haemoglobin, causing the amino acid glutamic acid to be replaced with the hydrophobic amino acid valine in the sixth position. The  $\beta$ -globin gene is found on the short arm of chromosome 11. The association of two wild-type  $\alpha$ -globin subunits with two mutant  $\beta$ -globin subunits forms HbS. Under low-oxygen conditions (being at high altitude, for example), the absence of a polar amino acid at position six of the  $\beta$ -globin chain promotes the non-covalent polymerization (aggregation) of haemoglobin, which distorts red blood cells into a sickle shape and decreases their elasticity (Desai & Hiren Dhanan, 2004).

Due to the presence of this abnormal haemoglobin in patients suffering from sickle cell anaemia, HbA<sub>1c</sub> cannot be used for the monitoring of diabetes, as the HbS peak on the HPLC interferes with the HbA<sub>1c</sub> peak (Smaldone, 2008).

#### **1.6.1.3 Thalassaemia**

Thalassaemia is an inherited autosomal co-dominant blood disease. It can also be classified as an inherited haemolytic anaemia. In thalassaemia, the genetic defect results in reduced rate of synthesis of one of the globin chains that make up haemoglobin. Reduced synthesis of one of the globin chains can cause the formation of abnormal haemoglobin molecules. Thalassaemia produces a deficiency of  $\alpha$ - or  $\beta$ -globin, unlike sickle-cell disease which produces a specific mutant form of  $\beta$ -globin.  $\alpha$ -Globin chains are encoded by two closely linked genes on chromosome 16. Thus in a normal person with two copies of each chromosome, there are two loci encoding the  $\beta$ -chain, and four loci encoding the  $\alpha$ -chain (Thalassaemia International Federation, 2009).

The thalassaemias are classified according to which chain of the haemoglobin molecule is affected. In  $\alpha$ -thalassaemias, production of the  $\alpha$ -globin chain is affected, while in  $\beta$ -thalassaemia production of the  $\beta$ -globin chain is affected.

#### **1.6.1.4 Patients Receiving Blood Transfusions**

When a patient suffers trauma with severe blood loss such as a car accident, they will require a blood transfusion to replace the blood lost. In other words, their blood is being replaced by a donor's blood. Their blood volume is being supplemented by a donor's blood to increase their blood volume, including the erythrocytes. This means that a portion of the circulating blood is not the patient's own and HbA1c analysis will not be of any use.

#### **1.6.1.5 Iron Deficiency Anaemia**

Iron deficiency anaemia is classified as a nutritional anaemia due to insufficient intake of iron in the diet, which is critical in red blood cell formation. It is characterized by an anaemic state (low haemoglobin concentration) with the morphology of the erythrocytes being hypochromic (light in colour) and microcytic (small in size). The same morphology is seen in patients suffering chronic blood loss and other haematological diseases such as thalassaemia and patients who suffered lead poisoning. It can also occur in vegetarians whose diet does not contain sufficient iron.

The main source of iron in the diet is red meat. Iron absorption takes place in the duodenum (first section of the small intestine in higher vertebrates) and is highly dependent on gastric hydrochloric acid, which is produced by the stomach. The latter ensures that iron is available in the ferro ( $\text{Fe}^{2+}$ ) form which is the absorbable form of iron. In the circulation, iron is transported by a transporter protein known as transferrin and is stored as ferritin (Killip, Bennet, & Chambers, 2007).

#### **1.6.1.6 Megaloblastic Anaemia**

Megaloblastic anaemia is anaemia of macrocytic (large) and hypochromic classification that results from inhibition of DNA synthesis in red blood cell production. This is often due to deficiency of vitamin B<sub>12</sub> and/or folic acid. The cause of megaloblastic anaemia is not only due to hypovitaminosis but may be due to antimetabolites which inhibit DNA production, such as alcohol and some chemotherapeutic or antimicrobial agents, for example azathioprine or trimethoprim (Savage *et al.*, 1994).

It is characterized by many large immature and dysfunctional erythrocytes known as megaloblasts in the bone marrow and also by hypersegmented or multisegmented neutrophils (Savage *et al.*, 1994).

### **1.6.1.7 Polycythaemia**

Polycythaemia is a condition in which there is an increase in the erythrocyte volume of the blood, which is measured as the haematocrit.

It can be due to an increase in the mass of erythrocytes, otherwise known as absolute polycythaemia or to a decrease in the volume of plasma, known as relative polycythaemia.

Absolute polycythaemia can further be classified into 3 subtypes: 1) polycythaemia vera, 2) secondary polycythaemia and 3) Chuvash polycythaemia.

Polycythaemia vera is characterized by excess erythrocytes production as a result of an abnormality of the bone marrow. In most cases, excess white blood cells and platelets are also produced (Wallash, 2007).

Secondary polycythaemia is caused by either natural or artificial increases in the production of erythropoietin (the hormone responsible for red blood cell production in the bone marrow), causing an increased production of erythrocytes. There may be 6 to 8 million and occasionally 9 million erythrocytes per cubic millimeter (microliter) of blood (Wallash, 2007).

Chuvash polycythaemia is a familial form of erythrocytosis different from classical polycythaemia vera. It involves patients from Chuvashia and is associated with a C598T mutation in the von Hippel-Lindau gene. A cluster of patients with Chuvash polycythaemia have been found in other populations, such as on the Italian island of Ischia, located in the Bay of Naples (Niu *et al.*, 2008).

Relative polycythaemia is an apparent rise of the erythrocyte level in the blood. The underlying cause is reduced blood plasma, often caused by loss of body fluids, such as through burns, dehydration and stress (Wallash, 2007).

### **1.6.1.8 Pregnancy**

One of the complications women might develop during pregnancy is iron deficiency due to an increase in demand for the development of the foetus. This will result in anaemia during middle to late pregnancy. Research has shown that HbA<sub>1c</sub> was elevated in relationship to glycaemia in late pregnancy but not glycated serum proteins (GSP's). This was due to iron deficiency which normally occurs in late pregnancy. It has been suggested that GSP's offer a better index for glycaemic control in late pregnancy (Hashimoto *et al.*, 2008). In 2006, a

study was done to determine if the serum concentration of fructosamine can be of diagnostic use in pregnant patients with abnormal glucose tolerance. It was found that it could be used to monitor these patients, and to identify which patients were at high risk of an abnormal glucose tolerance. The test, however, could not be used to predict if these patients will develop gestational diabetes in early pregnancy (Li & Yang, 2006).

#### **1.6.1.9 Foetal Haemoglobin or HbF**

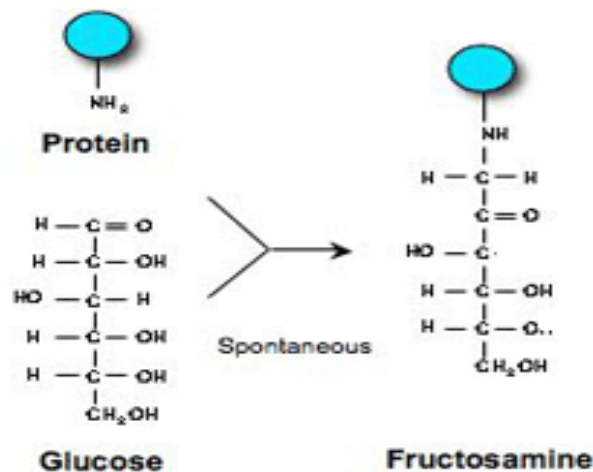
HbF is foetal haemoglobin found in the foetus before birth. In HbF, the  $\beta$ -chains are substituted by  $\gamma$ -chains. During pregnancy, easy transition of oxygen from the mother's haemoglobin across the placenta to the foetus's haemoglobin occurs due to HbF. Under normal conditions, the HbF concentration rapidly decreases to concentrations less than 2 % after 2 years of age. This concentration will, under normal conditions, be 1 – 7% once a patient reaches adult life (Wood, 1993).

There are, however, some individuals who suffer from a hereditary condition where HbF persists into adult life. In some cases, the HbF concentration may be 70 – 80 % of the total haemoglobin concentration, and this will cause an interference in the quantitation of HbA1c (Higgins, Stewart, & Boehr, 2008).

#### **1.6.2 Fructosamine**

Fructosamine is a generic name given to a compound known as plasma protein ketoamines. It is formed by a spontaneous non-enzymatic reaction between a carbonyl group of a glucose molecule with an amino group of a protein (Burton, Ashwood, & Burns, 2006). In blood, fructosamine is primarily glycated albumin, as it is the most abundant protein present. It is also known as glycated serum proteins (GSP) or glycated albumin, and is used to monitor the plasma glucose concentration over a shorter period of time (usually 4 to 6 weeks) so as to assess diabetic control. The plasma or serum half-life of albumin is 17-20 days.

Figure 1.1 explains the basic structure and formation of fructosamine. It shows how a glucose molecule will react spontaneously with the  $\text{NH}_2$  terminus of a protein molecule, such as albumin, to form the fructosamine molecule.



**Figure 1.1:** Basic structure and formation of fructosamine (de Duve Institute, 2009).

Over the years, the use of HbA<sub>1c</sub> as a marker for glucose control has been well established. However the use of HbA<sub>1c</sub> is limited in certain disease conditions. Several patients with raised plasma glucose levels, but with normal HbA<sub>1c</sub> concentrations showed elevated fructosamine concentrations, which indicated poor glycaemic control (Youssef, El Abbassi, & Peiris, 2008). It has been suggested that fructosamine testing should be performed as a routine check on diabetic patients with normal HbA<sub>1c</sub> concentrations to confirm their glucose is under control. Fructosamine assays are cheap and fast assays which can be performed in normal routine laboratories and is currently an underutilized assay (Youssef, El Abbassi, & Peiris, 2008).

Babu *et al.* (2006) suggested that plasma fructosamine levels were closely associated with malondialdehyde and glutathione in patients suffering from rheumatoid arthritis, as these 2 substances can modulate the glycation process. The authors recommended that fructosamine concentrations should be interpreted with caution in these patients (Babu *et al.*, 2006).

HbA<sub>1c</sub> and fructosamine must be used with caution as tools for monitoring chronic glycaemia in patients suffering from Acquired Immunodeficiency Syndrome (AIDS) due to an enhancement of protein glycation induced by an unknown protein enhancer (Kabadi & Kabadi, 2008). This is an important fact to consider when interpreting fructosamine results, as South Africa has the largest population of people living with HIV, which was 5.7 million in 2009 (World Health Organization, 2009).



A direct correlation exists between serum fructosamine and albumin concentrations. Patients suffering from nephrotic syndrome and cirrhosis of the liver have low serum concentrations of albumin due to their disease. It was established that serum fructosamine measurement in these patients as a tool for glycaemic control was not useful. Fructosamine measurements should also be used with caution in patients who suffer from other clinical conditions where circulating plasma protein and albumin concentrations are altered (Constanti *et al.*, 1992).

It was shown that a single serum fructosamine assay at any given time of the day can be used as a tool for the screening and diagnosis of GDM, instead of making use of the OGTT (Weerasekera & Peiris, 2000).

A study was conducted to compare the correlation of fructosamine and HbA<sub>1c</sub> with carbohydrate intake on the same group of participants. It concluded that there was a stronger relationship between dietary intake of carbohydrates, especially simple sugars, and fructosamine, than HbA<sub>1c</sub> (Misciagna *et al.*, 2004).

Stress hyperglycaemia with random serum glucose of > 11.0 mmol/L in non-diabetic adults was monitored in a study conducted between 1994 and 1995. This study tried to establish whether random serum glucose measurement and/or fructosamine measurements can be used as a marker of risk for diabetes mellitus. These patients came for follow-up blood testing 5 years after this initial baseline blood analysis was performed. Random plasma glucose showed that it could not be used as such a marker. However, fructosamine showed to be a more reliable marker than plasma glucose for predicting this risk (Wahid *et al.*, 2002).

Patients with diabetes mellitus suffer from many complications. One of these complications is dyslipidaemia (abnormal lipid profile), which is an independent risk factor for the development of heart disease and atherosclerosis. A study was conducted to establish whether the lipid profile in patients suffering from diabetes mellitus differed amongst groups of patients who had been identified as having good, satisfactory or poor glycaemic control, by measuring each individual's lipid profile and serum fructosamine concentrations. It concluded that improving glycaemic control in patients with diabetes mellitus also improved dyslipidaemia in patients with poor glycaemic control, thus lowering the risk of atherosclerosis. Fructosamine gives a good index of glycaemic control and its value will also be a reflection of the serum lipid profile in patients with poor glycaemic control (Abdel-Gayoum, 2004).

Research done in Singapore in 1988 showed that serum fructosamine correlates well with HbA<sub>1c</sub> and serum lipids (Tho *et al.*, 1988). A random lipogram was done together with the fructosamine in this study to determine whether this correlation exists within the apparently healthy sample population group, since abnormalities in plasma lipid concentration are common amongst diabetic patients (Kithier & Cejka, 1987).

In animals, it was established that normal serum fructosamine concentration in dogs with normal glycaemic values indicated a normal protein status, or a very transient albumin disturbance. Abnormally low fructosamine values in normoglycaemic dogs in the same study indicated that there might be a long term albumin disturbance, or the recovery of such a condition. This study went on to suggest that fructosamine might even be used as a tool to monitor a hypoproteinaemic or hypoalbuminaemic state in dogs and their recovery there-of as well as treatment progression (Thoresen & Bredal, 1998). Total serum protein and albumin was done on the sample population to see whether this correlation exists in a human based study.

In 2006, a study was done to determine if the serum concentration of fructosamine can be of diagnostic use for pregnant patients with abnormal glucose tolerance. It was found that it could be used to monitor these patients, and to identify which patients were at high risk of an abnormal glucose tolerance. The test, however, could not be used to predict if these patients will develop gestational diabetes in early pregnancy (Li & Yang, 2006).

Fructosamine concentration should be assessed continuously together with blood glucose concentrations to establish the correlation between the two analytes (Post *et al.*, 2000).

### **1.7 Total Serum / Plasma Protein and Albumin**

Total protein, also known as serum total protein or plasma total protein, is a biochemical test used to measure the total amount of protein present in serum/plasma. Protein in plasma consists of albumin as well as a myriad of other proteins present, some in very low concentrations. Albumin is present in the largest concentration. Globulins, in turn, consist of the following 4 subfractions:  $\alpha_1$ ,  $\alpha_2$ ,  $\beta$  and  $\gamma$ . Each of these globulin fractions and albumin can be separated and quantitated using serum protein electrophoresis where separation of particles occurs, based on their electric charge and size through an agarose gel medium. They are then visualized by fixation and staining of the agarose gel. The gel is then placed through a process of densitometric scanning to quantitate the stained bands of subfractions on the agarose gel. Another popular method currently in use for protein electrophoresis is the capillary zone electrophoresis, which is based on high performance liquid

chromatography methodology. Total protein determination however is a faster and cheaper way to quantitate albumin and all the globulin fractions using automated analysers in the laboratory. One of the methods for protein analysis usually employs the Biuret reagent (Beckman Coulter, 2000). However, other methods are also available which include: Kjeldahl method, dye-binding and refractometry.

Most of the proteins found in plasma are synthesized and secreted by the liver cells (or hepatocytes) into circulation, except for immunoglobulins, which are synthesized by plasma cells. Currently, more than 500 different plasma proteins have been identified (McPhee *et al.*, 1995).

The main function of plasma proteins is to act as transporter molecules of various water insoluble substances, such as metals, certain drugs and unconjugated bilirubin. Other functions of plasma proteins include: 1) maintaining the water distribution between cells and tissues, interstitial compartments, and the vascular system, 2) it acts as a buffer to maintain physiological pH, 3) their participation in the immune system as antibodies, 4) it acts as hormones in various physiological- and body functions, 5) its role as biocatalysts or enzymes in chemical reactions and 6) the participation in blood homeostasis and clotting factors in the coagulation of blood (Bishop, Fody, & Schoeff, 2005).

Hyperproteinaemia (increase in total protein concentration) is seen in conditions such as paraproteinaemia (monoclonal gammopathy) where there is an increase in the concentration of a single monoclonal gammaglobulin. The most well known examples are: 1) Multiple Myeloma and 2) Waldenström's Macroglobulinaemia. Hyperproteinaemia has also been observed in dehydration due to water loss from the vascular system, resulting in concentration of the proteins remaining behind due to their large molecular size. It is not seen as often as hypoproteinaemia (Al-Sobhi *et al.*, 2009).

Hypoproteinaemia, a total protein concentration less than the reference interval, occurs in conditions where a negative nitrogen balance exists. One condition where this occurs is excessive loss due to plasma protein being excreted via the kidneys in renal disease, such as acute- or chronic renal failure or nephrotic syndrome. Other disease states associated with hypoproteinaemia include: inflammation of the gastrointestinal tract, blood loss due to excessive bleeding as a result of deep wounds or severe trauma, liver diseases such as cirrhosis or hepatitis due to decreased protein synthesis by the liver, and burns (Kennish, 2005). Decreased total protein may also be observed in patients with a poor diet due to decreased protein intake. This study hypothesized that there will be a positive correlation between fructosamine concentration and total protein concentration of all the participants.

Fructosamine measurements have shown to vary with the concentration of serum protein present, the proportion of serum protein and with the lifespan of serum protein. The most important factor was proven to be the concentration of total protein. Therefore, when interpreting fructosamine concentration, the concentration of total protein should also be considered (Oimomi & Masuta, 1989).

Albumin is the major component of plasma proteins and is produced by the liver. It is soluble and monomeric (a small molecule that can bind chemically to other monomeric substances to become a polymer). One albumin molecule has 585 amino acids in its sequence (Bar-Or *et al.*, 2006).

Hypoalbuminaemia (a decrease in plasma albumin concentration) is caused by many conditions and they are: 1) insufficient amino acid supply for albumin synthesis due to malnutrition or muscle wasting diseases such as muscular dystrophy, 2) liver disease such as hepatitis; however, during liver cirrhosis, there is an increase in plasma globulins that will mask the hypoalbuminaemia, because of a concomitant normal reference range, 3) gastrointestinal loss due to inflammation of the mucosal lining of the gastrointestinal tract and 4) glomerular damage in renal disease causing albumin loss. It is not abnormal to find small amounts of albumin in normal urine. A study performed in 2008 indicated that hypoalbuminaemia is an independent predictor of cardiovascular disease and mortality in patients suffering from stage IV chronic kidney failure (Shah & Dumler, 2008).

A condition also exists where there is an abnormal structure of albumin in plasma due to unusual molecular structure, resulting in a decrease in albumin concentration. This condition is known as dysalbuminaemia and is characterized by two albumin bands when doing densitometric tracing after serum protein electrophoresis has been performed (Kalambokis *et al.*, 2002).

Hypoalbuminaemia and hyperglycaemia may be possible indices of mortality and the metabolic status of critically ill patients (Safavi & Honarmand, 2009). It was hypothesized that the fructosamine concentration and the albumin concentration in this study will show a positive correlation in all the participants.

Due to the high turnover of human serum albumin in blood (half life of 17 - 20 days), the degree of glycation in serum proteins, which is mostly albumin, provides the clinician with an index of glycaemia over a shorter period of time. Since the measurement of GSPs correlate well with HbA<sub>1c</sub>, it was suggested as an alternative method for the routine monitoring of glycaemia and glycaemic control in patients with diabetes (Goldstein *et al.*, 2004).

Since albumin is the most abundant plasma protein, it has been shown to correlate well with the concentration of fructosamine present (Oimomi & Masuta, 1989).

### 1.8 Fructosamine versus HbA<sub>1c</sub>

A comparative study was done with HbA<sub>1c</sub> and fructosamine to determine a relationship between the 2 analytes. The results showed that there is evidence for a glycosylation gap and its relationship to diabetic neuropathy. The glycosylation gap can be a useful tool in diabetic and glycaemic control (Cohen *et al.*, 2003). Formulas indicating the relationship between the 2 analytes have been summarized as follows (National Quality Measures Clearinghouse, 2008):

$$\text{HbA}_{1c} = (0.017 \times \text{Fructosamine}) + 1.61$$

AND

$$\text{Fructosamine} = (\text{HbA}_{1c} - 1.61) \times 58.82$$

A recent study was done to compare the clinical impact of variability in HbA<sub>1c</sub> and fructosamine. The conclusion of that study was that HbA<sub>1c</sub> alone may not accurately reflect glucose control. A suggestion was made that fructosamine together with HbA<sub>1c</sub> should be used routinely to monitor glycaemic control (MacDonald *et al.*, 2008).

In 2007, a project was launched in the United States to compare the differences in HbA<sub>1c</sub> by race and ethnicity among patients with impaired glucose tolerance. The outcome showed that HbA<sub>1c</sub> was higher among U.S. racial and ethnic minority groups with IGT after adjustment of factors affecting glycaemia such as age, sex, education, marital status and blood pressure. It was concluded that HbA<sub>1c</sub> may not be a valid method for assessing and comparing glycaemic control across racial and ethnic groups. It was then questioned whether raised HbA<sub>1c</sub> can be used as diagnostic test for diabetes (Herman *et al.*, 2007).

HbA<sub>1c</sub> is used to monitor the adequacy of diabetes management and medication adjustments over a longer period. The day-to-day management of glucose is done by means of fingerprick testing and measurement of capillary glucose. This is mostly performed by the patients themselves. A mathematical relationship has been developed between these two parameters to express HbA<sub>1c</sub> values as average glucose concentration (Nathan *et al.*, 2008). The mathematical formula is as follows:

$$\text{Estimated Average Glucose (mmol/L)} = (1.59 \times \text{HbA}_{1c}) - 2.59$$

## **1.9 Impaired Lipid Metabolism and Hyperglycaemia**

Impaired lipid metabolism in patients suffering from uncontrolled diabetes has been shown to be an independent risk factor for the development of cardiovascular disease (CVD) complications. Research has shown that diabetic patients have a two-fold mortality rate and risk of developing serious CVD than non-diabetic individuals. Diabetic patients usually exhibit an atherogenic lipid profile, with an increase in triglyceride concentration and a decreased high-density lipoprotein (HDL) concentration. This will greatly increase their risk for CVD compared to non-diabetic individuals. Patients with type 2 diabetes who carry the apolipoprotein E 4 genotype were shown to have a greater CVD risk, owing to a metabolic alteration in lipid metabolism. This will cause an increase in total cholesterol and low-density lipoprotein (LDL) concentrations (Khan, Sobki, & Khan, 2007).

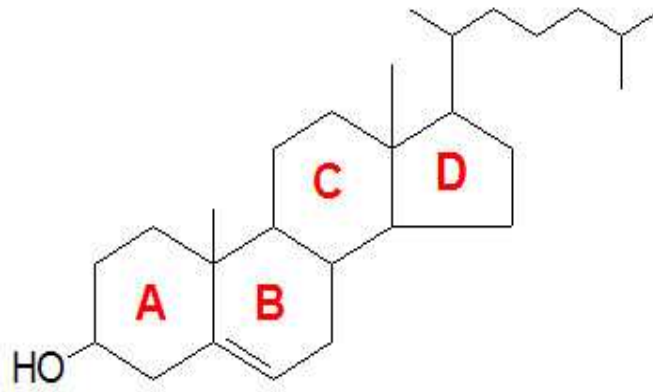
A lipogram consists of serum cholesterol, HDL, LDL and triglycerides. Due to the association between hyperlipidaemia, CVD and diabetes, it was measured on all the participants in this study.

### **1.9.1 Cholesterol**

Cholesterol is an unsaturated steroid alcohol molecule which contains 4 rings namely: A, B, C and D. It contains a single C-H side chain which is similar to a fatty acid in its physical properties. There is only one hydrophobic (water repelling) section on the cholesterol molecule which is found on the A-ring. We can thus deduce that cholesterol is an amphipathic molecule (one that contains hydrophobic and hydrophilic properties). The orientation of the cholesterol molecule is also in such a manner that it is organized in lipid layers, enabling the 4 rings and the side chain to be embedded in a parallel orientation to the fatty acid acyl chains on adjacent phospholipid molecules. The polar hydroxyl group found on the A-ring faces outwards, allowing it to interact with water by non-covalent hydrogen bonding (Masterjohn, 2010).

The cholesterol molecule can also exist as a cholesteryl ester, which is the esterified form of the molecule. This occurs when the hydroxyl group is conjugated with a fatty acid by means of an ester bond, the same as triglycerides, which will be discussed later on. In contrast to free cholesterol, cholesteryl esters have no polar groups making them very hydrophobic molecules. This is why they are not found on the surface of lipid membranes. Instead, they are found in the centre of a lipid drop, as seen with triglycerides (Bishop, Fody, & Schoeff, 2005).

Figure 1.2 shows the basic ring structure of a cholesterol molecule with the 4 rings named A, B, C and D with its relevant side chains.



**Figure 1.2:** The basic structure of a cholesterol molecule. (The Medical Biochemistry Page, 2009).

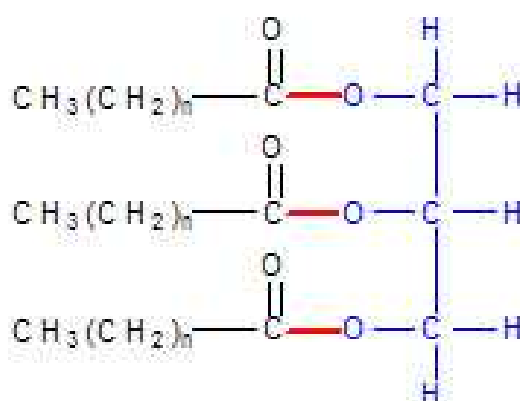
Cholesterol is almost exclusively synthesized by animals, however; plants synthesize esters which are similar in structure to cholesterol. It also has the unique property that it cannot be catabolized by most cells and can therefore not serve as a source of energy like glucose. The liver can convert cholesterol to primary bile salts like cholic acid. The function of bile salts are that they act as detergents in the gastrointestinal tract to help with the absorption of fat during the digestion process (Wolkoff & Cohen, 2003). A small amount can also be converted to vitamin D<sub>3</sub> after exposure to sunlight (Bogh *et al.*, 2010).

It is clear that cholesterol has many important functions. However, high cholesterol concentrations have been associated with an increased risk for cardiovascular disease. It therefore plays an important role in the early diagnosis of atherosclerosis, a condition where deposition of cholesterol in the arterial walls, leads to wall thickening and reduction of the arterial lumen, causing a decrease in normal bloodflow (Bishop, Fody, & Schoeff, 2005). Foods containing increased levels of saturated fats will increase this risk. Family history of hyperlipidaemia is also associated with an increased risk. Food such as eggs, meat and dairy products are high in cholesterol and should be consumed conservatively.

### 1.9.2 Triglycerides

Triglycerides are lipid structures which are composed of 3 molecules of fatty acids attached to a single molecule of glycerol by ester bonds. Due to the large number of different fatty acid structures, each fatty acid within the triglyceride molecule will have a different structure. This results in the triglyceride molecule having many different structural forms. Triglycerides can be solid at room temperature, because they contain tightly packed saturated fatty acids with no kinks in their structure. On the other hand, they can also be oils at room temperature, because these forms contain *cis* unsaturated fatty acids with bends in their structure. Liquid triglycerides mostly originate from plants such as corn and sunflower seeds, whereas the solid triglycerides are obtained from animals. It contains no charged groups or polar hydrophilic groups, making it a very hydrophobic molecule and virtually water insoluble (Zamora, 2010).

Figure 1.3 shows the structure of a typical triglyceride molecule, with the backbone, the ester linkages and three fatty acid side chains.



**Figure 1.3:** The basic structure of a triglyceride molecule (Washington University in St. Louis, 2009).

Triglycerides are measured to determine whether a patient suffers from hypertriglyceridaemia (increased concentration of serum triglycerides). This is often seen in patients who suffer from ischaemic vascular diseases (IVD) such as atherosclerosis. An increase in triglycerides has been associated with an increase in blood viscosity and a precipitation of platelet aggregates. This will result in a decrease in blood flow in the vascular system. In contrast, high density lipoproteins (HDL) will be decreased with an increase in triglyceride concentration (Chronolab, 2009). Triglycerides are not related to cardiovascular disease if it is raised alone. A low concentration of HDL and raised LDL



together with a high triglyceride concentration will be risk factor for the development of CVD. Other pathological conditions where triglycerides are increased include: acquired immune deficiency syndrome (AIDS), pernicious anaemia, acute- and chronic pancreatitis, diabetes mellitus, most heart-, renal and liver disease to name a few.

Research studies have identified a correlation between hyperglycaemia and hyperlipidaemia. One such study concluded that both male and female diabetic patients with uncontrolled diabetes had severe hyperglycaemia and increased concentrations of cholesterol, triglycerides and LDL, the latter being discussed later in this chapter. This paper also mentioned that, although mild hypertriglyceridaemia occurs commonly in type 2 diabetes, severe hypertriglyceridaemia occurs in patients with uncontrolled diabetes. It also showed significantly higher triglyceride concentrations in patients with diabetes suffering from chronic heart disease as compared to non-diabetic patients with chronic heart disease (Khan, 2007). An increase in triglyceride-rich lipoproteins has been identified as one of the main indicators of patients suffering from metabolic syndrome (Tada & Yoshida, 2007).

Triglyceride analysis for this study was of great importance as several papers have indicated its relationship to several disease states, including hyperglycaemia and diabetes (Abdel-Gayoum, 2004).

### **1.9.3 Lipoproteins: High-Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL)**

As indicated by the name, lipoproteins are made up of lipids and proteins. They are typically spherical in shape and range in size from 10 – 1200nm. The primary role of lipoproteins is for energy delivery to the cells of the body. Therefore, the core of a lipoprotein molecule will consist of the so-called “cargo” to be delivered to the cell. The size of the lipoprotein particle will also coincide with its lipid content. The amphipathic cholesterol and phospholipid are found in a single layer on the surface of a lipoprotein particle, while the hydrophobic triglyceride and cholesteryl esters are found in the core of the particle. If the lipoprotein particle is larger, it will have a larger core region containing more triglyceride and cholesteryl esters (The Biochemistry Questions Site, 2010). The two lipoprotein particles that were of interest to this study were HDL and LDL.

Apolipoproteins are found on the cell surface of a lipoprotein particle. One of their major functions is to assist in maintaining the structural integrity of the lipoprotein particle. They also serve as ligands to cell surface receptors and as activators and inhibitors to enzymes that will alter the structure of the lipoprotein molecule. Apolipoprotein contains a structural

motif which is called an amphipathic helix. Amphipathic helices are protein segments which are arranged in coil-like structures, enabling the hydrophobic amino acid residues to interact with lipids. The part of the helix containing the hydrophilic amino acids will face away from the lipids and towards the aqueous environment of the bloodstream. These helices thus enable these proteins to bind to lipids and making them more water soluble. (Gangani da Silva *et al.*, 2008).

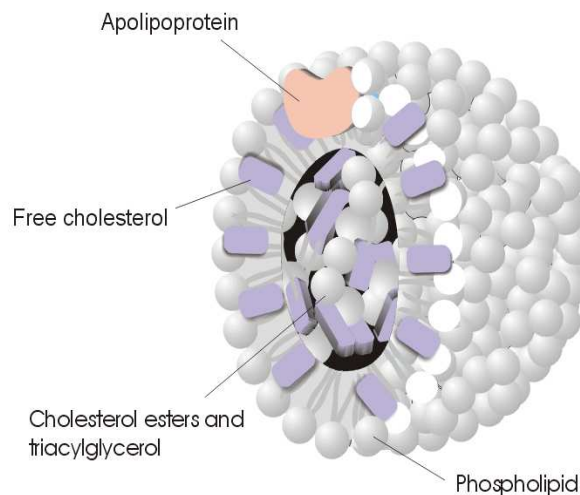
Apo A-I has a molecular weight of 28 kD and is the main apolipoprotein found on HDL. Apo A-II, A-IV, C-I, C-II, C-III and E are also found on HDL but in much smaller concentrations. Apo-B is a large protein found on LDL with a molecular weight of 500 kD. Apo-B also exists in 2 forms: apo B-100 and apo B-48 respectively. Apo B-100 acts as a ligand for the LDL receptor and therefore plays an important role in LDL uptake into body cells (Bishop, Fody, & Schoeff, 2005). The remaining apolipoproteins will not be discussed as it was not of importance to this study.

Table 1.4 below summarizes the difference in characteristics of HDL and LDL.

**Table 1.4:** Basic characteristics of HDL and LDL. Adapted from Bishop, Fody, & Schoeff (2005)

Characteristics	HDL	LDL
Density (g/ml)	1.063 – 1.21	1.019 – 1.063
Molecular weight (kD)	$(1.75 – 3.6) \times 10^5$	$2.75 \times 10^6$
Diameter (nm)	5-12	18 – 30
Total lipid (% by weight)	50	77
Total cholesterol (% by weight)	19	62
Total triglyceride (% by weight)	3	11

Figure 1.4 shows the basic three-dimensional structure of a lipoprotein, indicating the cholesterol and phospholipids on the outer layer and the triglyceride and cholesteryl ester in the core of the molecule.



**Figure 1.4:** Three-dimensional structure of a lipoprotein molecule (Kansas State University, 2009)

A correlation exists between hyperglycaemia and hyperlipidaemia as shown by past research. These studies concluded that both male and female diabetic patients with uncontrolled diabetes had severe hyperglycaemia and increased concentrations of cholesterol, triglycerides and LDL. In this same study, they compared HbA<sub>1c</sub> measurements with cholesterol, triglycerides, HDL and LDL. It showed a direct and significant correlation between LDL, triglycerides and cholesterol and an inverse correlation with HDL. Comparing male and female participants, there was no significant difference in LDL and triglyceride concentrations. However, females showed a higher HDL and cholesterol concentration versus males. The study proved that HbA<sub>1c</sub> showed the ability to predict dyslipidaemia in patients who have poor glycaemic control (Khan, 2007).

### 1.10 The Reference Value used for Fructosamine

Most of the reference values in use in South Africa today are derived from values used in overseas countries like the United States and Europe. These intervals should only serve as guidelines because each country's population will differ from the next due to the difference in lifestyle and diet. Chan *et al.*, 2008 concluded that obtaining reference values for each laboratory is essential for the local service population. However, this task can be difficult to

achieve because it can become a costly and time consuming exercise when compared to reference value validation from assay manufacturers. They found that the intervals set out by the manufacturers broadly correlated with their data and small changes were made to the reference intervals (Chan *et al.*, 2008).

Many factors should be kept in mind when doing analysis of data for the determination of reference intervals. One important factor is to derive the data from healthy individuals as set out by the National Committee for Clinical Laboratory Standards or NCCLS (National Committee for Clinical Laboratory Standards, 2001). The other factors will include: age, sex, race, diet and the amount of exercise the population group was exposed to. The screening process of apparently healthy individuals for the determination of a reference interval is therefore recommended (Horn & Pesce, 2003).

Test reference ranges are essential for any laboratory and it serves as an aid to the clinician to differentiate between the healthy and diseased patient. The establishment of such a range will depend on the size of the data set and the method of evaluation. Assuming the subjects of a sample population are all healthy, 5% of the results would be outside the reference limits, while the remaining 95% will be within normal limits. A sample size of about 120 will be acceptable for the determination of this reference range (Horn & Pesce, 2003).

The reference range used by the PathCare Pathology Group for fructosamine is 200 – 285  $\mu\text{mol/L}$  and was obtained from Tietz Textbook of Clinical Chemistry (Tietz, 1993).

It was hypothesized that the reference values for fructosamine for the South African sample population would differ from that in literature due to their lifestyle and diet.

### **1.11 Research Rationale and Hypotheses**

In subjects with diabetes, the plasma glucose concentrations need to be monitored in order to prevent and manage complications due to diabetes. Measurement of blood glucose is not ideal for glucose monitoring as it only relates to levels at that specific time. Hence, a method that can assess glucose control over a period of time is recommended. In this regard, the WHO recognises HbA<sub>1c</sub> as a gold standard in assessing the glycaemic control of patients suffering from diabetes, as well as assessing glucose control in patients without diagnosed diabetes (World Health Organization, 2006). However, HbA<sub>1c</sub> has limitations in certain disease conditions, particularly those that affect the red cells. In such cases, fructosamine can be used as an alternative method to monitor glycaemic control in diabetics.

Therefore, the objective of this study was to develop a new reference range for fructosamine in the South African population, based on the concentrations of fructosamine in apparently healthy individuals. Fructosamine was measured together with HbA<sub>1c</sub> and the other analytes mentioned before, to confirm that the study population were healthy individuals without diabetes or impaired glycaemic control.

The following hypotheses were addressed in this study:

#### **1.11.1 Hypothesis 1**

It was hypothesized that the fructosamine for the South African population would be different from that in literature due to their lifestyle and diet.

#### **1.11.2 Hypothesis 2**

It was hypothesized that fructosamine will correlate well with random or fasting blood glucose levels.

#### **1.11.3 Hypothesis 3**

It was hypothesized that fructosamine and random glucose values would correlate well with glycosylated haemoglobin (HbA<sub>1c</sub>) analysis.

#### **1.11.4 Hypothesis 4**

It was hypothesized that apparently healthy patients' lipogram (which included: cholesterol, triglycerides, high density lipoprotein and low density lipoprotein) correlated well with their fructosamine values and would reflect the overall fatty contents of the South African diet. It was further hypothesized that those participants, whose results were suggestive of diabetes mellitus, would have an abnormal lipid profile.

### **1.11.5 Hypothesis 5**

It was hypothesized that total serum protein and albumin concentrations, together with fructosamine would show a positive correlation, irrespective of age or ethnic group.

## **CHAPTER TWO: MATERIALS AND METHODS**

## **2.1 Ethical Issues and Approval**

Ethical Approval was obtained from the Faculty of Health and Wellness Sciences Research and Ethics Committee of Cape Peninsula University of Technology, Reference Number: CPUT/HW-REC 2009/H010 (Appendix A). Permission was also granted from the Executive Committee of the PathCare Pathology Group for this study.

## **2.2 Informed Consent**

Participants recruited to participate in the study were informed of the aims and objectives of the study through the circulation of information sheets. All procedures and implications were explained to the participants. Participation was entirely voluntary and participants were free to withdraw at any time with no obligations. All the relevant information was contained in the information sheet which was handed to the participants on the day of participation. Participants had the choice of having their blood samples destroyed immediately after analysis was completed, or that it could be stored with their permission for future studies. This had to be indicated on the consent form. The consent was signed at the participation venue. A copy of the information sheet and consent form in both English and Afrikaans appears in Appendix B and C.

## **2.3 Referral of Participants with Abnormal Results**

The criteria set out by the ADA was used to diagnose diabetes amongst the participants (random glucose concentrations of 11.1 mmol/L or greater and an HbA1c value 6.5% or greater as proposed by the ADA). The relevant participants were contacted by the researcher and was encouraged to go to a general practitioner (G.P.) elected by themselves or local clinic for further investigation. A copy of their blood results, together with a referral letter was sent with them for the attention of the healthcare practitioner taking over their management. A copy of the referral letters in both English and Afrikaans appear in Appendix D and E.

## **2.4 Research Design**

This was a cross sectional quantitative study aimed at determining a new reference range for fructosamine by sampling participants at the PathCare Laboratory, Vergelegen Mediclinic, Somerset West, Republic of South Africa. Both dependent and independent variables were obtained. Demographics included each participant's name, surname, contact



number and date of birth whilst biochemical data was obtained by analysis of blood collected.

## **2.5 Research Setting**

South Africa is one of the few countries in the world that has two health care systems in place: 1) the public sector and 2) the private sector. In the public sector health care varies from the most basic primary health care offered free of charge to the specialized hospital care, usually academic hospitals, at a nominal fee, depending on the socio-economic status of the patient. On the other hand, the private health care system, which includes PathCare, is mainly accessible to those who fall within the middle- to high-income earner's bracket as these individuals are likely to possess a medical insurance policy or medical aid. Also, the majority of health care professionals are attracted to the private sector due to a higher source of income (Big Media Publishers, 2009). The infrastructure of South Africa's private health care is considered to be of first world quality and of high standard, when compared to other African countries, including its neighbouring nations. The number of foreigners seeking top quality surgical procedures and paramedical services, such as pathology, at relative affordable prices is also on the increase. The Somerset West area, especially, has a large number of foreigners utilizing these services, as seen by the number of foreigners visiting the PathCare Laboratory at Somerset West (data from PathCare). PathCare is a large network of pathology laboratories responsible for analysis of patient pathology samples for medical diagnostic purposes. The practice is owned and led by a group of approximately seventy pathologists, whom were almost all trained in South Africa at some of the major medical training institutions in the country. The primary function of the company is to supply specialist interpretation of results and quality information obtained from analysis of patient samples.

The company has its origins as far back as 1922 when Dr. Patrick Mulligan, who emigrated from Northern Ireland to South Africa, established a private pathology firm in Cape Town. The practice grew at a steady pace over the years. Shortly after the World War II, Dr Mulligan was joined by his son, Dr Terence Mulligan. The Mulligan and Partners practice became a well known and respected name in the medical establishment at the time. In 1973 Dr. Bruce Dietrich joined the Mulligan practice and later became the CEO. Then the company became Dietrich, Street and Partners. The company showed a steady and strong growth throughout the 1980's and 1990's by amalgamations with other private pathology groups in almost all the provinces in the country. Today, the amalgamated group of

laboratories practise under the PathCare name, with a well respected reputation in South Africa, our neighbouring countries and the world (PathCare, 2009).

PathCare expanded deeper into Africa over the years and joined forces with local practitioners in Kenya and Nigeria. It has also established itself in the United Arab Emirates and the United Kingdom. It recently established PathCare International Limited in the United Kingdom, which is fully owned by PathCare.

PathCare, Somerset West, is one of the larger peripheral laboratories in the PathCare group. The laboratory serves as the main referral laboratory for part of the Boland area, including: Stellenbosch, Somerset West, Strand, Gordon's Bay, Hermanus and surrounding smaller towns. The laboratory and depot is situated at Vergelegen Mediclinic, Somerset West, and offers a 24 hour service to the hospital's level 1 emergency unit, hospital wards and surrounding supporters. The laboratory has a clinical pathology laboratory as well as a histopathology and cytology laboratory, with 3 permanent histo-pathologists on site. The remainder of the staff is made up of the managerial team, medical technologists and technicians, laboratory assistants, data capturing staff and administrative staff, nursing staff, phlebotomy technicians and the courier department.

### **2.5.1 Sample Size and Population**

The data presented here was collected from mid January 2010 to April 2010. PathCare, in addition to its core line of business, also offers a service to insurance companies. Apparently healthy subjects that visit the practice for screening tests by insurance companies or routine medical checkups were recruited to participate in the study. For the determination of reliable and accurate references values a sample size of  $\pm 120$  subjects is recommended (Horn & Pesce, 2003). The PathCare, Somerset West, clientele is mostly Caucasians and mixed ancestry population groups. Therefore, 446 apparently healthy subjects of these population groups participated in the study and their distribution is shown in table 2.1:

**Table 2.1:** Summary of the sample population and size

Caucasian Males (21 – 65 years)	117
Caucasian Females (21 – 65 years)	120
Mixed Ancestry Males (21 – 65 years)	95
Mixed Ancestry Females (21 – 65 years)	114
<b><u>TOTAL NUMBER OF PARTICIPANTS</u></b>	<b><u>446</u></b>

### **2.5.2 Inclusion Criteria**

The following participants participated in the study: 1) caucasian and mixed ancestry male and female adults aged between 21 – 65 years of age and 2) apparently healthy patients visiting the practice after referral from their doctor for a routine medical check- up, and those referred by any insurance companies for blood to be taken for policies were asked to participate in the study on a voluntary basis.

### **2.5.3 Exclusion Criteria**

The following patients were not eligible to participate in the study: 1) those under the age of 21 and above the age of 65, 2) those who suffer from any form of diabetes, 3) those who suffer from impaired glucose tolerance, 4) participants who suffer from hypertension or acute or chronic kidney failure and 6) any patient who is hospitalised as this is not a study to determine a reference range for hospitalised patients.

### **2.6 Sample Collection**

Venipuncture on the participant's arms was used as the method of choice for blood collection. Venipuncture was obtained using a closed system and collecting the blood into Vacutainer sample tubes. The following Vacutainer tubes were collected from each participant:

- 1) 1 x SST (orange top) vacutainer to obtain clotted blood
- 2) 1x EDTA (lavender top) to obtain whole blood
- 3) 1 x Fluoride (grey top) vacutainer to obtain plasma

## **2.7 Biochemical Analysis**

### **2.7.1 Glucose**

A random plasma glucose level was measured in all participants. Sample processing took place at the PathCare laboratory in Somerset West on a Beckman Coulter CX9 PRO analyser.

Glucose concentration was determined by an oxygen rate method using a glucose electrode on the Beckman Coulter CX9 PRO analyser (Beckman Coulter, 2000). 10 µL of sample was injected into the reaction cup and mixed with a glucose oxidase solution. The ratio of sample to reagent is 1: 100. Oxygen consumption is measured by circuits which is directly proportional to the concentration of the glucose in the sample (Beckman Coulter, 2000).

As an additional parameter, the Estimated Average Glucose concentration was calculated from the HbA<sub>1c</sub> value, using the calculation formula at the bottom of page 22.

Categories of glucose tolerance were defined applying the 1999 WHO criteria (World Health Organization, 1999). Any abnormal results were brought under the attention of the relevant participants and they were encouraged to take the matter up at their local clinic or general practitioner. The results together with a referral letter were sent with the participants if they decided to do so (Appendix D).

### **2.7.2 Fructosamine**

A random serum fructosamine concentration was measured in all participants making use of fresh serum samples. Sample processing took place on site at the PathCare Laboratory in Somerset West on a Beckman Coulter CX9 PRO analyser. The PathCare Pathology Group is a Beckman Coulter orientated practice, using this diagnostic firm as its main provider for chemistry analysis. The fructosamine test kit itself, however, is a Roche Diagnostics product. Thus, the fructosamine test was set up on the analyser as a user defined chemistry.

The fructosamine test principle is a colorimetric assay based on the ability of ketoamines to reduce nitrotetrazolium-blue to formazin in an alkaline solution. The rate for formazin formation is directly proportional to the concentration of fructosamine present in the sample. Uricase in the reagent serves to eliminate uric acid interference (as seen in patients with gout and renal failure) and the addition of a detergent in the reagent preparation serves to eliminate matrix effects. The reaction rate is measured spectrophotometrically at 546 nm.

The analytical range for the test is 10 – 1000  $\mu\text{mol/L}$  (ROCHE, Roche Diagnostics Technical Bulletin, 2000).

The calibration is a two-point calibration using Roche Diagnostics Precimath Fructosamine Calibrator and was performed on a daily basis.

### **2.7.3 HbA<sub>1c</sub>**

HbA<sub>1c</sub> analysis was performed on EDTA whole blood, using High Performance Liquid Chromatography or HPLC. Sample analysis took place within a one week time frame after sample collection. Samples were stored at 2 – 4 ° Celsius and transported on icepacks to the CPUT Campus in Bellville where sample processing took place.

HPLC is an improved form of normal liquid chromatography. Instead of the solvent being allowed to drip under gravitational force through a column, it is forced through the column under high pressure of up to 400 atmospheres, giving excellent separation of the particles in the solvent mixture. It speeds up the chromatography separation and the separation is much better than conventional liquid chromatography. It is an easily automated method and extremely sensitive (Clarke, 2010).

Quality control was done using a kit called Trinity Biotech Glycated Haemoglobin, lot number 3240. The kit consisted of 2 levels of controls. Level I had a mean value of 5.3%, with a reference range of 5.0 – 5.6%. Level II had a mean value of 10.9%, with a reference range of 10.1 – 11.7%. The Levy-Jennings charts for the respective control are shown in figures 2.12 and 2.13 respectively.

### **2.7.4 Total Protein**

Total protein measurement was done on random fresh serum samples of each participant. Sample processing took place on site at the PathCare Laboratory in Somerset West on a Beckman Coulter CX9 PRO analyser.

The SYNCRON CX9 Pro analyser determines total protein concentration by means of a rate Biuret reaction. 10  $\mu\text{L}$  of serum is injected into the reaction cup containing alkaline copper reagent. The sample to reagent ratio is 1: 100. Protein in the sample combines with the reagent, producing an alkaline copper chelate. The result is an increase in absorbance which is monitored at 545 nm. The rate of chelate formation is directly proportional to the total protein concentration in the sample (Beckman Coulter, 2000).

### **2.7.5 Albumin**

Serum albumin measurement was done on random fresh serum of each participant. Sample processing took place on site at the PathCare Laboratory in Somerset West on a Beckman Coulter CX9 PRO analyser.

The SYNCRON CX9 PRO analyser determines albumin by means of a timed end point reaction. In the reaction, serum albumin combines with bromocresol purple to form a coloured product. The sample to reagent ratio is 1: 100. The change in absorbance is monitored at 600 nm and the rate of change in absorbance is directly proportional to the concentration of albumin present in the sample (Beckman Coulter, 1995).

### **2.7.6 Total Cholesterol**

Total cholesterol measurement was done on random fresh serum of each participant. Sample handling did not require ultra-centrifugation of lipaemic samples. Sample processing took place on site at the PathCare Laboratory in Somerset West on a Beckman Coulter CX9 PRO analyser.

The SYNCRON CX9 PRO analyser determines cholesterol concentration by means of a timed endpoint reaction, whereby serum is mixed with cholesterol esterase (sample to reagent ratio is 1: 100). Cholesterol esterase hydrolyses cholesterol esters to free cholesterol and fatty acids. The free cholesterol is then oxidised to cholestene-3-one and hydrogen peroxide by cholesterol oxidase. Hydrogen peroxide, 4-aminoantipyrine and phenol are catalysed by peroxidase to produce a coloured quinoneimine product. The change of absorbance of this reaction is measured at 520 nm and the rate of change in absorbance is directly proportional to the cholesterol concentration in the sample (Beckman Coulter, 2000).

### **2.7.7 Triglyceride**

Triglyceride analysis was done on random fresh serum samples of each participant. Sample handling did not require ultra-centrifugation of lipaemic samples. Sample processing took place on site at the PathCare Laboratory in Somerset West on a Beckman Coulter CX9 PRO analyser.

The SYNCRON CX9 PRO system uses triglyceride GPO reagent by means of a timed endpoint reaction whereby the triglyceride in the serum sample is hydrolyzed to glycerol and free fatty acids with lipase as a catalyst (sample to reagent ratio is 1: 100). A sequence of 3

enzymatic coupled reactions occurs by using glycerol kinase, glycerophosphate and horseradish peroxidase causing an oxidative coupling of 3, 5-dichloro-2-hydroxybenzenesulfonic acid with 4-aminoantipyrine. The end product is a red quinoneimine dye. The rate of change of the reaction is monitored at 520 nm and this rate of change is directly proportional to the triglyceride concentration in the serum sample (Beckman Coulter, 2000).

### **2.7.8 HDL Cholesterol**

HDL cholesterol analysis was done on random fresh serum samples of each participant. Sample handling did not require ultra-centrifugation of lipaemic samples. Sample processing took place on site at the PathCare Laboratory in Somerset West on a Beckman Coulter CX9 PRO analyser.

The SYNCRON CX9 PRO uses the direct HDL cholesterol method, which is a homogenous assay without any offline sample pre-treatments or centrifugation steps. The method has a unique detergent, which solubilises only the HDL particles in the serum sample and allowing only the HDL to react with cholesterol esterase and cholesterol oxidase in the presence of chromogens to produce a coloured product. The same detergent used will inhibit the cholesterol enzymes to react with LDL, VLDL and chylomicrons in the sample by adsorbing them to the sample reaction surface. The ratio of sample used to reagent is 1:93 and reaction is monitored at 560 nm to determine the rate of change of absorbance in the reaction. This rate of change is directly proportional to the concentration of HDL cholesterol present in the serum sample (Beckman Coulter, 2000).

### **2.7.9 LDL Cholesterol**

LDL cholesterol analysis was done on random fresh serum samples of each participant. Sample handling did not require ultra-centrifugation of lipaemic samples. Sample processing took place on site at the PathCare Laboratory in Somerset West on a Beckman Coulter CX9 PRO analyser

The SYNCRON CX9 PRO uses the direct LDL cholesterol method for the determination of LDL cholesterol. It is also a homogenous assay with no sample pre-treatment or centrifugation steps required beforehand. A unique detergent will solubilise only the non-LDL lipoprotein and release cholesterol to react with cholesterol esterase and cholesterol oxidase, resulting in a non-colour forming reaction. The addition of a second detergent will solubilise the remaining LDL cholesterol particles in the reaction and a chromogenic coupler

will allow for a colour formation. The ratio of sample to reagent used is 1:93. The rate of colour change of the reaction is monitored at 560 nm. The rate of change in absorbance is directly proportional to the amount of LDL cholesterol present in the serum sample (Beckman Coulter, 2004).

## **2.8 Quality Control**

Quality control (QC) in medical pathology can be defined as the sum of analytical processes and techniques to detect, reduce and correct any deficiencies that exist in an analytical process (College of American Pathologists, 2010). Today, QC in medical pathology has become an integral part of any normal operational requirements for accredited laboratories. QC provides the laboratory with consistent routine checks of their test data to ensure that the results that they generate are of a high standard and to ensure the integrity of their data. This will ensure a high quality of patient's results and enables each laboratory to identify and act on any errors in a quick and timeous manner.

Having a good and effective QC program in place, is regarded as a key element for medical laboratories in South Africa to be accredited. PathCare is one of the pathology laboratories which obtained accreditation in this manner. Accreditation of medical laboratories in South Africa can be obtained via the South African National Accreditation System (SANAS) by applying the standards as set out in the International Organization of Standardization's (ISO) document 15189 of 2007: "Medical laboratories - Particular requirements for quality and competence" (International Organization of Standardization, 2010).

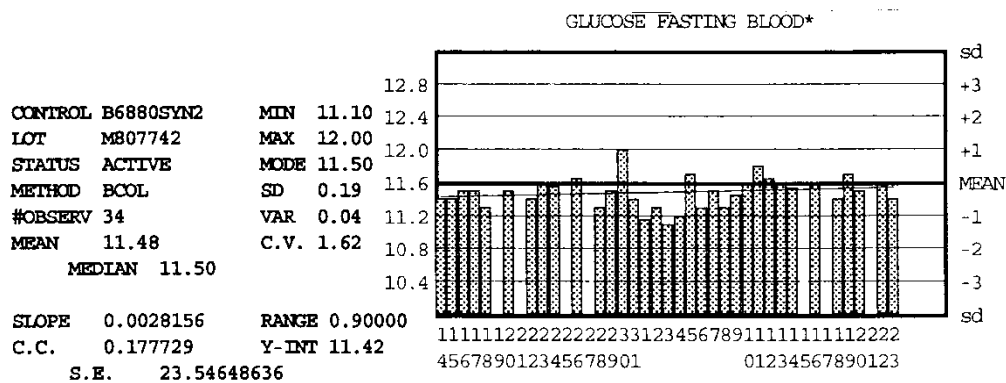
Application and processing of the data obtained from laboratory QC is done by making use of the multi-rule QC procedure, otherwise known as the Westgard rules. The Westgard rules are a set of decision criteria otherwise known as control rules that can be used to see if a control run is out-of-control or in-control. The data can be plotted on a Levy-Jennings chart to visualise the QC data obtained (Westgard QC, 2010).

QC was performed on a regular basis during a 24 hour period, as the laboratory itself is operated on a 24 hour basis. Beckman Coulter assayed Synchron level 1,2 and 3 were used as quality control material throughout the 24 hours on all the analytes, except for fructosamine and HbA1c. Fructosamine QC was performed on Precinorm fructosamine and Precipath fructosamine, which are Roche Diagnostics products. Analytical intervals of the Precinorm fructosamine and Precipath fructosamine were run on a daily basis when analyses was performed in parallel with sample analyses, as per vendor requirements (ROCHE, Roche Diagnostics Technical Bulletin, 2007). These controls were suitable for

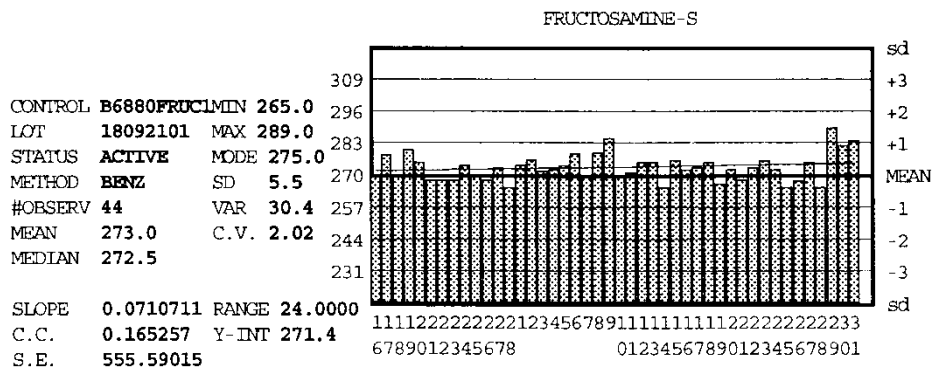


both the normal range of the analyte, as well as the pathological range of the analyte. In addition to the assayed Synchron controls for HDL and LDL, a Biorad product named Biorad Lipid Control level 2 was used for the control of the abnormal high ranges of these two analytes.

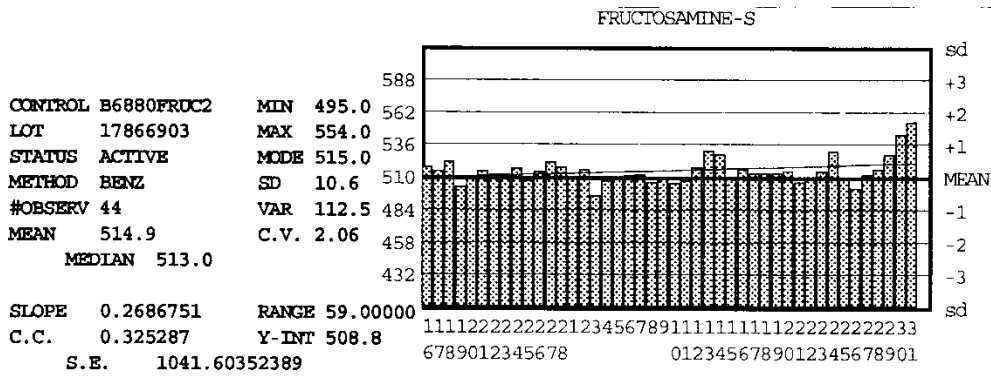
PathCare uses a laboratory information system called Meditech, version 3.26, supplied by Medical Information Technology. Examples of the Levy-Jennings charts for the analytes done during the study were taken from the Meditech system is shown in figures 2.1 to 2.11:



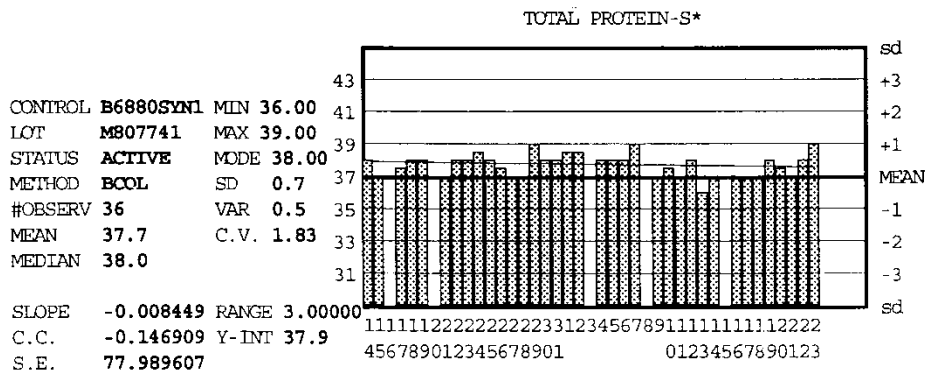
**Figure 2.1:** Levy-Jennings chart for assayed control Synchron 2 for glucose. The target mean was set at 11.60 mmol/L



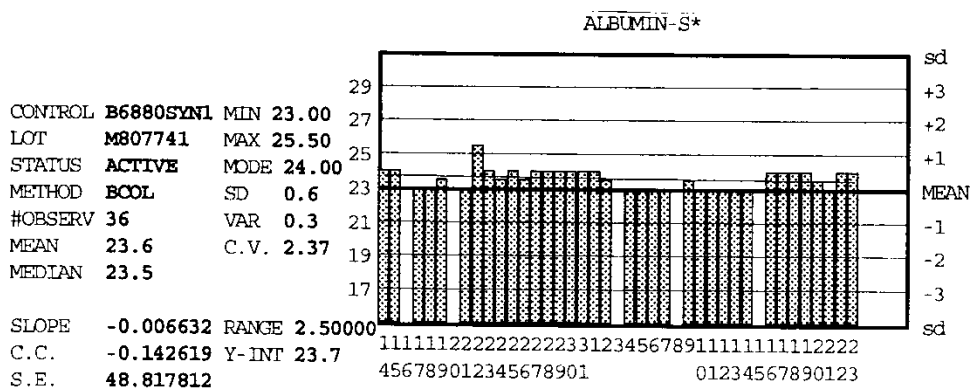
**Figure 2.2:** Levy-Jennings chart for assayed Precinorm fructosamine. The target mean was set at 270 µmol/L



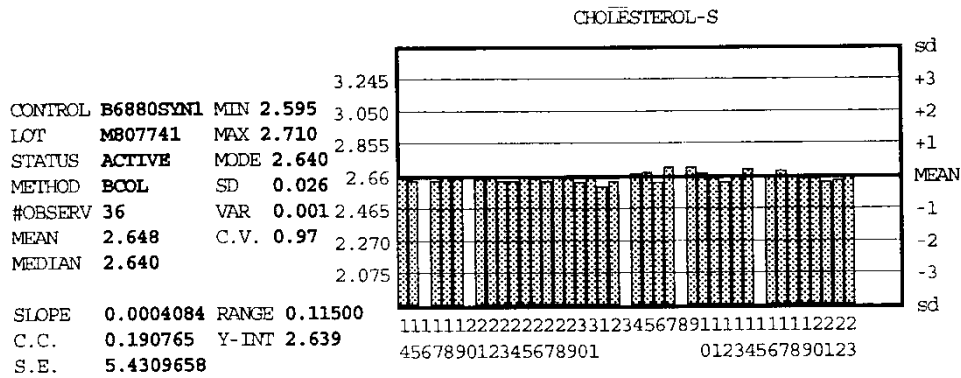
**Figure 2.3:** Levy-Jennings chart for assayed Precipath fructosamine. The target mean was set at 510  $\mu\text{mol/L}$



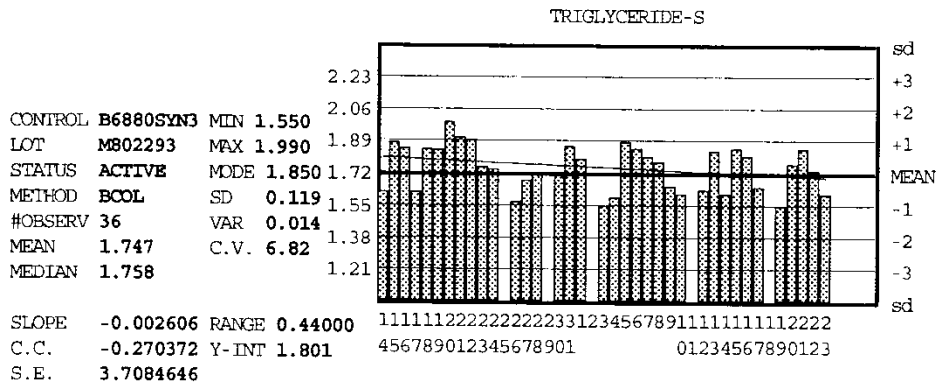
**Figure 2.4:** Levy-Jennings chart of assayed Synchron control level 1 for total protein. The target mean was set at 37.0 g/L



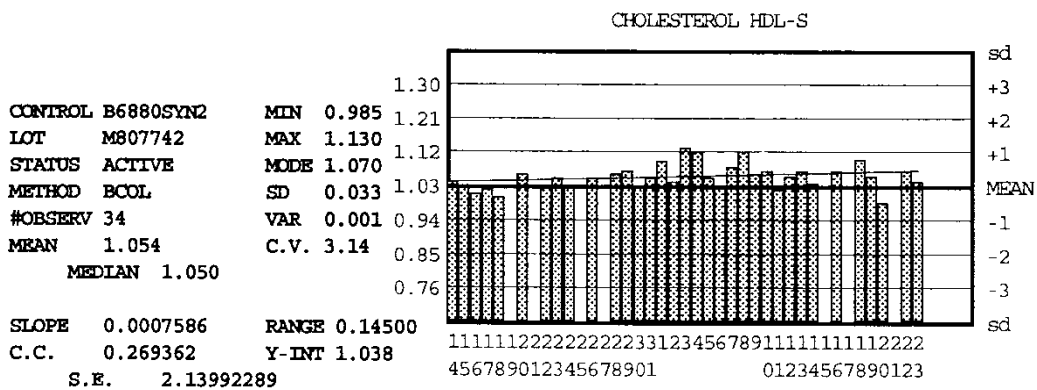
**Figure 2.5:** Levy-Jennings chart of assayed Synchron control level 1 for albumin. The target mean was set at 23.0 g/L



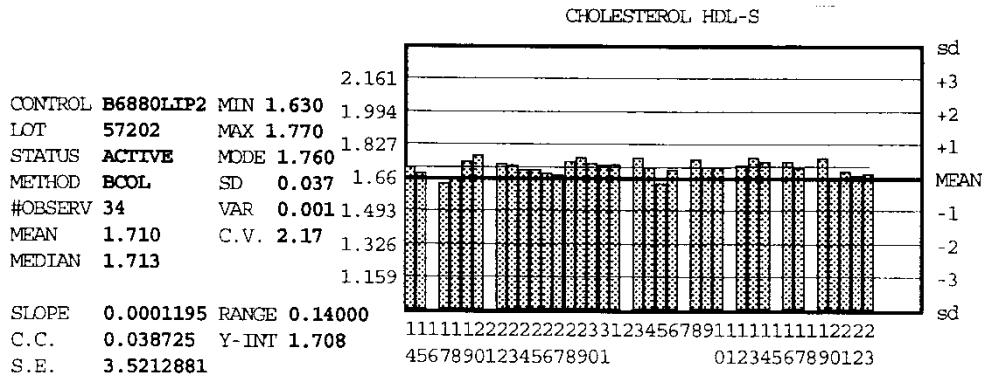
**Figure 2.6:** Levy-Jennings chart of assayed Synchron control level 1 for cholesterol. The target mean was set at 2.66 mmol/L



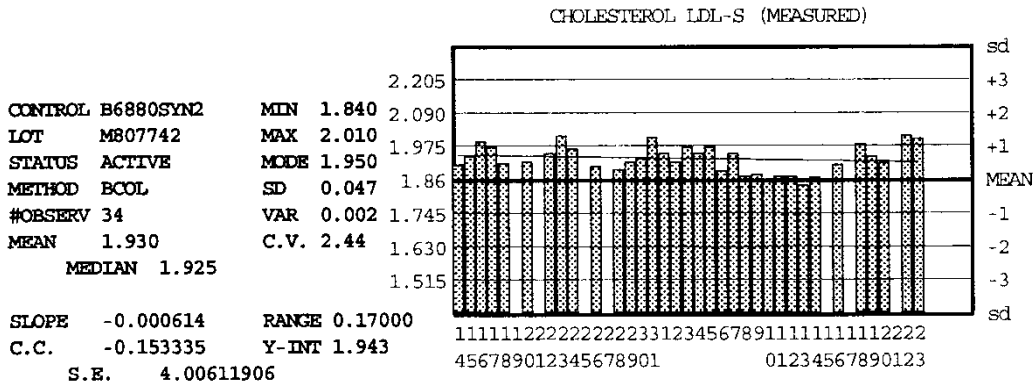
**Figure 2.7:** Levy-Jennings chart of assayed Synchron control level 3 for triglyceride. The target mean was set at 1.72 mmol/L



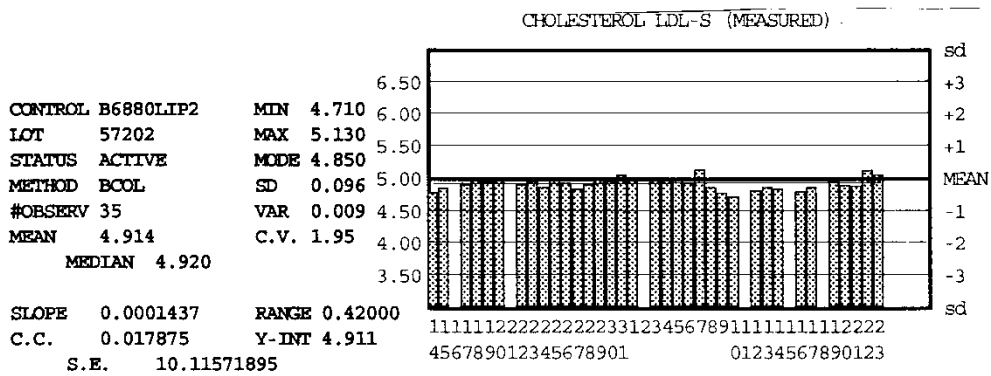
**Figure 2.8:** Levy-Jennings chart of assayed Synchron control level 2 for HDL. The target mean was set at 1.03 mmol/L



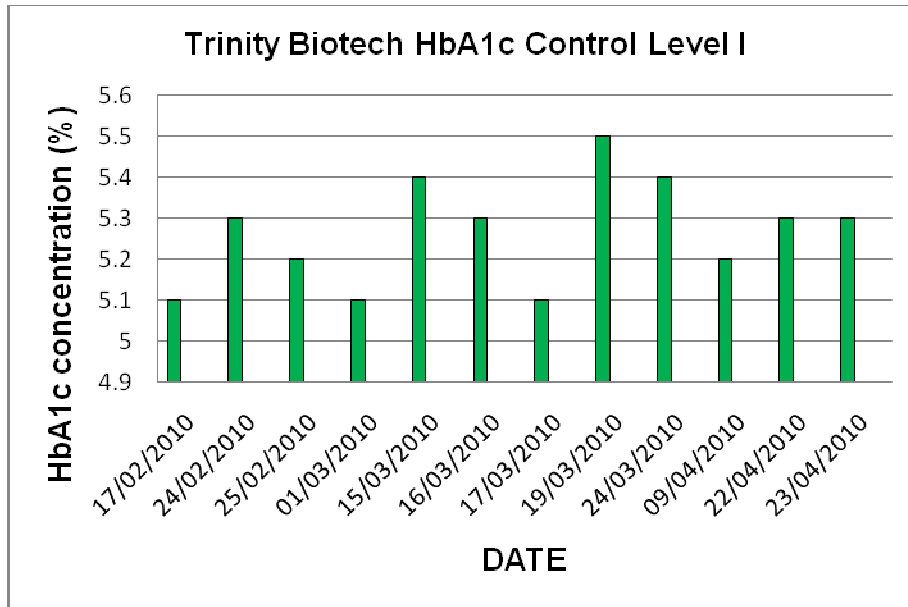
**Figure 2.9:** Levy-Jennings chart of the Biorad assayed Lipid Control Level 2 for HDL cholesterol. The target mean was set at 1.66 mmol/L



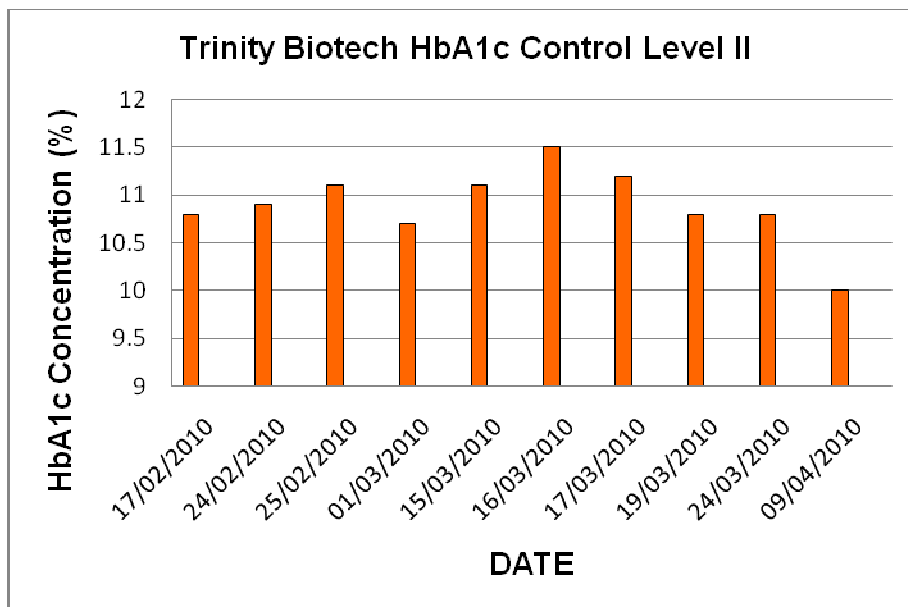
**Figure 2.10:** Levy-Jennings chart of assayed Synchron control level 2 for LDL. The target mean was set at 1.86 mmol/L



**Figure 2.11:** Levy-Jennings chart of assayed Biorad Lipid Control Level 2 for LDL. The target mean was set at 5.00 mmol/L



**Figure 2.12:** Levy-Jennings chart of the Biotech Trinity Glycated Haemoglobin (HbA1c) Control Level I. The mean was 5.3%. The 2SD range was 4.9 – 5.6%.



**Figure 2.13:** Levy-Jennings chart of the Biotech Trinity Glycated Haemoglobin (HbA1c) Control Level II. The mean was 10.9%. The 2SD range was 10.1 – 11.7%.

## **2.9 Statistical Analysis**

An Excel spreadsheet (Excel 2007, Microsoft Corporation) was used to capture the data. Statistical analyses of the data were performed using STATISTICA (STATISTICA 9, Stat Soft Inc. 1984 – 2009). The central tendencies of descriptive data are presented as median, or means, and the variability from central tendency is presented as interquartile range, 95% confidence interval and standard deviation (SD). For data where the normality assumptions were suspect, the Mann Whitney U test was used. Linear logistic regression analysis was used to study association between fructosamine or HbA1c and the other variables. A p-value of less than 0.05 was considered significant.

## **CHAPTER THREE: RESULTS**

### 3 Results

#### 3.1 Characteristics of the participants

The data collected from participants are summarised in table 3.1. Generally there were significant differences between male and female subjects except for serum fructosamine, total cholesterol and LDL-cholesterol levels. The participants were further categorised into racial groups and the data of the Caucasian and mixed ancestry participants are summarised in tables 3.2 and 3.3, respectively. In the Caucasian participants, no significant differences with respect to the age of participants were observed, however LDL-cholesterol was significantly higher in males than in females ( $p = 0.02$ ). In the mixed ancestry participants, significant differences were observed in fructosamine levels between male and female subjects ( $p = 0.01$ ).

**Table 3.1:** Biochemical data of the 446 healthy adult subjects.

	Male (n = 212)			Female (n = 234)			p-Value
	Median (IR)*	Mean $\pm$ SD	Confidence Interval	Median (IR)*	Mean $\pm$ SD	Confidence Interval	
AGE (years)	42.5 (18)	42.3 $\pm$ 11.4	40.7 – 43.8	39.0 (19)	39.8 $\pm$ 11.7	38.3 – 41.2	<b>0.0028</b>
TP (g/L)	71.0 (6)	71.0 $\pm$ 5.3	70.3 – 71.7	70.0 (6)	70.4 $\pm$ 5.0	69.7 – 71.0	<b>0.025</b>
ALB (g/L)	42.0 (4)	42.3 $\pm$ 2.9	41.9 – 42.7	40.5 (5)	40.4 $\pm$ 4.1	39.9 – 40.9	<b>&lt;0.001</b>
RBG (mmol/L)	5.6 (1)	5.8 $\pm$ 1.1	5.7 – 6.0	5.4 (1)	5.5 $\pm$ 0.8	5.4 – 5.6	<b>&lt;0.001</b>
EAG (mmol/L)	6.2 (0.9)	6.2 $\pm$ 0.70	6.1 – 6.3	6.0 (0.8)	6.0 $\pm$ 0.6	5.9 – 6.1	<b>&lt;0.001</b>
HbA1c (%)	5.5 (0.6)	5.6 $\pm$ 0.4	5.5 – 5.6	5.4 (0.5)	5.4 $\pm$ 0.4	5.3 – 5.4	<b>&lt;0.001</b>
FRUC ( $\mu$ mol/L)**	258 (25)	261 $\pm$ 22	258 – 264	261 (26)	258 $\pm$ 20	255 – 260	0.914**
TC (mmol/L)	5.0 (1.2)	5.1 $\pm$ 0.99	5.0 – 5.2	4.9 (1.4)	4.9 $\pm$ 0.96	4.8 – 5.0	0.10
TG (mmol/L)	1.62 (1.41)	1.93 $\pm$ 1.32	1.74 – 2.11	1.07 (0.74)	1.22 $\pm$ 0.70	1.13 – 1.31	<b>&lt;0.001</b>
HDL (mmol/L)	0.98 (0.39)	1.0 $\pm$ 0.4	0.97 – 1.1	1.3 (0.5)	1.3 $\pm$ 0.4	1.3 – 1.4	<b>&lt;0.001</b>
LDL (mmol/L)	3.1 (1.2)	3.2 $\pm$ 0.9	3.1 – 3.3	3.0 (1.1)	3.0 $\pm$ 0.8	2.9 – 3.1	0.056

TP, total protein; ALB, albumin; RBG, random blood glucose; EAG, estimated average blood glucose; FRUC, fructosamine, TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol. \* IR, interquartile range, \*\*p-Value is from the mean because results were normally distributed.



**Table 3.2:** Biochemical data of the 237 healthy adult Caucasian participants.

	Male (n = 117)			Female (n = 120)			p-Value
	Median (IR)*	Mean $\pm$ SD	Confidence Interval	Median (IR)*	Mean $\pm$ SD	Confidence Interval	
AGE (years)	44.5 (17)	43.9 $\pm$ 11.3	41.8 – 45.9	43.0 (18)	41.5 $\pm$ 11.5	39.5 – 43.6	0.16
TP (g/L)	71.0 (5)	70.3 $\pm$ 4.3	69.5 – 71.1	68.0 (5)	68.8 $\pm$ 4.1	68.1 – 69.5	<b>0.003</b>
ALB (g/L)	43 (4.5)	42.7 $\pm$ 2.7	42.2 – 43.2	42.0 (3.5)	41.5 $\pm$ 3.0	40.9 – 42.0	<b>0.002</b>
RBG (mmol/L)	5.6(1)	5.8 $\pm$ 0.9	5.6–5.9	5.4(0.9)	5.5 $\pm$ 0.8	5.3–5.6	<b>0.01</b>
EAG (mmol/L)	6.3 (0.8)	6.2 $\pm$ 0.6	6.1 – 6.3	5.8 (0.8)	5.9 $\pm$ 0.6	5.8 – 6.0	<b>&lt;0.001</b>
HbA1c (%)	5.6 (0.5)	5.5 $\pm$ 0.4	5.5 – 5.6	5.3 (0.5)	5.4 $\pm$ 0.4	5.3 – 5.4	<b>&lt;0.001</b>
FRUC ( $\mu$ mol/L)**	258 (24)	260 $\pm$ 20	256 – 263	261 (26)	260 $\pm$ 18	257–263	0.94
TC (mmol/L)	5.0 (1.1)	5.2 $\pm$ 1.0	5.0–5.4	5.0 (1.2)	5.0 $\pm$ 0.96	5.0–5.2	0.13
TRIG (mmol/L)	1.6 (0.4)	1.97 $\pm$ 1.3	1.7–2.2	1.04 (2.2)	1.2 $\pm$ 0.8	1.1–1.3	<b>&lt;0.001</b>
HDL (mmol/L)	1.0 (0.4)	1.0 $\pm$ 0.3	1.0–1.1	1.4 (0.5)	1.4 $\pm$ 0.4	1.3–1.5	<b>&lt;0.001</b>
LDL (mmol/L)	3.2 (1.1)	3.3 $\pm$ 1.0	3.1–3.5	3.0 (1.1)	3.0 $\pm$ 0.8	2.8–3.1	<b>0.02</b>

**Table 3.3:** Biochemical data of the 209 healthy mixed ancestry adult subjects.

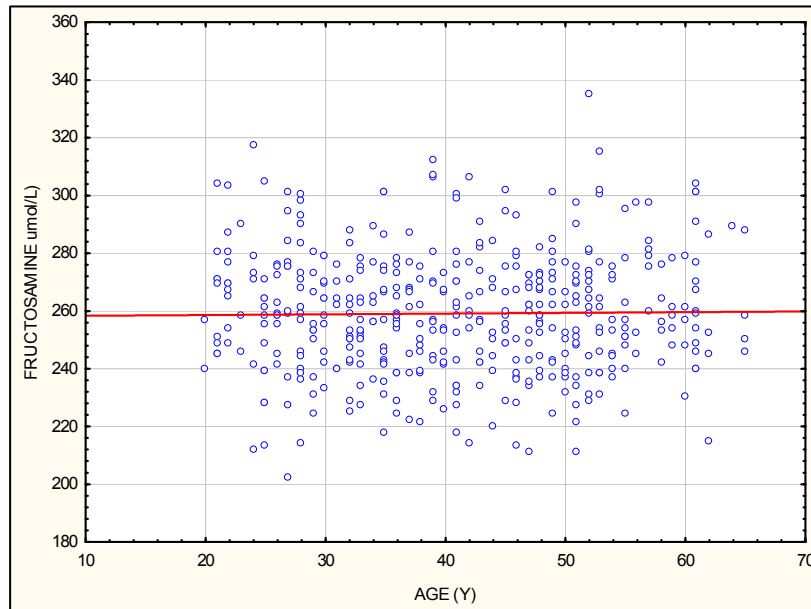
	Male (n = 95)			Female (n = 114)			p-Value
	Median (IR)*	Mean $\pm$ SD	Confidence Interval	Median (IR)*	Mean $\pm$ SD	Confidence Interval	
AGE (years)	38.5 (19)	40.3 $\pm$ 11.3	37.9 – 42.6	36.0 (17)	37.9 $\pm$ 11.0	35.8 – 40.0	0.14
TP (g/L)	72.5 (8)	71.9 $\pm$ 6.3	70.6 – 73.1	72.0 (5)	72.1 $\pm$ 5.4	71.9 – 73.1	<u>0.45</u>
ALB (g/L)	41.0 (4)	41.7 $\pm$ 2.9	41.1 – 42.3	39.0 (4.5)	39.2 $\pm$ 4.7	38.3 – 40.1	<b>&lt;0.001</b>
RBG (mmol/L)	5.6 (1.2)	5.9 $\pm$ 1.2	5.7 – 6.1	5.4 (0.90)	5.5 $\pm$ 0.8	5.3 – 5.6	<b>0.003</b>
EAG (mmol/L)	6.2 (1.3)	6.3 $\pm$ 0.82	6.1 – 6.5	6.0 (1.0)	6.0 $\pm$ 0.71	5.9 – 6.2	<u>0.05</u>
HbA1c (%)	5.5 (0.8)	5.6 $\pm$ 0.5	5.5 – 5.7	5.4 (0.6)	5.4 $\pm$ 0.4	5.3 – 5.5	<u>0.05</u>
FRUC ( $\mu$ mol/L)**	262 (33)	263 $\pm$ 23	258 – 268	254 (27)	255 $\pm$ 21	251 – 259	<b>0.01</b>
TC (mmol/L)	4.9 (1.40)	5.1 $\pm$ 0.9	4.7 – 5.1	4.8 (1.4)	5.0 $\pm$ 1.0	4.7 – 5.0	0.52
TRIG (mmol/L)	1.6 (1.2)	1.9 $\pm$ 1.4	1.6 – 2.2	1.1 (0.7)	1.2 $\pm$ 0.6	1.1 – 1.4	<b>&lt;0.001</b>
HDL (mmol/L)	1.0 (0.4)	1.1 $\pm$ 0.6	0.9 – 1.2	1.2 (0.5)	1.2 $\pm$ 0.3	1.1 – 1.3	<b>&lt;0.001</b>
LDL (mmol/L)	3.0 (1.3)	3.1 $\pm$ 0.9	2.9 – 3.3	3.0 (1.2)	3.0 $\pm$ 0.9	2.9 – 3.2	0.82

### 3.2 Fructosamine Reference Values

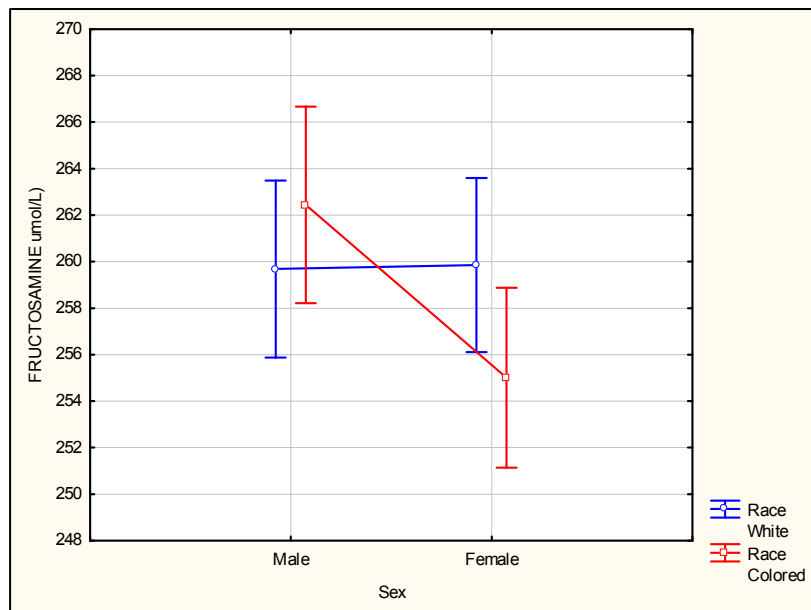
To obtain the 95% reference interval, nonparametric methods were used, employing the 2.5 and 97.5 percentiles to determine the reference values for fructosamine as follows: The 2.5 and 97.5 percentiles are first determined and used to form the 95% confidence interval from which the reference values are determined from the mean  $\pm$  1.96 multiply by the standard deviation of the newly constructed 95% confidence interval. Table 3.4 shows the reference values obtained for all participants as well as the different racial groups according to gender. Whilst the age was significantly different in the population groups as seen in Table 3.1 ( $p = 0.0028$ ), no significant correlation was observed between fructosamine levels and age (figure 3.1). However, in the mixed ancestry group there was a significant correlation between fructosamine levels and gender (figure 3.2).

**Table 3.4:** Fructosamine reference values according to gender and race. Units are in  $\mu$ mol/L.

	Male	Female	Total
<b>Mixed ancestry</b>	222 - 304	217 - 293	219 - 297
<b>Caucasian</b>	223 - 296	228 - 291	226 - 293
<b>Total participants</b>	223 - 299	222 - 293	223 - 295



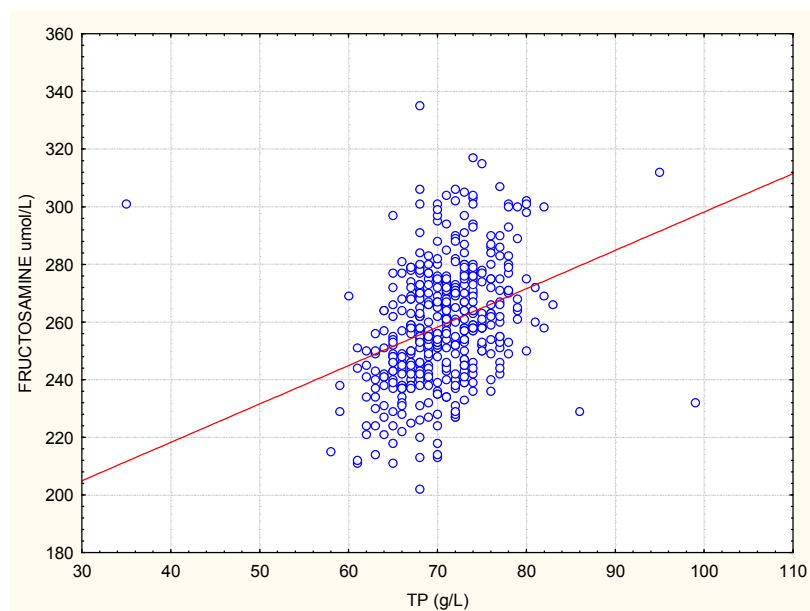
**Figure 3.1:** Spearman correlation between fructosamine and age in the total study group,  $r = 0.00$ ,  $p = 0.96$ .



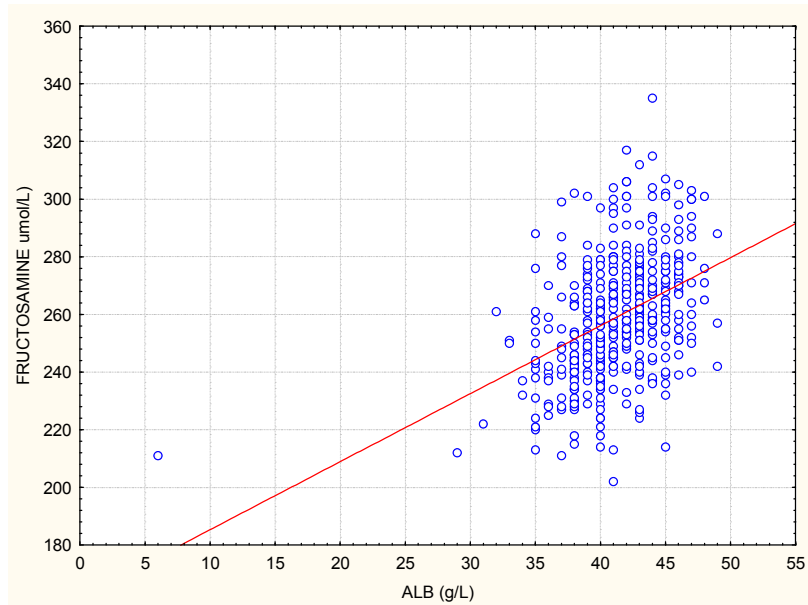
**Figure 3.2:** Relationship between fructosamine levels and gender in Caucasian and mixed ancestry participants. Significant differences are observed in mixed ancestry males and females,  $p = 0.01$ .

### 3.3 Correlations between Fructosamine and Other Biochemical Test

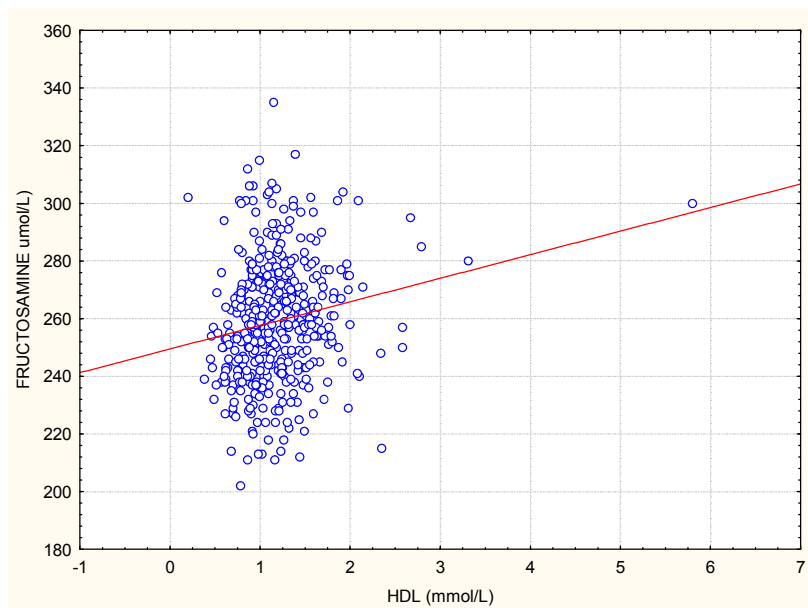
Fructosamine showed a positive correlation with total protein, albumin and HDL-cholesterol, ( $p < 0.0001$ ) in the total study population as well as in the mixed ancestry males, Caucasian males and females. Figures 3.3 – 3.5 illustrate the significant correlation between fructosamine and total protein, albumin and HDL-cholesterol of the whole study group. However, in the female mixed ancestry participants, the correlation between fructosamine and HDL-cholesterol was absent,  $p > 0.05$  (figure 3.6).



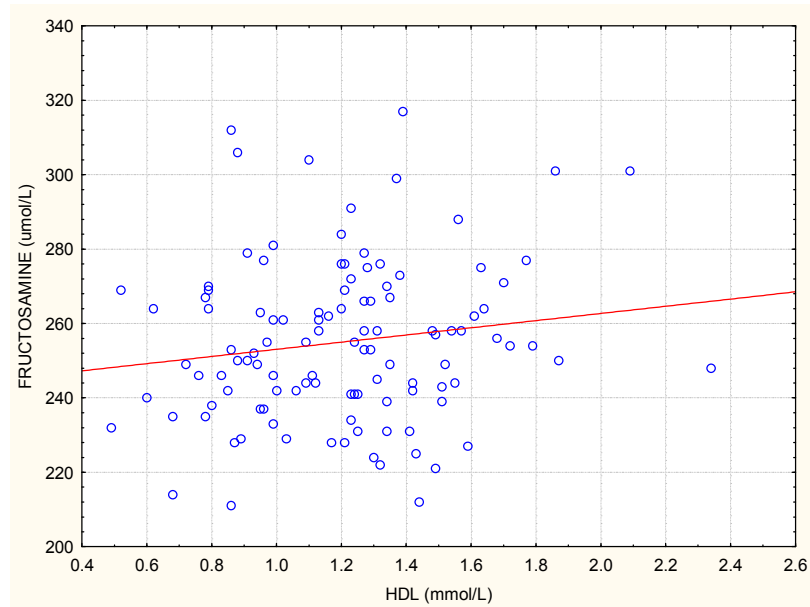
**Figure 3.3:** Correlation between total protein and fructosamine,  $r = 0.39$ ,  $p < 0.00001$



**Figure 3.4:** Correlation between albumin and fructosamine,  $r = 0.42$ ,  $p < 0.00001$



**Figure 3.5:** Correlation between HDL-cholesterol and fructosamine,  $r = 0.19$ ,  $p < 0.0001$ .



**Figure 3.6:** Correlation between HDL-cholesterol and fructosamine in the female mixed ancestry participants,  $r = 0.11$ ,  $p = 0.23$ .

### 3.4 Regression Analysis

Linear logistic regression analysis was used to study association of fructosamine and HbA1c with the other variables. The models containing either fructosamine or HbA1c as the dependent variables were tested for association with age, total protein, albumin, lipids and random blood glucose levels, but HbA1c was excluded in the fructosamine model and vice versa. Both models revealed significant differences,  $p < 0.0001$ . In the fructosamine model the effect of all the variables contained in the model on fructosamine levels was 27.6%,  $R^2 = 0.276$ , but the significant contributors were age, total protein, albumin and HDL-cholesterol,  $p < 0.05$  (Table 3.6). On the other hand, the effect of the variables on HbA1c levels was 22.6%,  $R^2 = 0.226$ , and the significant contributors were age, random plasma glucose and triglycerides,  $p < 0.05$  (Table 3.7).

**Table 3.5:** Fructosamine model: linear regression analysis

<b>Predictors</b>	<b>B</b>	<b>Beta</b>	<b>P-value</b>
AGE (years)	0.198	0.109	<b>0.015</b>
TP (g/L)	1.137	0.281	<b>&lt;0.0001</b>
ALB (g/L)	1.937	0.341	<b>&lt;0.0001</b>
RBG(mmol/L)	1.531	0.070	0.099
CHOLESTEROL (mmol/L)	3.716	0.175	0.076
HDL (mmol/L)	5.607	0.121	<b>0.026</b>
LDL (mmol/L)	-3.491	-0.148	0.107
TG (mmol/L)	-1.474	-0.078	0.152

**Table 3.6.** HbA1c model: linear regression analysis

<b>Predictors</b>	<b>B</b>	<b>Beta</b>	<b>P-value</b>
AGE (years)	0.010	0.276	<b>&lt;0.0001</b>
TP (g/L)	-0.006	-0.074	0.107
ALB (g/L)	-0.003	-0.029	0.525
RBG(mmol/L)	0.117	0.262	<b>&lt;0.0001</b>
CHOLESTEROL (mmol/L)	-0.055	-0.134	0.186
HDL (mmol/L)	-0.071	-0.079	0.162
LDL (mmol/L)	0.053	0.114	0.225
TG (mmol/L)	0.045	0.123	<b>0.029</b>

## **CHAPTER FOUR: DISCUSSION**



## 4.1 Discussion

Due to advances in medical laboratory diagnostics, it has become an integral part of the diagnostic tools used by healthcare workers to make decisions regarding their patients' care and treatment. Medical laboratory science has evolved from time consuming, laborious manual methods to the fast, highly specific and sensitive auto-analysers used today in most laboratories worldwide. Laboratories can choose from a myriad of instruments and methods available from the different medical diagnostic companies. Since each medical diagnostic company has its own technology for a particular test, the laboratory might have difficulty in deciding which method and instrument would satisfy their needs. These developments, pose new challenges to medical laboratories and their staff. One of these would be to establish reference values for pathology tests used for the population they serve.

The concept "Reference Values" is often incorrectly referred to as "normal values". In the clinical laboratory, however, normal has become obsolete and found to be imprecise to satisfy modern demands (Solberg, 1989; Burtis, 2001). This resulted in the creation of the expert panel on Theory of Reference Values in 1970. The International Federation of Clinical Chemistry (IFCC) recommends the use of the term "Reference Values" and related terms, such as reference individual, reference limit, reference interval and observed values to describe normal in a clinical laboratory (Burtis, 2001). Normal practice for the estimation of reference values involves the calculation of the mean and 2 standard deviations (SD), or to be more precise 1.96 SD, of all the data from the results obtained from the test under investigation. Using this calculation would yield the 2.5% and 97.5% reference interval. This method can only be considered if the data from the population has a Gaussian distribution. If the data from the population group is skewed, normal practice would be to adjust this data by doing a log-transformation to obtain a Gaussian distribution. Furthermore, it is recommended that at least 120 healthy individuals from a population group should be used. However, several other statistical methods are available, particularly for smaller sample sizes, which will make certain assumptions with regards to the sample population data obtained. These include truncation methods and robust methods. The truncation method makes an assumption that approximately 20% of the data are outliers, thus using 80% of the sample population data obtained to derive the 95% reference interval. In the case where no outliers are present in a sample population, only 76% of the sample population data is used (Horn & Pesce, 2003). On the other hand, robust methods are prone to outliers (Horn, Pesce, & Copeland, 1998), therefore it is recommended that outlier detection should be performed before reference interval values are calculated and implemented (Horn *et al.*, 2001).

In the present study, we used non-parametric methods to determine the reference values in 446 (120 white females, 117 white males, 114 coloured females, 95 coloured males) healthy patients visiting the Somerset West PathCare practice, Western Cape, South Africa. This study provides evidence that the reference range of 200 – 285  $\mu\text{mol/L}$  currently used at PathCare Laboratories is not appropriate for the Caucasian and mixed ancestry population groups in South Africa. Overall, the reference values for the entire sample were 223 – 295  $\mu\text{mol/L}$ . However, differences in the reference values were noted in the different population groups (white females = 228 – 291  $\mu\text{mol/L}$ ; white males = 223 – 296  $\mu\text{mol/L}$ ; mixed ancestry females = 217 - 293  $\mu\text{mol/L}$  and mixed ancestry males = 222 – 304  $\mu\text{mol/L}$ ). In the present study, fructosamine levels were determined on a Beckman Coulter CX9 PRO automated analyser, using a second-generation assay from Roche Diagnostics, and the 95<sup>th</sup> percentile obtained (295  $\mu\text{mol/L}$ ) is higher than previously reported using the second generation assays (Cefalu *et al.*, 1991; Henny & Schiele, 1990). In 529 non-diabetic and diabetic subjects the 95<sup>th</sup> percentile upper limit was reported to be 289  $\mu\text{mol/L}$  and this is similar to the upper limit observed in the Caucasian females in this study (Cefalu *et al.*, 1991). However, in the article by Cefalu *et al.* (1991), 230 of the subjects that were non-diabetic, their mean  $\pm$  1.96SD was  $227 \pm 35$   $\mu\text{mol/L}$ . If we were to literally use the simple normal practise for the estimation of reference values, that is, mean  $\pm$  2SD, the upper limit in those 230 non-diabetic subjects would be 296  $\mu\text{mol/L}$ , which is similar to that found in the present study. On the other hand, the upper limit of 276  $\mu\text{mol/L}$ , reported by Henny and Schiele, is in contrast with those found in this study. The findings of our study and the others attest to the notion that population differences are a major determinant of reference values.

The differences observed between this newly developed reference values and that currently used could be due to the differences in the population and methods used. The reference values presently used at PathCare was obtained, using a first-generation fructosamine assay that employed the nitro-blue tetrazolium colorimetric method (Johnson, Metcalf, & Baker, 1983; Tietz, 1993). The major drawback of these first-generation fructosamine assays is the interference by uric acid, bilirubin, lipids, and is affected by the albumin concentration in the sample (Baker *et al.*, 1985). Assays that improved on the concerns of the first-generation assays were introduced in 1989 by Vogt *et al.*, (1989) to eliminate the interference of uric acid. Uricase was included in the reagent, and the effects of lipaemia and protein were improved by exploiting non-ionic surfactants (Vogt *et al.*, 1989). Furthermore, the second-generation assays were standardised against glycated polylysine by replacing synthetic standard by deoxymorpholinofructose (Schleicher & Vogt, 1990).

Other variables that would play an important role in the determination of laboratory test reference values would include: age, sex, diet, circadian rhythm, race, posture, medication, physical activity, socio-economic status, medical history and fasting status. These variables must be carefully controlled and assessed when interpreting results for the compilation of reference values (Boyd, 2010). A study by Henny and Schiele, involving 1114 non-diabetics of both sexes, including children, found fructosamine reference values to be slightly affected by age and sex (Henny & Schiele, 1990). However, in our study, slight ethnic differences were evident as the 95<sup>th</sup> percentile was highest in the mixed ancestry group, 297  $\mu\text{mol/L}$  compared to 293  $\mu\text{mol/L}$  in Caucasians, but no correlation was observed between fructosamine and age. In contrast, a previous report on children found no sex-related differences for fructosamine values, while the fructosamine concentrations in the current study were significantly higher in males of mixed ancestry origin compared to their female counterparts as seen in figure 3.2. In addition, the influence of these factors has been reported in a study that determined the reference interval for HbA1c (Herman *et al.*, 2007). The authors conducted the study and processed their results using several statistical models, which included: normal-errors multiple linear regression, single multiple-regression, Shapiro-Wilks test of the residuals and the White's test of homoscedasticity. They found that co-variables such as age, sex, race, systolic blood pressure, body mass index (BMI), corrected insulin response and insulin resistance do play a role when determining reference values for HbA1c in these racial and ethnic minority groups (Herman *et al.*, 2007).

Although fructosamine and HbA1c are used to monitor glycaemic control, no correlation was observed between fructosamine and HbA1c. Similarly, other studies have reported poor correlation between serum fructosamine and glycated haemoglobin (MacDonald *et al.*, 2008; Dominiczak *et al.*, 1988). This is probably due to the differences in half-lives of the major proteins and erythrocytes for fructosamine and HbA1c respectively. Glycated haemoglobin is a product of non-enzymatic linkage of glucose to free amino acid on the globin protein of haemoglobin. In humans, the average lifespan of red blood cells is 120 days, hence the measurements of HbA1c reflecting the concentration of glucose over the last 2 months, with low intra-individual variability in non-diabetic subjects (Selvin *et al.*, 2007). On the other hand, fructosamine is the name given to a group of stable carbohydrate-protein complexes formed by an irreversible enzymatic reaction between glucose and serum proteins, mainly albumin (Bishop, Fody, & Schoeff, 2005). It is controlled by the rate of synthesis and elimination. The rate of fructosamine synthesis is therefore directly dependent on the rate of protein synthesis and composition as well as the concentration of glucose during the lifetime of the circulatory proteins. Therefore, the rate of fructosamine synthesis is directly

proportional to persistent hyperglycaemia and protein concentration. This is further supported by the positive correlation between fructosamine and total serum protein or albumin concentration observed in this study. The half-life of albumin is 17 – 20 days; therefore, fructosamine correlates well with glycaemic control in this same time period in diabetic patients (Goldstein, 2004). Thus, measurement of fructosamine allows more frequent assessment of glycaemic control than glycated haemoglobin usually assessed every 3 to 6 months, and is sometimes used as an intermediate glycation index for diabetes control (Roohk & Zaidi, 2008). The value of an intermediate glycation index is that it allows retrospective evaluation of changes in diet and exercise habits as well as faster evaluation of changes in medication dosages and other control measures (Carter *et al.*, 2000). However, in the present study, fructosamine levels did not show any association with non-fasting plasma glucose levels whilst HbA1c positively correlated with plasma glucose levels ( $r = 0.32$ ,  $p < 0.0001$ ) as seen in the regression analysis of the fructosamine and HbA1c models. Therefore, its superiority in the assessment of glycaemia needs to be further investigated.

Diabetes mellitus is a known strong risk factor for development of CVD. Research has shown that diabetes mellitus patients, who suffer from CVD, with no prior myocardial infarctions, have a high risk of mortality equating to non-diabetic patients who have suffered prior myocardial infarctions. Furthermore, treatment of CVD in diabetes mellitus patients should be just as aggressive as in non-diabetic patients with prior myocardial infarctions (Haffner *et al.*, 1998). HbA1c and plasma glucose levels have been associated with risks of CVD and death (Sarwar *et al.*, 2010; Selvin *et al.*, 2010). Furthermore, HbA1c measurement in a community-based population of non-diabetic adults showed an association with a risk of developing CVD and diabetes mellitus (Selvin *et al.*, 2010). In our study, HbA1c positively correlated with random plasma glucose, age and triglycerides, whilst negatively correlated with HDL-cholesterol, further supporting the role of glycated haemoglobin in the prediction of the development of diabetes-related complications. Research has shown that diabetes, especially when under poor control, exhibited elevated levels of total cholesterol, triglycerides and LDL with decreased concentrations in HDL (Khan, Sobki, & Khan, 2007). In healthy individuals with normoglycaemic and normolipidaemic blood profiles, HDL counteracts the atherogenic effects of LDL on the epithelial cell wall of arteries. While in diabetes mellitus, the apolipoproteins within the HDL molecule becomes glycated and more triglyceride rich, resulting in an alteration in their structure and inability to perform their normal protective function against oxidised LDL (Perségol *et al.*, 2006). Unlike HbA1c, fructosamine correlated positively with HDL-cholesterol. It is not clear why fructosamine showed a positive correlation with HDL-cholesterol, but we speculate that the positive

correlation seen between fructosamine and HDL in our study is a compensatory process to up-regulate HDL production due to the alteration in the HDL structure that also becomes glycated (Perségol *et al.*, 2006). These findings therefore suggests that fructosamine is unlikely to be associated with risks of cardiovascular diseases in nondiabetic subjects as has been observed with HbA1c (Selvin *et al.*, 2010).

Diabetes is diagnosed using the criteria as described by the ADA and WHO (American Diabetes Association, 2008; World Health Organization, 2006). Both the ADA and WHO state that a patient is diagnosed with diabetes mellitus when a fasting blood glucose concentration exceeds 7.0 mmol/L. Furthermore, the WHO criteria requires a 2 hour plasma glucose of  $\geq 11.1$  mmol/l after a 75g oral glucose challenge whilst the ADA accepts a random blood glucose concentration  $\geq 11.1$  mmol/L. The oral glucose tolerance test (OGTT) is more sensitive and slightly more specific than the fasting plasma glucose in the diagnosis of diabetes mellitus. However, reproducibility is poor and therefore less commonly used in practice than the fasting plasma glucose which on the other hand underestimates the prevalence of diabetes mellitus and misses those individuals with impaired glucose tolerance (IGT), a pre-diabetic state also associated with cardiovascular diseases (Melchionda, 2002; Temelkova-Kurktschiev, 2000; Peterson, 2005) .

The disadvantage of both these diagnostic approaches is that they require the patient to be fasting and if need be confirmed with a second fasting sample. Other disadvantages are that glucose has a large biological and diurnal variation and depends on recent carbohydrate intake while the OGTT is fairly invasive (Sacks, 2009). HbA1c and fructosamine do not require a fasting sample and have much less intra-individual variation. In this study, 5 subjects were excluded because their random blood glucose was  $\geq 11.1$  mmol/L and was therefore diabetic according to the ADA criteria. Interestingly, their mean fructosamine level was 367  $\mu\text{mol/L}$ , which exceeds the 95<sup>th</sup> percentile of this study. These findings suggest a potential role for fructosamine in the screening or diagnosis of diabetes mellitus. The use of fructosamine in screening for diabetes has previously been reported (Hughes, 1995; Roberts, 1986), however its sensitivity and specificity have been found to be poor in the diagnosis of diabetes (Baker, 1983; Tsuji, 1991). Similarly HbA1c, another measure of glycaemic control, has recently been proposed as a diagnostic tool for diabetes mellitus. The International Expert Committee of the ADA has recommended an HbA1c value of  $\geq 6.5\%$  to be a diagnostic level for the diagnosis of diabetes based on the established association between glycated haemoglobin and microvascular complications (ADA, 2010). However, conflicting cut-off values have been proposed in different studies. Rowley *et al.*

(2005) found an HbA1c of 7.0% to be optimal for identifying diabetes mellitus in a population with a prevalence of 12-22% (Rowley *et al.*, 2005). On the other hand, Bennett *et al.* (2007) reviewed 9 studies examining HbA1c as a screening tool and found a value of 6.1% to be optimal (Bennett *et al.*, 2007). Furthermore, HbA1c values greater than 6.5% have been found in individuals without diabetes mellitus. In the United States of America more than 2.4 million are reported to have a glycated hemoglobin value higher than 6.5%, whilst 7 million have a value higher than 6.0% (Selvin *et al.*, 2009). However a recent study has shown that these individuals are at high risk for the development of diabetes mellitus (Selvin *et al.*, 2010). Similarly, in our study, HbA1c levels greater than 6.5% were observed in some subjects. In such individuals the ADA recommends the diagnosis of diabetes mellitus should be confirmed by repeating the HbA1c together with either a fasting plasma glucose concentration of  $\geq 7.0$  mmol/L, or the 2 – hour glucose concentration of  $\geq 11.1$  mmol/L after a standard OGTT (American Diabetes Association, 2010).

According to the WHO, the current estimate of patients suffering from diabetes worldwide is 80 million. This figure is estimated to increase to 366 million by the year 2030. The WHO's most updated figure of individuals suffering from diabetes mellitus in South Africa is 814 000, and the estimate for 2030 is about 1.2 million (World Health Organization, 2008). Studies carried out in South Africa have shown marked geographical and ethnic variations in the prevalence of diabetes (Motala *et al.*, 2003; Erasmus *et al.*, 2001). The mixed ancestry population of South Africa has the second highest prevalence of diabetes preceded by that of the Indians (Motala *et al.*, 2003). In developing countries, diabetes is often undiagnosed and the complications of diabetes may thus far exceed that of known cases due to the unmanaged glycaemic state. Five participants were excluded in this study due to undiagnosed diabetes. Four out of the five participants (80%) were of mixed ancestry, further highlighting the high incidence of diabetes in this population group of South Africa. In a recent study, the prevalence of diabetes in the mixed ancestry population from Cape Town, South Africa was 25.6% and nearly half were undiagnosed diabetic cases (Soita, 2009).

## **4.2 Limitations**

The limitations of this study included the use of random blood glucose to exclude subjects with diabetes mellitus. In the present study, we proved that a correlation between total protein and fructosamine ( $p = 0.025$ ) as well as albumin and fructosamine ( $p < 0.001$ ) does exist. Similar studies proved the same trend and suggested that fructosamine values should be corrected for total protein and albumin concentrations (McCance *et al.*, 1986; Van Dieijen-Visser, Seynaeve, & Brombacher, 1986). However, we did not deem it necessary, as all of our participants' total protein and albumin concentrations were within normal reference values.

## **4.3 Conclusion and Recommendations**

In conclusion this study has conclusively shown that the reference range that is currently used in the PathCare Laboratories for fructosamine is not appropriate for this population using the methods currently available in the practise. The single reference range (226 - 293  $\mu\text{mol/L}$ ) for the Caucasian males and females is recommended. However, due to the significant differences observed between the mixed ancestry males and females it is recommended that gender specific references ranges be used for this population group, which are: 1) 222 - 304  $\mu\text{mol/L}$  for the mixed ancestry males and 2) 217 - 293  $\mu\text{mol/L}$  for the mixed ancestry females respectively. Due to a lack of correlation between fructosamine and blood glucose we do not recommend the use of an independent fructosamine assay in the diagnosis of diabetes, but rather to take these parameters into consideration in the assessment of diabetes mellitus. Further studies including the black African population group of South Africa are required to confirm these findings. We further recommend the establishment of a separate reference value for diabetic patients to determine whether their glycaemia is under control or not.

## **CHAPTER FIVE: REFERENCE LIST**



## 5.1 Reference List

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## **APPENDIX DOCUMENTS**



## **APPENDIX A**



17 August 2009

### **CPUT/HW-REC 2009/H010**

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Tel: +27 21 442 6162 Fax +27 21 447 2963

Symphony Road Bellville 7535

OFFICE OF THE CHAIRPERSON:  
HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE (HW-REC)  
Registration Number NHREC: REC- 230408-014

At the meeting of the Health and Wellness Sciences-REC on the 8 May 2009 approval was granted to Francois Christiaan Smit, pending additional information and amendments now received. This approval is for research activities related to an M Tech: Biomedical Technology at this institution.

TITLE: The development of a new reference range for fructosamine for the PathCare Pathology Group, Somerset West, South Africa

INTERNAL SUPERVISOR: Prof. T Matsha

INTERNAL CO-SUPERVISOR: Dr. Oluwafemi Oguntibeju

EXTERNAL CO-SUPERVISOR: Drs. Cor Aalbers and Esmè Hitchcock

Comment:

This ethics approval is supported by written permission from the research site where the study will be conducted.

Research activities are restricted to those detailed in the proposal and application submitted in August 2009.

Approval will not extend beyond 16 August 2010. An extension must be applied for should data collection for this study continue beyond this date.

Prof. Penelope Engel-Hills  
CHAIR: HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE  
E-mail: [engelhillsp@cput.ac.za](mailto:engelhillsp@cput.ac.za)

**APPENDIX B**



**THE PATHCARE, SOMERSET WEST, FRUCTOSAMINE STUDY CONSENT FORM.**

**Principal Investigator: Francois Christiaan Smit**

**Principal Supervisor: Prof. Tandi Matsha (CPUT)**

**Co-Supervisors: Dr Oluwafemi Oguntibeju (CPUT)  
Dr Cor Aalbers (PathCare)**

**Collaborator: Dr Esmè Hitchcock (PathCare)**

**Address: Faculty of Health and Wellness Sciences, Cape  
Cape Peninsula University of Technology (CPUT), Bellville  
Campus  
Symphony Way, 7535**

**PathCare Laboratories  
Mediclinic Vergelegen  
Main Road  
Somerset West  
7140**

**Contact Numbers: Mr. Francois Smit – (021) 852 3144  
Dr T. Matsha – (021) 959 6366  
Dr C. Aalbers – (021) 596 3400**

## **Dear Participant,**

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied and that you clearly understand what this research entails and how you could be involved. Also, your participation is entirely voluntary and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do initially agree to take part.

This study has been approved by the Cape Peninsula University of Technology's Health and Wellness Science Research Ethics Committee and will be conducted according to the ethical guidelines and principles of the International Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

### **What is this research study all about?**

Worldwide new causes of certain diseases or conditions are continuously being discovered by research on the cells and molecules of the body. For research to be carried out on certain diseases it is necessary to first establish the incidence and prevalence of the disease and what the blood results should look like in a healthy population. This project aims to determine a new reference range in apparently healthy participants for an analyte called fructosamine. Fructosamine, together with blood glucose, is used as a tool to monitor glucose levels in the blood over a period of one month. Fructosamine is not routinely analysed and is underutilized. Not many diagnostic companies offer the test. If this study proves that the test itself is more useful as its current status, diagnostic companies may approach the researcher to manufacture a kit which is more readily available and easier to use than the current one in the market.

### **Why have you been invited to participate?**

The prevalence of diabetes in South Africa is not well documented and few studies have been conducted since 1994. Many subjects with diabetes are unknown to the health service, often because they are not yet diagnosed. In order to assess the magnitude of the problem, you have been approached to participate in this project to determine a new reference range for fructosamine, which will later be implemented as the new reference range in the PathCare Laboratories practice worldwide.

You are thus hereby invited to participate in this study, with no obligation, free of charge. Coloured and Caucasian males and females between the ages of 21 and 65 are welcome to participate.

### **What will your responsibilities be?**

The participant will be requested to provide information about his/her medical history. Participants suffering from any of the following medical conditions will not, unfortunately, be able to participate:

- kind of pre-existing diabetes or any form of impaired glucose intolerance
- high blood pressure
- any form of kidney problems

Completion of the required documentation will take no longer than 10 minutes. Random venous blood will be collected, and the blood will be used to determine your glucose, fructosamine, total protein, albumin, glycosylated haemoglobin (HbA<sub>1c</sub>) and lipogram concentrations. The remainder of the blood sample will be used for genetic and future research studies, with your permission. The DNA may be stored for several years until the technology for meaningful analysis becomes available. No pharmaceutical agent (medication) will be tested in the study.

### **Will you benefit from taking part in this research?**

You will be able to collect your results after about 1 week free of charge. If there seems to be a problem with your blood results, you will be encouraged to go with your results to your general practitioner for further investigation and treatment, together with a specially compiled letter explaining the nature of the study and why you participated.

In the unlikely event that the research may lead to the development of commercial applications, the participant or the participant's heirs will not receive any compensation, but profits will be reinvested into supporting the cause of further research which may bring benefits to my/the participant's family and to the community, such as health screening, medical treatment, educational promotions, etc.

### **Are there any risks involved in my taking part in this research?**

A slight bruising might occur after blood has been drawn from the arm but this will heal quickly. A medical nursing sister/staff nurse or phlebotomist will be present on all occasions taking the blood samples. Three blood collection tubes will be taken from you in total.

**Who will have access to your medical records?**

The participant's identity will be kept confidential throughout. Information will not be associated with the participant's name. The research staff will use only a bar-coded number, access will be limited to authorized scientists and any scientific publications, lectures or reports resulting from the study will not identify me/\*the participant.

Some insurance companies may mistakenly assume that taking part in research indicates a higher risk for disease. Thus no information about you or your family will be shared with such companies.

**Will you or your child be paid to take part in this study and are there any costs involved?**

You will not be paid or gain any financial or any kind of other compensation to take part in the study. There will be no costs involved for you if you take part in the project.

**Is there any thing else that you should know or do?**

You can contact **Francois Smit at Tel (021) 852 3144 or francois.smit@pathcare.co.za** if you have any further queries or encounter any problems.

You will receive a copy of this information and consent form for your own records if it is requested

**DECLARATION BY PARTICIPANT:**

**AFFIX BARCODE HERE**

I declare that:

- 1) I have read or had read to me this information and consent form and that it is written in a language with which I am fluent and comfortable.
- 2) I have had a chance to ask questions and all my questions have been adequately answered.
- 3) I understand that taking part in this study is voluntary and I have not been pressurized to take part.
- 4) I may choose to withdraw from the study at any time and will not be penalized or prejudiced in any way.
- 5) I may be asked to leave the study before it has finished if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan as agreed to.

**I give consent that the sample of my blood may be (please tick appropriate box):**

- **Used for this study and stored for future research studies**
- **Used for this study only and any blood remaining to be discarded immediately**

Signed at (*place*) ..... on (*date*) ..... 2010

**Signature of participant:** ..... **Signature of witness:** .....

**DECLARATION BY THE INVESTIGATOR**

I, Francois Christiaan Smit, declare that:

- 1) the nursing sister / phlebotomist explained the information in this document to (*Names of participant*)..... in my absence as the researcher is not there at all times and that the participant may contact the researcher at any time if he /she has any further questions
- 2) the nursing sister / phlebotomist encouraged him/her to ask questions and took adequate time to answer them.
- 3) that he/she adequately understood all aspects of the research, as discussed above in this document

Signed at (*place*) ..... on (*date*) ..... 2010.

**Signature of investigator / nursing sister / phlebotomist:** .....

## **APPENDIX C**



### **DIE PATHCARE, SOMERSET WES, FRUKTOSAMIENE STUDIE TOESTEMMINGSVORM**

<b>Navorser:</b>	<b>Francois Christiaan Smit</b>
<b>Hoof Toesighouer:</b>	<b>Prof. Tandi Matsha (CPUT)</b>
<b>Mede Toesighouers:</b>	<b>Dr Oluwafemi Oguntibeju (CPUT) Dr Cor Aalbers (PathCare)</b>
<b>Raadgewer:</b>	<b>Dr Esmè Hitchcock (PathCare)</b>
<b>Adres:</b>	<b>Fakulteit Gesondheids Wetenskappe Cape Peninsula Universiteit van Tegnologie (CPUT) Bellville Kampus Symphony Weg, 7535</b>  <b>PathCare Laboratories Medikliniek Vergelegen Hoofweg Somerset Wes 7130</b>
<b>Kontak Besonderhede:</b>	<b>Mnr F.C. Smit- (021) 852 3144 Dr. T. Matsha – (021) 959 6966 Dr. C. Aalbers – (021) 596 3400</b>

#### **Geagte Deelnemer,**

U word hiermee uitgenooi om aan 'n navorsingsprojek deel te neem. U word vriendelik versoek om deur hierdie informasie brojure te lees wat die hele projek se besonderhede uitstipuleer. Indien u enige vrae het rondom die studie, onseker is wat die studie behels, of meer inligting rakende die studie wil bekom, is u meer as welkom om die navorser of enige ander van die spanlede te kontak met u vrae. Dit is van kardinale belang dat u heetmal moet seker wees en verstaan wat die studie behels en hoe u deelname kan bydra tot die studie. U deelname aan die studie is geheel en al vrywillig en as u voel u wil nie aan die studie deelneem nie, is u meer as welkom om te nee te sê. Dit sal u op geen wyse negatief benadeel nie. U is ook welkom om aan die studie te onttrek op enige stadium, al het u toestemming gegee aan die begin.

Hierdie studie is goedgekeur deur die Cape Peninsula Universiteit van Tegnologie se Health and Wellness Departement Etiese Navorsings Kommitte. Die studie word uitgevoer volgens die etiese riglyne en metodes, soos uiteengesit in die Internasionale Verklaring van Helsinki, asook die Suid Afrikaanse Mediese Navorsingsraad (MRC) se Etiese Riglyne vir Navorsing.

### **Wat behels die studie?**

Wêreldwyd word daar daagliks nuwe siekte toestande ontdek deur navorsing wat uitgevoer op molekules en selle van die menslike liggaam. Vir dië tipe navorsing om plaas te vind, moet daar eers vasgestel word hoe gereeld 'n siekte toestand voorkom onder 'n populasie groep, en hoe die bloed resultate sou lyk in 'n gesonde groep mense. Hierdie studie se hoof doelwit is om 'n nuwe reikwydte uit te werk vir analiet fruktosamiene in 'n populasie gesonde individue, wat dan as die nuwe reikwydte ingespan gaan word in die PathCare praktyk . Fruktosamiene, in samewerking met bloed glukose, word gebruik om diabetese glukose inname oor 'n periode van 1 maand te monitor en daarvolgens die behandeling te bepaal. Die projek behels die versameling van genetiese materiaal (bloed), wat dan bloodgestel gaan word aan 'n paneel bloedtoese. Die oorblywende bloed word dan gestoor vir 'n periode vir moontlike toekomstige navorsingsprojekte. Wanneer 'n groot groep gesonde deelnemers se bloed geneem is, word die data van die uitslae as 'n geheel ingespan om die nuwe reikwydte te bepaal.

### **Hoekom word u genader om deel te neem?**

Die voorkoms van diabetes onder Suid Afrikaners is ongelukkig nie goed gedokumenteer nie en sedert 1994 tot hede is die hoeveelheid studies rakende diabetes in Suid Afrika baie min. Baie individue het onbewustelik onderliggende glukose intolleransie of diabetes wat telke male lank ongediagnoseerd aangaan voor dit per toeval ontdek word deur roetine mediese ondersoek.

Vir ons om die probleem aan te spreek en die nuwe reikwydte vir fruktosamiene te bepaal, word deelnemers genader om aan die projek deel te neem. Kleurling en blanke manlike en vroulike deelnemers, tussen die ouderdomme van 21 en 65, word vriendelik uitgenooi om vrywillig, sonder enige verpligting, aan die projek deel te neem, sonder enige finansiële uitgawes of wins.

### **Wat word van u verwag?**

Deelnemers gaan gevra word om hulle mediese geskiedenis weer te gee. As u aan een van die volgende mediese toestande ly, word u ongelukkig uitgeskakel om aan die studie deel te neem, aangesien die reikwydte bepaal moet word van deelnemers wat as gesond voorkom:

- \* enige vorm van diabetes of glukose intolleransie
- \* hoë bloeddruk (hipertensie)
- \* enige vorm van nierversaking

Dit gaan u nie langer as 10 minute neem om die nodige dokumentasie te voltooi nie. Die flebotomie personeel gaan lukraak (nie-vastende) bloed monsters by u neem. Dit gaan dan getoets word vir: glukose, fruktosamiene, totale proteïen, albumien, geglukosileerde haemoglobien (HbA<sub>1c</sub>) en 'n lipogram. Die oorblywende bloed gaan dan, met u toestemming, gestoor word vir moontlike toekomstige projekte en genetiese analise. Die DNA mag vir 'n paar jaar gestoor word todat beter tegnologie beskikbaar gaan wees wat beter toets metodes en uitslae sal bepaal. Geen farmaseutiese middels (medikasie) word in die studie bepaal nie.



### **Hoe gaan u deelname u positief beïnvloed?**

Na omtrent 1 week, is u welkom om u resultate te kom afhaal, sonder enige kostes verbonde aan u kant. As daar gedurende die analsie van u bloed abnormal uitslae verkry word, sal u aangemoedig word om na u algemene parktisyn toe te gaan vir verdere ondersoek. 'n Brief sal ook aan u gegee word om saam met u te vat na u algemene praktisyn toe, om aan hom/haar te verduidelik hoekom u deelgeneem het aan die studie.

Geen finansiële kompensasië vir die navorser of enige van die toesighouers of medewerkers van die projek, sal plaasvind indien die projek in die toekoms sal oorgaan in 'n kommersiële aplikasië nie. Alle winste sal dan gevat word en gekanaliseer word vir die opbou en ontwikkeling van gemeenskapsgesondheid en bevordering van mediese dienste.

### **Wat is die risiko's verbonde van deelnemers aan die projek?**

Kneusing van die wond area waar die bloed getrek gaan word mag voorkom. Maar dit sal weggaan en gesond word na n paar dae. Die phlebotomie personeel sal teenwoordig wees ten alle tye wat u bloed getrek gaan word. Drie bloed monsters sal by u gekollekteer vir die studie.

### **Wie gaan toegang hê tot u mediese rekords en geskiedenis?**

Alle deelnemers aan die studie se mediese geskiedenis en mediese rekords sal dwarsdeur die studie konfidensieël gehou word. Geen informasie rakende die studie sal gekoppel wees aan enige van die deelnemers nie. U besonderhede sal gekoppel wees aan 'n strepieskode, en slegs die navorser en die hoof toesighouer sal toegang hê tot die deelnemers se kontakbesonderhede. Geen publikasie of lesing sal u naam en ander besonderhede hê wat u sal koppel aan die studie nie.

Sekere assuransie en versekering firma's sal die deelname aan so 'n studie verkeerdelik beskou as iemand wat aan 'n hoë risiko siekte toesand lei, dus geen informasie rakende enige deelnemer of hulle direkte familie sal aan die firma's gegee word nie.

### **Sal u finansiële kompensasië ontvang vir deelname aan die studie?**

Geen finansiële kompensasië of enige ander tipe kompensasië sal aan u gegee word om aan die studie deel te neem nie. Daar sal ook geen finansiële onkoste vanaf u kant wees om aan die studie deel te neem nie.

### **Is daar enige its anders wat u wil weet of moet doen rakende die studie?**

U kan die navorser, **Francois Smit**, kontak gedurende kantoor ure by **(021) 852 3144** of via epos: [francois.smit@pathcare.co.za](mailto:francois.smit@pathcare.co.za) as u enige verdere vrae het of enige probleme ondervind.

U sal 'n kopie van die informasie en toestemming ontvang vir u eie rekords as u daarvoor vra.

**VERKLARING VAN DIE DEELNEMER:**

**PLAK STREPIES KODE HIER**

Ek, die deelnemer verklaar dat:

- 1) Ek die informasie brosjure gelees het, of dat dit aan my gelees was en dat die toestemmingsvorm in 'n taal was wat vir my verstaanbaar en duidelik was.
- 2) Ek die geleendheid gehad het om enige vrae rakende die studie te vra en dat my vrae, al dan nie, vir my bevredigend beantwoord was.
- 3) Ek verstaan dat die deelname aan die studie vrywillig is en dat daar geen druk op my toegepas was om aan die studie deel te neem nie.
- 4) Ek op enige tydstip aan die studie mag onttrek en die daarvoor gepenaliseer sal word nie.
- 5) Ek die studie moet verlaat indien die navorser sou dink dit is 'n goeie idee, of dat u nie aan die kriteria voel doen het wat gestipuleer was in die informasie brosjure nie.

**Ek gee ook hiermee toestemming dat my bloed (merk af die toepassende blokkie):**

- **Gebruik mag word vir die studie en gestoor mag word vir toekomstige navorsing**
- **Gebruik mag word vir die studie en dan dadelik verwoes moet word.**

Geteken te (pleknaam)..... op(datum)..... 2010

**Handtekening van Deelnemer:.....**

**Handtekening van Getuie:.....**

**Verklaring deur Navorser:**

Ek, Francois Christiaan Smit, verklaar hiermee dat:

- 1) Die suster / phlebotomist die informasie in die brosjure stuk uiteengesit het aan: (*Naam van Deelnemer*)..... aangesien die navorser nie teenwoordig kan wees ten alle tye nie.
- 2) Die suster / phlebotomist vir hom/haar aangemoedig het om enige vrae te vra en hy / sy die tyd geneem het om dit te beantwoord.
- 3) Die suster / phlebotomist tevrede is dat hy/sy die aspekte rondom die projek verstaan, soos uiteengesit in die informasie brosjure.

Geteken te (pleknaam)..... op(datum)..... 2010

**Handtekening van Navorser/ Suster / Phlebotomist: .....**

**APPENDIX D**



**Dear Doctor**

..... participated in a research study entitled:

*“The development of a new reference range for fructosamine for the PathCare Pathology Group, Western Cape, South Africa”*

The study was a research project undertaken by Cape Peninsula University of Technology (CPUT) and PathCare as part of a Master’s degree dissertation.

A random blood sample of this participant was drawn for the following set of tests: Glucose, Fructosamine, Lipogram, Total Protein and Albumin.

Please find the results attached for your attention. We feel that the results obtained from this participant warrants further medical investigation. Please feel free to contact the researcher or any of the supervisors in this study using the contact details given below, should you have any questions in this regard.

Thanking you.

.....

**Signature**

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.....

**Date**

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.....

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## APPENDIX E



### **Geagte Dokter**

..... was 'n deelnemer aan 'n navorsingsprojek met die titel:

*“The development of a new reference range for fructosamine for the PathCare Pathology Group,  
Western Cape, South Africa”*

Die projek was 'n gesamentlike onderneming tussen die Cape Peninsula Universiteit van Tegnologie (CPUT) en PathCare wat deel uitgemaak het van 'n tesis vir 'n Meestersgraad.

'n Lugraak (nie vastende) bloedmonster was op die deelnemer getrek gewees en getoets vir die volgende: Glukose, HbA1c, Fruktosamiene, Lipogram, Totale Protein en Albumien.

Vind asseblief die resultate van die deelnemer aangeheg. Ons voel dat die deelnemer verdere mediese ondersoeke benodig deur u na aanleiding van die bloed uitslae verkry in die studie. Indien u enige verdere navrae het rakende die uitslae, is u meer as welkom die navorser of enige van die projekbestuurders te nader by die kontak besonderhede hier onder verskaf.

Baie dankie

.....  
**Handtekening**

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