THE EFFECT OF A COMBINATION OF SHORT-CHAIN FATTY ACIDS ON GLYCOMETABOLIC CONTROL IN MEN

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SUMMARY

Dietary fibre has revealed benefits for health maintenance and disease prevention and as a component of medical nutrition therapy. Dietary fibre forms an important part of the Westernised diet, which is characterised by low-fat, low-carbohydrate and low-fibre intake. A high-fibre diet may favourably influence glycometabolic control. It is believed that short-chain fatty acids (SCFAs) may partially be responsible for some of the beneficial effects of dietary fibre on metabolism. These SCFAs namely, acetate, propionate and butyrate are the major products of colonic fibre fermentation. Some of the SCFAs have been shown to improve blood glucose and insulin levels. However, the effect of a combination of SCFAs on glycometabolic control is still unclear.

The main aim of the study was to determine the effect of a combination of SCFAs (acetate: propionate: butyrate in the ratio of 70:15:15, respectively) and (acetate & propionate: in the ratio of 50:50, respectively) on glycometabolic control in men.

The study was a randomised, placebo-controlled, double-blinded clinical trial. Voluntary subjects were recruited for this study using a very strict set of inclusion criteria. All subjects received a placebo for a period of one week following the collection of baseline blood samples and other information. A second baseline blood sample was collected from each individual at the end of this period to ensure accurate reflection of the variables and a stable baseline. Subjects were randomly assigned to three different intervention groups and consumed the different mixtures of either placebo, acetate-propionate-butyrate or acetate-propionate supplement for a period of four weeks following the second baseline blood collection. Supplementation of eight capsules daily was sustained for four weeks. Metabolic indicators (serum glucose, serum insulin, serum albumin, total protein, total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, non-esterified fatty acids (NEFA), anthropometric status and blood pressure were measured at baseline two (day 8) and after supplementation (day 36). A wash-out period of one week following the supplementation period measured any changes in the metabolic indicators (day 43). The usual dietary intake of the subjects was obtained using a food frequency questionnaire (FFQ) at baseline one (day 0) and after

supplementation (day 36). Anthropometric status included body mass index (BMI) and waist-to-hip ratio (WHR), which were measured by means of standardised methods (on days 1, 8 and 36). The BMI and WHR fell within the normal range, and remained within the normal range during the study. This indicated that the subjects were apparently healthy. The study group was also of homogeneous nature, mainly as a result of the strict inclusion criteria applied at the time of recruitment of the subjects.

The fasting serum glucose levels were within the higher normal range (5.1 – 5.7mmol/L). No statistically significant changes were observed in any of the glycometabolic parameters following supplementation with the different SCFAs regimens (acetate, propionate and butyrate; acetate and propionate). Total cholesterol (TC) levels of the subject group as a whole fell within the normal range of the population (3.0 – 5.2mmol/L). However, the observed levels fell in the higher normal range (4.1 – 4.8mmol/L). The HDL-C levels increased slightly in group three (acetate and propionate) and slightly decreased in group two (acetate, propionate and butyrate), however not significantly. The LDL-C significantly decreased in group two (acetate, propionate and butyrate). The observed decreased in systolic blood pressure were statistically significant after the intervention period in group two (acetate, propionate and butyrate). However, observed changes in LDL-C and systolic blood pressure were of no clinical importance. The FFQ indicated a tendency towards the adoption of an atherogenic Westenised diet.

This study could not shown that a combination of short chain fatty acids have a beneficial effect on glycometabolic control. The findings of this study are supported by other studies, which indicate that acetate, propionate and butyrate do not improve glucose metabolism in healthy subjects. In contrast, other studies indicated a decrease in fasting serum glucose concentration from propionate (Todesco *et al.*, 1991) and acetate (Jenkins *et al.*, 1991) as well as from a combination of acetate, propionate and butyrate (De Wet, 1999).

The controversial results regarding the effect of short chain fatty acids on glucose metabolism emphasize the importance of further investigation about the association between physical characteristics and formation of SCFAs, as well as the different combinations of SCFAs over a longer period of time.

Key words: Westernised diet, dietary fibre, SCFAs, serum glucose, serum insulin.

OPSOMMING

Dieetvesel toon dat dit voordele inhou vir die instandhouding van gesondheid, voorkomende funksies het teen siektes en ook 'n deel vorm van mediese voedingsterapie. Dieetvesel vorm 'n belangrike deel van die Westerse dieet, wat gekenmerk word deur 'n lae-vet, lae-koolhidraat en lae-vesel inname. 'n Hoë-vesel dieet mag die glukometaboliese kontrole voordelig beïnvloed. Verder mag kort-ketting vetsure (KKV) gedeeltelik verantwoordelik wees vir sommige voordelige effekte van dieetvesel op metabolisme. Hierdie KKV naamlik, asetaat, propionaat en butiraat is die hoofprodukte van fermentasie van vesel in die kolon. Sommige van die KKV het 'n verbetering op bloedglukose en insulienvlakke getoon. Die effek van 'n kombinasie van KKV op glukometaboliese kontrole is nog onduidelik.

Die hoofdoelwit van die studie was om te bepaal watter effek 'n kombinasie van KKV (asetaat: propionaat: butiraat in kombinasie van 70:15:15 onderskeidelik) en (asetaat en propionaat: in kombinasie van 50:50 onderskeidelik) op glukometaboliese kontrole in mans het.

Die studie was 'n ewekansige plasebo-gekontroleerde dubbelblinde kliniese proef. Vrywillige proefpersone is geselekteer vir hierdie studie volgens baie streng insluitingskriteria. Alle proefpersone het 'n plasebo vir 'n periode van een week ontvang nadat 'n basislyn vir bloedwaardes en ander informasie ontvang is. 'n Tweede basislyn van bloedwaardes is van elke individu aan die einde van hierdie periode geneem om 'n akkurate refleksie van die veranderlikes en 'n betroubare basislyn te verky. Proefpersone is ewekansig in drie verskillende intervensiegroepe verdeel en het verskillende kombinasies ontvang: of 'n plasebo, asetaat, propionaat en butiraat of asetaat-propionaat supplement vir 'n periode van vier weke gevolg deur 'n tweede basislyn van bloedwaardes. Supplementasie van agt kapsules daagliks het vir vier weke plaasgevind. Metaboliese parameters (serum glukose, serum insulien, serum albumien, totale proteine, totale cholesterol (TC), hoë-digtheid lipoproteine (HDL-C), lae-digtheid lipoproteine (LDL-C), nie-veresterde vetsure), antropometriese status en bloeddruk is gemeet op

basislyn twee (dag 8) en na supplementasie (dag 36). 'n Uitwasperiode van een week na die supplementasie periode is gedoen om enige veranderinge in die metaboliese parameters te meet (dag 43). Die gebruiklike dieetinname van die proefpersone is verkry deur gebruik te maak van 'n voedselfrekwensievraelys op basislyn een (dag 0) en na supplementasie (dag 36). Antropometriese status het liggaamsmassa-indeks (LMI) en middel-tot-heup verhouding (MHV) ingesluit en is gemeet deur middel van gestandaardiseerde metodes (dag 1, 8 en 36). Die BMI en MHV het in die normale grense geval en so gebly deur die studie. Dit het dus getoon dat die proefpersone oënskynlik gesond was. Die studiegroep was ook homogeen van aard hoofsaaklik as gevolg van die streng insluitingskriteria gedurende die seleksieperiode.

Die vastende serumglukose vlakke was binne die hoë normale vlakke (5.1 – 5.7mmol/L). Geen statisties betekenisvolle veranderinge is waargeneem in enige van die glukometaboliese parameters na supplementasie met die KKV (asetaat, propionaat en butiraat ; asetaat en propionaat). Totale cholesterol (TC) vlakke van die groep as 'n geheel het egter binne die normale vlakke van die populasie geval (3.0 – 5.2mmol/L). Die waargenome vlakke het in die hoog normale vlak geval (4.1 – 4.8mmol/L). Die HDL-C vlakke het gestyg in groep drie (asetaat en propionaat) en gedaal in groep twee (asetaat,propionaat en butiraat). Die LDL-C vlakke het betekenisvol gedaal in groep twe (asetaat, propionaat en butiraat). Die waargeneemde daling in sistoliese bloeddruk was statisties betekenisvol groep twee (asetaat, propionaat en butiraat). Alhoewel, die veranderinge in LDL-C en sistoliese bloeddruk was nie van kliniese waarde. Die voedselfrekwensievraelys het 'n neiging tot die aankweek van westerse eetpatrone aangetoon.

Hierdie studie kon egter nie toon dat 'n kombinasie van KKV a voordelige effek op glukometabolisme het nie. Die bevindinge van hierdie studie word ondersteun deur ander studies wat ook aangedui het dat asetaat, propionaat en butiraat nie glukosemetabolisme in gesonde persone verbeter nie. In teenstelling hiermee het ander studies getoon dat 'n verlaging in vastende serumglukose konsentrasies verkry is na propionaatsupplementasie

(Todesco *et al.*, 1991) en asetaatsupplementasie (Jenkins *et al.*, 1991), asook 'n kombinasie van asetaat, propionaat en butiraat (De Wet, 1999).

Die teenstrydige resultate van KKV met betrekking tot KKV op glukose metabolisme beklemtoon die belangrikheid van verdere navorsing om die verband tussen fisiese eienskappe en die vorming van KKV, asook die verskillende kombinasies van KKV oor 'n langer periode aan te toon.

Sleutelwoorde: Westerse dieet, dieetvesel, KKV, serumglukose, serum insulien.

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LIST OF ABBREVIATIONS

Acetyl-Coa	Acetyl coenzyme A
ADP	Adenosine diphosphate
AI	Adequate intake
BP	Blood pressure
BMI	Body mass index
BRISK	Coronary Heart Disease Risk Factor Study in the African Population of the Cape Peninsula
°C	Degree celcius
Cat. No.	Catalogue number
CHD	coronary heart disease
CHO	carbohydrates
CoA	coenzyme A
cm	centimeter
code no.	code number
CV	coefficient of variation
DP	degree of polimerisation
DRI	Dietary Reference Intake
FFA	free fatty acids
FFQ	food frequency questionnaire
g	gram
g/day	grams per day
g/L	grams per liter

HDL-C	high density lipoprotein cholesterol
hr	Hour
IBW	ideal body weight
kJ	kilojoules
kg	kilogram
LDL-C	low-density lipoprotein cholesterol
L	Litre
Max	Maximum
Med	Median
mg	milligram
mg/day	milligrams per day
Min	Minimum
mL	milliliter
mmol/L	millimol per liter
N/A	not applicable
NEFA	non-esterified fatty acids
Nm	Nanometer
NSP	non-starch polysaccharide
pH	percentage hydrogen
RDA	Recommended Dietary Allowance
RE	Retinol Equivalents
SANDF	South African National Defence Force
SBP	systolic blood pressure

SCC	short-chain carbohydrates
SCFAS	short-chain fatty acids
SD	standard deviation
TE	total energy
TC	total cholesterol
TG	triglycerides
THUSA	Transition and Health during Urbanisation of South Africans
TP	total protein
UFS	University of the Free State
µg	micro gram
µIU/mL	micro international units per milliliter
µmol	micromol
W/H^2	W is weight in kilograms and H is height in square meters
WHR	waist-to-hip circumference ratio
>	bigger than
<	smaller than

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CHAPTER 1

PROBLEM STATEMENT

1.1 INTRODUCTION

The relationship between dietary fibre intake and reduced risk of certain diseases has become more evident each year (ADA, 2002). Dietary fibre which is ingested from natural sources are of very high value to the Western diet as the latter is poor in dietary fibre. Dietary fibre is also used as a therapeutic treatment of disorders such as atherosclerosis and colon cancer (Savage, 1987).

A certain amount of ingested carbohydrates, which are not absorbed in the small intestine reaches the colon (Nordgaard *et al.*, 1995; Treem *et al.*, 1996). This includes not only fibre, but also starch, so called "resistant starch" derived from corn, potatoes, oats and wheat as well as small amounts of unrefined carbohydrates. Certain carbohydrates may promote fermentation in the colon, increasing the production of short-chain fatty acids. This may alter bacterial flora in the small bowel and colon (Vanderhoof, 1998). The short-chain fatty acids, (SCFA), namely acetic, propionic and butyric acids, are major products of bacterial fermentation of the carbohydrate that enters the colon (Anderson & Bridges, 1984; Wolever *et al.*, 1997). The SCFA are rapidly absorbed from the colonic lumen (McNeil *et al.*, 1978; Reckemmer *et al.*, 1988) and transported directly to the liver, except for some butyrate which is used by colonic epithelial cells as respiratory fuel (McNeil *et al.*, 1978; Roediger, 1980). Acetate largely bypasses colonic and liver metabolism but is metabolized by peripheral tissue (i.e. muscle) (Cummings *et al.*, 1987a) and brain (Juglin-Dannfelt, 1977). Approximately 75% of acetate is extracted during a single pass of blood through the human liver (Dankert *et al.*, 1981; Peters *et al.*, 1992). Bacterial fermentation of one gram of monosaccharide yields approximately ten mmol of organic acid (Scheppach *et al.*, 1992). The SCFAs that escape colonic metabolism enter the hepatic portal blood, where their concentration varies considerably, depending on production rate and, therefore on the diet (Cheng *et al.*, 1987). Soluble fibre is fermented

within six hours by human fecal bacterial into short-chain fatty acids (Lahaye *et al.*, 1993). It is widely believed that SCFAs derived from colonic fermentation of dietary fibre may partially be responsible of the metabolic effects caused by dietary fibre in human subjects. Interest in the effects of short-chain fatty acids on carbohydrate metabolism in humans was prompted by the suggestion that these acids may play a role in the mediating effects of dietary fibre in glucose control. It is widely accepted that these acids have a beneficial effect on glycometabolic control (Anderson & Bridges, 1984). The oxidation of fatty acids inhibits glycolysis and stimulates glyconeogenesis in muscles (Williamson, 1964) as well as the liver (Anderson & Bridges, 1984). It has also shown that dietary supplementation of soluble fibre has been shown to improve glucose tolerance (Wolever & Jenkins, 1986). Mann (1987) found that the fasting blood glucose concentration was lowered in response to a high-fibre diet. Simpson *et al.* (1981) suggested that a high-carbohydrate, leguminous diet containing significant amounts of slowly absorbed, highly fermentable carbohydrate decreases insulin requirements in non-insulin dependent diabetic subjects. The intake of oats (Rytter *et al.*, 1996; Wood *et al.*, 1994) and guar (Kirsten *et al.*, 1991; Chuang *et al.*, 1992) reduced plasma glucose and insulin levels.

A study by Wolever *et al* (1991) indicated that where acetate and propionate were given simultaneously by inducing a solution of sodium propionate with neutralised vinegar solution into the rectum plasma glucagon concentration increased significantly. In contradiction, Todesco *et al.* (1991) pointed out that glucose concentration was lowered significantly when only propionate was administered. The results were confirmed by Venter *et al.* (1989). Furthermore they also indicated that propionate decreased the maximum insulin increments during a glucose tolerance test. This may be an indication of improved insulin sensitivity. Alamowitch *et al.* (1996) demonstrated that acute administration of SCFAs (60% acetate; 25% propionate and 15% butyrate) over a period of 12 hours does not significantly alter glucose metabolism in healthy subjects. Furthermore, Brighenti *et al.* (1994) also found that the ingestion of 16mmol of vinegar-derived acetic acid with carbohydrate-rich food (bread) flattened postprandial glycemia in healthy subjects. Heaton *et al.* (1988) also found that whole oats and oat bran have a relatively low glycemic effect. However, Jenkins *et al.* (1991) found no evidence of decreased blood glucose and insulin concentrations, or improved glucose tolerance after

serum acetate concentrations were raised.

To achieve the positive effects of fibre on carbohydrate metabolism it is suggested that a fibre intake of double the average intake (45g) should be provided by the diet (Alamowitch *et al.*, 1996; Ebihara & Nakajima's 1998; Mann, 1987; Venter *et al.*, 1990; Wolever *et al.*, 1989). However, this high-fibre intake may be responsible for some side-effects, such as abdominal distension, a bloated feeling, pain and increased flatus (Cummings, 1987; Muir *et al.*, 1995). To overcome this problem, Crouse *et al.* (1968) and Wolever *et al.* (1989) suggested the possibility that acetate, the main fermentation product of dietary fibre, could also reduce blood glucose levels.

It has repeatedly been shown that a high-carbohydrate/high fibre diet improves blood glucose control (Riccardi & Rivellese, 1991). It is also important that not only the direct effect of the fibre itself, but also the indirect effect of SCFAs on carbohydrate metabolism must be considered (Anderson & Bridges, 1984; Asplund *et al.*, 1985; Venter *et al.*, 1990; Todesco *et al.*, 1991).

The question arises whether the SCFAs if given orally, could be used for their beneficial effect on carbohydrate metabolism. This could partially replace a high-fibre diet, reducing some of its side-effects (Cummings, 1987; Muir *et al.*, 1995).

Recent studies have revealed that various third-world populations are in the process of transition from a traditional, low fat high-fibre diet, to a westernised high-fat low-fibre diet which increases their risk of developing degenerative diseases (O'Dea, 1991; Popkin *et al.*, 1993). South Africans have also adopted typical Western lifestyles and eating patterns (Vorster *et al.*, 1999). The urban African population in South Africa is presently experiencing rapid urbanization, as evident in the black population in the Cape Peninsula who shows a tendency towards a progressively atherogenic western diet (Bourne *et al.*, 1993). Slabber *et al.* (1997) also indicated that urban African men at the UFS show a tendency towards an atherogenic westernised diet. Furthermore, the Black population in the South Western Cape (Oelofse *et al.*, 1996) and in the Free State Province (Mollentze *et al.*, 1995) showed a tendency towards an atherogenic lipid profile with lipid

concentrations in the higher normal levels. Slabber *et al.* (1997) also found that urban African men on the UFS campus have a moderate to high hypercholesterolemia.

Members of the South African National Defence Force (SANDF) are also exposed to a westernised lifestyle and eating habits, cigarette-smoking and alcohol use due to their higher income and western diets. The study by De Wet (1999) also showed a tendency towards an atherogenic diet and lipid profile in a similar population group in the SANDF.

Taking into account the above mentioned, it was decided to undertake this study in a attempt to determine the effect of oral SCFAs on the glycometabolic control in African males who are exposed to a westernised atherogenic diet.

1.2 AIM OF THE STUDY

The main aim of the study is to determine the effect of a combination of short-chain fatty acids (acetate: propionate: butyrate:- 70:15:15) and (acetate and propionate:- 50: 50) on glycometabolic control in men.

1.2.1 OBJECTIVES

1.2.1.1 To determine glycometabolic indicators: serum glucose and insulin levels

1.2.1.2 To determine background information regarding other metabolic parameters, anthropometric status and blood pressure as well as the dietary intake.

1.3 STRUCTURE OF THE STUDY

A short summary of the study is given in the beginning. The first chapter of the study consists of an introduction stating the motivation for and aim of the study. Chapter two gives a literature review of the most critical information required for background information and in order to understand and interpret the study. The methodology used in the study is discussed in chapter three, and the results are given in chapter four. The

results are discussed in chapter five, followed by conclusions and recommendations in chapter six. Examples of the questionnaires used in the study are given as appendices.

CHAPTER 2

LITERATURE SURVEY

2.1 INTRODUCTION

Glycometabolic control can be defined as the maintenance of blood glucose homeostasis. The blood glucose homeostasis is affected by glucose absorption and insulin secretion. Glucose absorption takes place in the digestive tract, is transported into cells and oxidised in the cells as a source of energy, and stored in the liver and muscles as glycogen (Sherwood, 1997). Insulin secretion is influenced by the capacity of the pancreas and the ability of insulin to suppress hepatic glucose production as well as the reaction of skeletal muscle and liver to insulin (Turner & Clapham, 1998).

Carbohydrates are not just an important source of energy to humans, but also consist of important food components such as dietary fibre (Ettinger, 2000, p.39).

Fibre was originally described as plant cell wall material, which passed through the gut unchanged and provided bulk to the faeces (Smolin & Grosvenor, 2000, p.98), which is known to be beneficial to health (Englyst & Hudson, 2000, p. 75). Today it is known that fibre plays a role during digestion in the small bowel and is a substrate for fermentation in the colon, where non-starch polysaccharides of the plant cell wall are metabolised to short-chain fatty acids (ADA 2002; Cummings, 1995). These short-chain fatty acids (SCFAs) may play a role in glycometabolic control by influencing blood glucose homeostasis.

The relationship between carbohydrates, including non-starch polysaccharides (NSP) and SCFAs, will be discussed in this chapter. Factors influencing glycometabolic control including the effect of SCFA on glycometabolic control will also be reviewed.

2.2. DIETARY CARBOHYDRATES

Carbohydrates are divided into three groups according to the degree of polymerisation (DP), i.e. the number of monosaccharide units joined together (Cummings, 1997): monosaccharides, oligosaccharides with DP of two to about ten ; and the polysaccharides, i.e. those carbohydrates with DP greater then approximately ten (Englyst & Hudson, 2000, p. 62). Nutritionists traditionally regarded monosaccharides (DP 1) and disaccharides (DP 2) as free sugars. The dividing point between oligosaccharides and polysaccharides on the basis of DP is admittedly arbitrary and has not proved convenient for nutritionists or food analysts. Englyst and Hudson (2000) proposed that dietary carbohydrates should be classified as free sugars, short-chain carbohydrates, starch and non-starch polysaccharides (Table 2.1).

2.2.1 FREE SUGARS

As indicated in Table 2.1 free sugars may be divided into mono- and disaccharides as well as their acid and alcohol derivatives. They can be measured accurately (Cummings *et al.*, 1997; Englyst & Hudson, 2000, p. 63).

2.2.1.1 Monosaccharides

All carbohydrates can be broken down into free sugars, the form in which they are used in the body. Free sugars or monosaccharides (one sugar) consist of glucose, fructose and galactose.

Glucose is the most widely distributed sugar in nature (Ettinger, 2000, p. 33). Glucose is found in varying amounts in honey, maple syrup, fruits, berries and vegetables. Glucose is often formed from the hydrolysis of sucrose, as in honey, maple sugar and invert sugar. It is also present in foods containing starch hydrolysis products, such as corn syrups and high-fructose corn syrups (FAO, 1997, p. 68).

TABLE 2.1: TYPES, SOURCES AND END PRODUCTS OF CARBOHYDRATES

(Ettinger, 2000, p. 34; Englyst & Hudson, 2000, p.63 ; Cummings *et al.*, 1997).

TYPE OF CARBOHYDRATE	SOURCES	END PRODUCTS
FREE SUGARS		
• Monosaccharides		
Glucose	Fruits, honey, corn syrup	Glucose
Fructose	Fruits, honey	Fructose
• Disaccharides		
Sucrose	Cane and beet sugar	Glucose & fructose
Lactose	Milk & milk products	Glucose & galactose
Maltose	Malt products	Glucose
• Sugar alcohols		
Sorbitol	Dietetic products	
Mannitol		
Xylitol		
SHORT-CHAIN CARBOHYDRATES		
• Oligosaccharides	Malt products, onions, leeks	Fructose
• Inulin	Onions,garlic, mushrooms	
STARCH	Grains, vegetables	Glucose
• Digestible		
• Resistant		
NON-STARCH POLYSACCHARIDES		
• Soluble fibre	Plant cell wall Pectin, gums, hemi-cellulose, storage poly-saccharides	
• Insoluble fibre	Lignin, cellulose, hemi-cellulose	

Fructose is the sweetest of all monosaccharides. Fruits contain from 1% - 7% fructose. Some fruits may contain considerably greater concentrations of fructose. Fructose makes up about 3% of the dry weight in vegetables and about 40% in honey (Ettinger, 2000, p. 33).

2.2.1.2 Disaccharides

When two free sugars are hooked together they form a disaccharide; glucose and fructose form sucrose, glucose and galactose form lactose (milk sugar) and glucose and glucose form maltose when starch ferments or is digested.

Sucrose is present in honey, maple sugar, fruits, berries and vegetables. It may be added to food products as liquid or crystalline sucrose or as invert sugar. It is commercially prepared from sugar cane or sugar beets (FAO, 1997, p. 68).

2.2.1.3 Sugar alcohols

Monosaccharides and disaccharides in which the aldose and ketose functional groups have been reduced to hydroxyl groups are known as sugar alcohols. Sugar alcohols such as sorbitol, mannitol and xylitol occur in small amounts in fruits (FAO, 1997, p.74 ; Robinson *et al.*, 1986, p.66). Xylitol is equal in sweetness to sucrose. Inositol is an alcohol related to hexoses and occurs in the bran of cereal grains (Robinson *et al*, 1986, p. 66).

Xylitol and mannitol are absorbed more slowly than glucose and sucrose, causing lower blood glucose and insulin responses (Robinson *et al.*, 1986, p. 66).

2.2.2 SHORT-CHAIN CARBOHYDRATES (SCC)

Short-chain carbohydrates (SCC) are dietary carbohydrates other than free sugars that are soluble in 80% ethanol. They consist of oligosaccharides (raffinose, stachyose and verbascose) and inulin. Fructo-oligosaccharides and inulin have been shown to selectively

stimulate the growth of bifidobacteria, which is potentially beneficial to health (Englyst & Hudson, 2000, p. 64).

2.2.2.1 Oligosaccharides

Oligosaccharides is composed of two to 20 monosaccharides joined together (British Nutrition Foundation, 1990; Ettinger, 2000, p. 36). Smolin and Grosvenor (2000) and Cummings and Englyst (1995) classified an oligosaccharide with a molecular size of 10. The most common oligosaccharides are sucrose, lactose and maltose, which is also classified under disaccharides. Oligosaccharides are not very common except for a series of galactosylsucroses and fructo oligosaccharides. Maltodextrins are industrially derived from starch and most are readily digested and absorbed in the small bowel (Cummings *et al.*, 1997). The galactosylsucrose family in oligosaccharides include raffinose, stachyose and verbascose. Fructo-oligosaccharides have been commercially prepared by the action of a fructofuranosyl furanosidae from Aspergillus niger on sucrose (FAO, 1997, p. 69).

2.2.2.2 Inulin

Inulin is found in artichokes, chicory, onions and asparagus and it is a polymer of fructose in β (2-1) linkage (small polysaccharides). It is rapidly fermented in the caecum and colon and has recently been shown to have a lipid-lowering effect (Johnson, 2000, p. 3).

2.2.3 STARCH

When many sugars are linked together in either straight or branched chains, the substance thus obtained is called a polysaccharide which can be very complex.

Starch, a carbohydrate formed by plants, is a polysaccharide that has over 300 simple sugars hooked together. When starch is broken down it forms an intermediary polysaccharide called dextrin. Dextrin in turn breaks down into maltose and finally into glucose (Ettinger, 2000, p.37). Starch is the major carbohydrate in the human diet and

consists of 80 - 90 % of all polysaccharides eaten. All starch can ultimately be degraded by human alpha-amylase. It occurs as the reserve polysaccharide in the leaf, stem, root, seed, fruit and pollen of many higher plants (FAO, 1997, p. 69). Common food starches are derived from seed (wheat, maize, rice, barley) and root (potato, cassava/tapioca) sources (FAO,1997, p. 69) and are glucose polymers with similar chemical composition (Ettinger, 2000, p. 38).

The rate and extent to which starch is digested in the small intestine is determined by its physiological properties (Cummings *et al.*, 1997). Slowing starch digestion or modifying other factors such as lipid and protein content of the meal and thus slowing gastric emptying reduces the glycaemic index and insulin responses. Some starches are rapidly digested and give rise to blood glucose responses similar to or even greater than sugars (Wolever & Miller, 1995).

If starch or its hydrolysis products escape digestion they pass into the large intestine where they may be fermented. The poorly digested starch is known as resistant starch (Brand Miller, 2000, p. 19; Ettinger, 2000, p. 38).

2.2.4 NON-STARCH POLYSACCHARIDES (NSP)

Dietary fibre is generally defined as plant material, mainly derived from plant cell walls, that is resistant to digestion by human gastro-intestinal enzymes (Hunt *et al.*, 1993). Food chemists prefer to define fibre as lignin and non-starch polysaccharides (NSP). NSP consist of polysaccharides other than starch that are insoluble in 80% ethanol (Englyst & Hudson, 2000, p. 66).

NSP are also commonly classified by their water solubility. This may also explain their mechanical and physiological effect (Hunt *et al.*, 1993). Therefore, NSP can be categorised as soluble and insoluble fibre (Ettinger, 2000, p.41). The insoluble and soluble components of dietary fibre, their function and sources are summarised in Table 2.2.

2.2.4.1 Soluble fibre

Soluble fibre includes pectin, gums, certain hemicelluloses and mucilages. Fruit, oats, barley and legumes contain more soluble fibre than other foods do (Hunt *et al.*, 1993; Walker, 1993). Soluble fibre tends to increase intestinal transit time, delays gastric emptying, slows glucose absorption and lowers serum cholesterol levels. Soluble fibre is almost completely fermented in the colon to SCFAs (Walker, 1993). The fibre in fruits, vegetables and grains is never exclusively insoluble or soluble.

Pectin has a galacturonic acid structure absorbs water and forms a gel. Food sources of pectin include apples, citrus fruits, strawberries and carrots. Pectin is also added to fat-free yogurt and other products to provide texture and stability.

Gums are similar in structure to pectin except that the galactose units are combined with other sugars (glucose) and polysaccharides. Food sources of gums include oats, legumes, guar and barley. The specific textural qualities of these fibres are commercially useful when added to processed foods such as ice cream (Ettinger, 2000, p.40).

Mucilages are a mixed group of complex polysaccharides which are not generally part of the cell wall, and which are often associated with the endosperm and mixed with starch (Ettinger, 2000, p.40).

2.2.4.2 Insoluble fibre

Water-insoluble fibre includes lignin, cellulose, and many hemicelluloses. Examples include wheat, most grain products and vegetables. Insoluble fibre shortens bowel transmit time, increases faecal bulk, renders faeces softer and delays glucose absorption and starch hydrolysis (Walker, 1993).

Table 2.2 Components of NSP (Adapted from Procter & Gamble, 1991)

Fibre component and type	Function	Source
Pectin (soluble)	Binds adjacent cell walls and holds water in networks	Apples Bananas Citrus fruit Strawberries Carrots
Gums (soluble)	Gelatinous exudate from stems or seeds	Oat bran Legumes Guar Barley
Mucilage (soluble)	Viscous water-holding substance similar to gum	Seeds Seaweeds Psyllium
Lignin (insoluble)	Along with cellulose, forms the woody cell walls of plants	Mature vegetables Cereal grains Wheat Fruits with edible seeds such as strawberries
Cellulose (insoluble)	Basic structural material of cell walls	Whole-wheat flour Wheat bran Peels of apples and pears Vegetables
Hemicellulose (insoluble and soluble)	Surrounds skeletal material of cell walls and acts as cement between them	Wheat bran Wholewheat

Lignin is a woody fibre found in the stems and seeds of fruits and vegetables and in the bran layer of cereals. It is a polymer composed of phenylopropyl alcohols and acids (ADA, 2000). Lignin may have properties that are useful in preventing cancer. Food sources of lignin include mature vegetables, wheat, fruits and edible seeds such as strawberries.

Plant structural carbohydrate is formed largely from cellulose, a simple polymer of glucose in $\propto$ (1-4) glycosidic linkage. Cellulose is the most abundant organic compound in the world, constituting 50% or more of all the carbon in vegetation. Food sources of cellulose include whole-wheat flour, bran and vegetables.

Hemicellulose fibres contain cellulose molecules substituted with other sugars. Hemicellulose is named for the predominant sugar in the backbone, xylan, galactan or mannan or in the side chain arabinose or galactose. Food sources of hemicellulose are bran and wholegrain products.

Table 2.3 summarizes the fibre content of some foods.

Table 2.3 Dietary fibre (insoluble) content of food commonly served in portions

(Adapted from Ettinger, 2000, p.40)

Food Group	<1g	1-1.9g	2-2.9g	3-3.9g	4-4.9g	5-5.9g	>6g
Breads	Bagel	Whole Wheat	Bran	NA	NA	NA	NA
1 slice	White French		muffin(1)				
Cereals	Rice	Oatmeal	Shredded	Honey	Raisin	Corn Bran	All Bran
28g	Crispies	Nutri-Grain	wheat	Bran	Bran		
	Special K Corn flakes						
Pasta	NA	Macaroni	NA	Whole	NA	NA	NA
(1cup)		Spaghetti		wheat			
				Spaghetti			
Rice (½	White	Brown	NA	NA	NA	NA	NA
cup)							
Legumes	NA	NA	NA	Lentils	Lima	NA	Kidney
½ cup cooked					beans		beans
					Dried beans		Baked beans
Vegetable	Cucumber	Asparagus	Broccoli	Peas	NA	NA	NA
½ cup unless stated	Lettuce (1 cup) Green Pepper	Green Beans Cabbage Cauliflower Potato without skin(1) Celery	Brussels sprouts Carrots Corn Potato with skin (1)				
Fruits	Grapes-20	Apricots-3	Apple	Apple	NA	NA	NA
1 medium fruit unless stated	Watermel on (1 cup)	Grapefruit (½) Peach with skin Pineapple (½ cup)	without skin Banana Orange	w/skin Raspberri es (½ cup)			

NA – Not Applicable

2.3 SHORT-CHAIN FATTY ACIDS

2.3.1 Definition

Fatty acids are classified according to the number of carbons in the chain, the number of double bonds and the position of the first double bond. Short-chain fatty acids can be described as saturated unbranched alkyl monocarboxylic acids of 2-4 carbon atoms as shown in Table 2. 4 (Wrong, 1995, p.2).

The fermentation of dietary fibre carbohydrates results in the formation of short-chain fatty acids of which acetate, propionate and butyrate are the major components (Bourquin *et al.*, 1992; Dreher, 1987, p. 230). It is believed that these by-products have beneficial effects on the gastrointestinal tract (Ettinger, 2000).

Table 2.4 Short-chain fatty acids (Mortensen & Clausen, 1996)

Chemical formula	Trivial name
CH_3-COOH	Acetate
CH_3-CH_2-COOH	Propionate
CH_3-$(CH_2)_2$-COOH	Butyrate

2.3.2 Production of short-chain fatty acids

The production of SCFAs or fermentation is the process whereby anaerobic bacteria break down carbohydrates and other substrates to obtain energy for growth and maintenance of cellular function. It is an important component of normal large-bowel activity (Mortensen & Clausen, 1996). Bacterial fermentation of dietary fibre components can be a major source of gas in the bowel of healthy humans. The three most common gases formed are hydrogen, carbon dioxide and methane (Brand-Miller, 2000, p.19). The production of hydrogen in the colon depends on the concentration of fermentable carbohydrates such as hemicellulose and soluble fibres. Methane is only produced by colonic bacteria, but is only

moderately affected by the type of dietary fibre (Brand-Miller, 2000, p.19).

The main substrates for microbial fermentation in healthy individuals are resistant starch and plant cell wall polysaccharides currently referred to as dietary fibre or non-starch polysaccharides. Other substrates may come from endogenous sources, i.e., sloughed epithelial cells, mucus, intestinal enzymes and other intestinal excretions (Mortensen & Clausen, 1996). The chemical structure of dietary fibre varies depending on its botanical origin; and its effects in the large bowel depend on the rate and degree of fermentation. Water-soluble fibre contributes less to faecal bulk than insoluble fibre because of its more rapid and complete fermentation (FAO, 1997).

Various amounts of starch up to 20% escape digestion in the small intestine and pass into the colon and become available as substrate for microbial fermentation (Mortensen & Clausen, 1996). This part of dietary starch called resistant starch acts as fermentable fibre. Simple sugars such as lactose, raffinose and stachyose may also fail to be absorbed by the small intestine. Some dietary protein may also escape absorption in the small intestine which may be named resistant peptides. In diseases with intestinal malabsorption mono- and disaccharides may pass into the colon. Semi-synthetic disaccharide lactulose and sugar alcohols are also poorly absorbed.

The initial step in protein breakdown involves hydrolysis of polypeptides to peptides and amino acids, which then become available for assimilation or deamination to yield SCFAs.

There is some variation in the percentage of SCFAs produced from a single polysaccharide. SCFAs vary widely in their relative proportions, depending on the fibre source in the diet (Bugaut & Bentèjac, 1993). SCFAs in human faeces, following consumption of different defined polysaccharides, have been measured on the average, in the molar ratio of acetate:propionate:butyrate of 53:27:20 (Savage,1987; Bugaut & Bentejac, 1993). The specific molar percents from different dietary fibres are presented in Table 2.5. Patterns are obvious because of the monosaccharide composition of the polysaccharides and the rate of hydrolysis (Mortensen *et al.*, 1988). For example, high

levels of butyrate are formed during *in vitro* fermentation of starch and sorbitol (Mortensen *et al.*, 1988) and high levels of acetate are formed when pectin and lactulose are consumed (Mortensen *et al.*, 1991).

Table 2.5 SCFA molar percents from 24hr fermentation of dietary fibre *in vitro* (Bugaut & Bentejac, 1993)

Substrate	Acetate	Propionate	Butyrate
Pectin	81	11	8
Gum arabic	68	23	9
Oat bran*	65	19	16
Wheat bran*	63	16	21
Cellulose #	53	21	26

* alpha-cellulose and hemicellulose are 7% and 19% dry total dietary fibre respectively, in oatbran, and 19% and 38%, respectively, in wheatbran.

48-hr fermentation

Colonic production of SCFAs represents an important symbiosis between humans and intestinal microbial organisms. As mentioned earlier, the SCFAs concentration depends on the production rates, therefore on the diet (Cheng *et al.*, 1987). SCFAs may influence carbohydrate metabolism (Wolever *et al.*, 1991) and may therefore contribute to the protective effect of NSP against degenerative western diseases

2.3.3 Metabolism of short-chain fatty acids

Some forms of carbohydrate cannot be digested by humans. Cellulose, hemicelluloses, pectin, gums and other forms of fibre pass relatively unchanged to the colon where they are partially fermented by bacteria in the colon. Neither salivary nor pancreatic amylase have the ability to split the cellulose bond. Some "resistant" starches and sugars are less well digested or absorbed than others, and consumption of large amounts may result in the passage of significant amounts of these into the colon where they, like fibre, are fermented

to SCFAs and gases. Starches resistant to digestion tend to include uncooked starchy foods and plant food with high protein and fibre content, such as legumes and whole grains (Bjorck *et al.*, 1994; Cummings and Englyst, 1995).

In a healthy person, 70% to 80% of dietary fibre is metabolised in the colon to carbon dioxide, hydrogen, methane and short-chain fatty acids (SCFAs). Acetate, butyrate and propionate account for approximately 85% of all SCFAs produced in the human colon. SCFAs are readily absorbed by the intestinal and colonic mucosa and have the following effects: (1) enhance sodium and water absorption; (2) increase colonocyte proliferation; (3) increase metabolic energy production; (4) enhance colonic blood flow; (5) stimulate the autonomic nervous system, and (6) increase the gastrointestinal hormone production (Ettinger, 2000, p.42).

Over 70% of the fuel for coloncytes is derived from the SCFA butyrate (Krishnan *et al.*, 1988). The preference of coloncytes for butyrate was found even in the neonatal rat in the immediate postnatal period before butyrate is available from bacterial fermentation. Propionate is absorbed and cleared by the liver and may be important in hepatic lipid or glucose metabolism. Acetate is rapidly metabolized to carbon dioxide by peripheral tissues and can serve as substrate for lipid and cholesterol synthesis. Small quantities of short-chain fatty acids are absorbed directly into the portal blood rather than being converted into triglycerides and absorbed into the lymphatics.

SCFAs are mainly metabolised in the liver. Propionate and butyrate are almost entirely taken up, but the percentage of acetate uptake is lower (frequently less than 50%). However, due to its higher concentration in the portal vein, acetate uptake generally exceeds that of propionate and butyrate (Remesy *et al.*, 1995, p.171). The metabolisms of acetate, propionate and butyrate will now be discussed.

2.3.3.1 Acetate metabolism

Acetate largely bypasses colonic and liver metabolism but is metabolised by peripheral tissue (i.e. muscle) (Cummings *et al.*, 1987a) and brain (Juhlin-Dannfelt, 1977). For metabolism, acetate requires activation with coenzyme-A (CoA), which is variably distributed in the cytosol and mitochondria of many tissues (Ballard, 1972; Wolever, 1995, p.484).

Based on the concentration in peripheral and portal blood, approximately 75% of acetate is extracted during a single pass of blood through the human liver (Dankert *et al.*, 1981; Peters *et al.*, 1992). It can be calculated that complete oxidation of SCFAs in the liver could account for more than a third of energy expenditure there. However, not all SCFAs taken up by the liver are oxidised there. After ethanol administration, studies in arteriovenous differences implicate the liver to be a net producer of acetate. Under these conditions a variety of human tissues, including skeletal muscle and brain, utilise considerable quantities of acetate. When the circulating acetate concentration is increased from 0.8 to 1.1mmol/L by the consumption of alcohol (25g), acetate taken up by the tissues contributes 12% to 22% of energy expenditure of muscle both at rest and during exercise (Lundquist *et al.*, 1973).

In humans, only acetate, one of the end-products of SCFAs, reaches the circulation beyond the liver in appreciable quantities. The peripheral venous plasma concentration of acetate in normal humans, as measured by gas-liquid chromatography, is about 50 μmol/L in the 12-hour fasting state (Scheppach *et al.*, 1991). Acetate in peripheral blood is not entirely derived from the colon since several tissues both produce and consume acetate simultaneously (Bleiberg *et al.*, 1992). Acetate can also stimulate gluconeogenesis from lactate (Rèmèsy *et al.*, 1995, p.177) which may influence glycometabolic control.

2.3.3.2 Propionate metabolism

Propionate is utilised primarily by the liver where it is used as substrate for gluconeogenesis (Bugaut & Bentejac, 1993). Under normal conditions, propionate is totally taken up by the liver (Rèmèsy *et al.*, 1995, p.177). Propionate metabolism depends on the bioavailability of vitamin B_{12} or biotin (Chiang & Mistry, 1974). Propionate metabolism increases the requirements for vitamin B_{12}, which could be critical with dietary fibres such as pectin, as it may interfere with vitamin B_{12} reabsorption during its enterohepatic cycle (Cullen & Oace, 1989).

2.3.3.3 Butyrate metabolism

A small percentage of plasma butyrate is bound to albumin ranging from 10% in the rat to 30% in sheep (Remesy & Demigne, 1974). Hepatic uptake of butyrate is practically 100 % under any physiological conditions. Butyrate is a preferred energy source for coloncytes and is thus extensively metabolised in the colon (Roediger, 1982). Butyrate uptake could be facilitated by the presence of a butyrate-binding protein in the cytosol (Remesy *et al.*, 1995 p.182). Butyrate is exclusively metabolised in the mitochondria and is a potentially ketogenic substrate during the postabsorptive period. Butyrate activation is probably mediated by medium-chain acyl-CoA syntheses (Remesy *et al.*, 1995. p. 182). Because of the provision of acetyl-CoA in mitochondria, butyrate is an effective activator of gluconeogenesis from lactate, and of ureogenesis. Butyrate also leads to ketone body production and is used as an important respiratory fuel rather than acetate, propionate and even glutamine, glucose and ketone bodies (Bugaut & Bentejac, 1993). High concentrations of butyrate inhibit propionate utilisation (Demignè *et al.*, 1986), thus butyrate probably thwarts some of the inhibitory effects of propionate.

2.4 GLYCOMETABOLIC CONTROL

As mentioned earlier, glycometabolic control refers to the maintenance of blood glucose homeostasis. The concentration of glucose in the blood, set within the normal adult range of 3.9 to 5.8mmol/L (Levin, 1999, p.56).

2.4.1 Regulation of glycometabolism

The level of glucose concentration in the blood is regulated by both metabolic and hormonal mechanisms.

2.4.1.1 Metabolic mechanisms

The metabolism of nutrients may take one of two pathways: (1) Anabolic pathways or anabolism in which molecules are built up and energy is stored, and (2) catabolic pathways or catabolism in which molecules are broken down and energy is released (Ettinger, 2000). The chemical pathways of glucose metabolism are shown in Fig 2.1. According to this figure glucose may be converted to glycogen either by the liver or by muscles for storage as a quick energy reserve. Glucose may also be converted to fat by the liver and stored as adipose (fat) tissue (Donelly, 1996, p. 229). Glucose can also be oxidised by muscles and red blood cells to form lactate, which on entering the lever is resynthesized into glucose (Levin, 1999). The steps in these processes are very complex and controlled by various hormones.

By means of the catabolic pathways glucose is oxidised in order to release energy (Sherwood, 1997). This process is highly complex and takes place not as one explosive reaction but rather as a series of stages and steps in which many intermediate compounds are formed.

In the cells the oxidation of glucose takes place in one of two ways depending on how

much oxygen is available. As a rule the necessary amount of oxygen is present and glucose is first broken down into a three-carbon substance called pyruvic acid and two molecules of high-energy compound called ATP (adenosine triphosphate). ATP is the

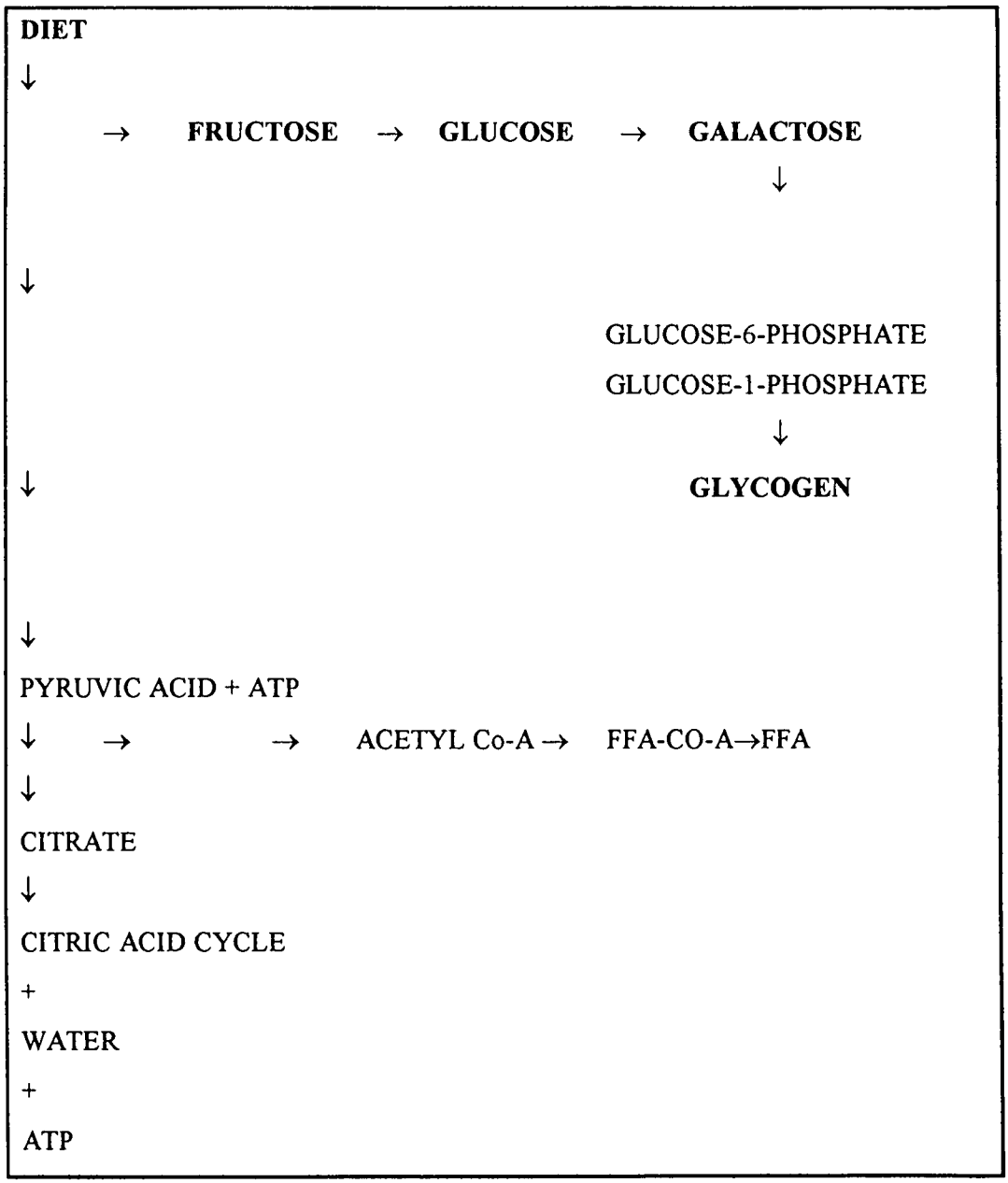

Figure 2.1: Chemical pathways of glucose metabolism (Berne *et al.*, 1998, p. 801 & Wenck *et al.*, 1983, p. 135).

most energy-rich of several high-energy phosphate compounds found in the body and is the most important substance formed when glucose is broken down. By releasing phosphorus, ATP supplies energy in almost every cell in the body where energy is needed (for example in muscles). When ATP releases energy, it becomes ADP (adenosine diphosphate) (Berne *et al.*, 1998).

After pyruvic acid and ATP are formed from glucose, the pyruvic acid is further broken down: first to the two-carbon substances called acetyl CoA (CoA stands for coenzyme a) and then to citrate. The citrate goes into a major cycle called the citric acid cycle, the Krebs cycle, or the tricarboxylic cycle in which many intermediate compounds are formed. The end result is carbon dioxide and water (Ganong, 1993, p 261). The total process from pyruvic acid to carbon dioxide and water releases 36 ATP molecules, illustrating its high-energy potential.

Approximately one quarter of glycogen stores is in the liver and about three quarters are in the muscle mass (Berne *et al.*, 1998). Liver glycogen can be made available to other tissues by the process of glycogenolysis and glucose release. Muscle glycogen can be used only by muscle, because this tissue lacks the enzyme glucose-6-phosphatase which is required for release of glucose into the bloodstream (Ganong. 1993, p. 260). Glycogen can be formed from all three major dietary sugars.

2.4.1.2 Hormonal mechanism

The major hormones controlling the glucose level are insulin, glucagon and epinephrine (adrenaline), but others such as thyroid hormone, glucocorticoids and growth hormone also play a role.

(a) Insulin

Insulin has a primary influence on glycometabolic control. It also influences many other

cellular functions (Table 2.6). Glucose has a profound effect on the secretion of insulin. The glucose level in the blood controls insulin release. High blood glucose levels (hyperglycemia) cause secretion of insulin whereas low levels inhibit it. Other compounds also increase insulin secretion namely amino acids, free fatty acids, ketone bodies, and the hormones glucagon and secretin (Levin ,1999).

Table 2.6 The metabolic effect of insulin (Levin, 1999)

Positive effects	**Negative effects**
Glucose uptake	Pyruvate → glucose
Amino acid uptake	Apoptosis
Acetyl CoA → fatty acid	Gene expression
Glucose → glycogen	
Protein synthesis	
DNA synthesis	
Na^+ K^+ pump	
Gene expression	

(b) Glucagon

Low blood sugar levels stimulate the secretion of glucagon. Glucagon acts on the hepatic cells of the liver to cause glycogenolysis and also enhances the formation of glucose from amino acids and lactate (Levin, 1999, p.58).

When plasma glucose levels increase approximately two fold, glucagon secretion is inhibited by concurrent changes in the β cell activity, rather than by the direct effects of glucose or insulin on the α cells.

(c) Epinephrine

Epinephrine favours the breakdown of liver and muscle glycogen to yield blood glucose and decreases the release of insulin from the pancreas, thus raising the blood sugar levels

(Ettinger, 2000, p. 39).

(d) Thyroid hormones

Severe lowering of blood glucose concentration increases thyroxine secretion. Hepatic glycogenolysis and gluconeogenesis are increased, leading to a rise in blood glucose concentration (Levin, 1999, p.58).

(e) Glucocorticoids

Glucocorticoids and steroid hormones influence blood glucose levels by stimulating gluconeogenesis. These hormones reduce glucose utilisation, and increase the rate at which protein is converted to glucose, thus counteracting the action of insulin (Ettinger, 2000, p. 39).

(f) Growth hormone

Growth hormone also raises the blood glucose levels by increasing amino acid uptake and protein synthesis by all cells, diminishing cellular uptake of glucose, and increasing the mobilization of fat for energy (Ettinger, 2000, p.39).

2.5 THE EFFECT OF SCFAS ON GLYCOMETABOLIC CONTROL

Many studies have shown that SCFAs have a beneficial effect on gly-cometabolic control (Anderson & Bridges, 1984).

2.5.1 The effect of SCFAs on glucose metabolism

The oxidation of fatty acids inhibits glycolysis and stimulates gluconeogenesis in muscles (Williamson, 1964). This includes acetate, which is rapidly activated to acetyl-CoA by acetate thiokinase . The intracellular concentration of citrate is increased by acetate

(Garland & Randle, 1964). Fatty acid oxidation also increases levels of intracellular acetyl Co-A and citrate (Garland & Randle, 1964). An increased citrate concentration inhibits glycolytic flux by inhibiting phosphofructokinase activity (Newsholme & Start, 1973; Anderson & Bridges, 1984). Gluconeogenesis may be stimulated by high concentration of acetyl-CoA, which inhibits pyruvate oxidation to acetyl-CoA.

Acetate in the presence of insulin may enhance the activity of the pentose phosphate pathway (Flatt & Ball, 1966). This enhancing effect of acetate and insulin provides the reducing equivalents necessary to synthesize fatty acids from acetate. A study indicated that the addition of 25g/day of lactulose, a non-absorbed sugar, for a period of two weeks, to healthy subjects is fermented to yield a high proportion of acetate (Mortensen, Holtug & Rasmussen, 1988). The results indicated a significant increase in serum triglyceride levels (Jenkins *et al.*, 1990). Thus, acetate may increase net glucose utilization by the liver (Flatt & Ball, 1966) by increasing flux through the pentose phosphate pathway.

Acetate and longer chain fatty acids have been shown to reduce glucose uptake and oxidation by isolated muscle preparation *in vitro*. However, Scheppach *et al.* (1988) found that oral acetate has no effect on glucose tolerance or glucose turnover. According to Crouse *et al.* (1968), acetate may influence glucose utilization indirectly since oral and rectal acetate promptly reduce free fatty acid levels in serum (Wolever *et al.*, 1991). Physiological increases in free fatty-acid concentrations in the serum have been shown to reduce glucose utilization in humans (Ferrannini *et al.*, 1983; Jenkins *et al.*, 1990). The infusion of SCFAs or the fermentable fibre guar gum into the caecum of healthy subjects increased glucagon levels in five of six subjects (Stephen et al., 1989), whereas glucagon increases blood glucose levels via glucose production (Lins *et al.*, 1983).

Propionate and other fatty acids with an odd number of carbon atoms are gluconeogenic substrates (Newsholme & Start, 1973). Rectal infusion of 180mmol (17.5g) sodium propionate has been shown to increase blood glucose in human subjects (Wolever *et al.*, 1991). However, Berggren *et al.* (1996) found that sodium propionate in overweight rats reduce fasting blood glucose levels and urinary glucose excretion. Dietary propionate has been shown to reduce fasting blood glucose levels and maximum serum insulin increments

(Venter *et al.*, 1990) which could be consistent with reduced glucose production or enhanced utilization. Todesco *et al.* (1991) showed that propionate reduced the rate of digestion of starch by nearly 50% which could explain the effects of oral propionate on blood glucose and insulin levels.

Table 2.7 gives a summary of studies indicating the effect of SCFAs on serum glucose. These studies also indicate that fibre decreases glucose levels, with special reference to soluble fibre and NSP.

Table 2.7 Studies indicatiing the effect of SCFAs on glucose

Reference	Effect of SCFAs on glucose
Jenkins *et al.*, 1991; Scheppach *et al.*, 1988	No evidence of lower blood glucose levels where serum acetate concentration was elevated.
Venter *et al.*, 1990	Propionate decreased fasting serum glucose and maximum insulin increments during glucose tolerance tests.
Akanji and Sacks, 1991	High acetate levels did not effect glucose utilization during dialysis.
Anderson and Bridges, 1984	In liver cells acetate inhibits glycolysis.
Thornburn *et al.*, 1993	Carbohydrate fermentation decreases hepatic glucose output in healthy subjects.
Feldman *et al.*,1995	Dietary fibre from locust bean decreased glucose and insulin levels in NIDDM subjects.

Table 2.7 (continued) **Studies indicating the effect of SCFAs on glucose**

Reference	Effect of SCFAs on glucose
Alamowitch *et al.*, 1996	SCFAs do not significantly alter glucose metabolism in healthy subjects.
Akanji *et al.*, 1990	Acetate production increased and glucose levels decreased in diabetic subjects following a high fibre diet.
Liljeberg *et al.*,1999	Porridge and bread products based on a high fibre barley genotype, favourably reduced glucose and insulin responses.
Onyechi, Judd and Ellis, 1998	African plant foods rich in non-starch polysaccharides reduce postprandial blood glucose and insulin concentrations in healthy human subjects.
Thorsdottir *et al.*, 1998	Sugar beet fibre in formula diet reduces postprandial blood glucose and serum insulin.
Fairchild *et al.*, 1996	A new breakfast cereal containing guar gum reduces postprandial plasma glucose and insulin concentrations in normal-weight human subjects.
Mcburney *et al.*, 1995	Increases in SCFA delivery to the splanchic bed do not directly affect plasma insulin or glucose levels.
Wolever *et al.*,1991	Rectal infusions of propionate showed an increase in glucose levels

However, whether acetate reduces glucose levels in humans is contradicted (Jenkins *et al.*, 1991; Alamowitch *et al.*, 1996). Propionate revealed a decrease in glucose levels (Venter *et al.*, 1990). On the other hand, a study by Wolever *et al.* (1991) showed an increase in glucose levels after rectal infusions of propionate. Furthermore, a combination of two SCFAs (acetate and propionate) given rectally, have an effect on the lipid profile, but the effect on glucose is not yet clear (Wolever *et al.*, 1991). When a combination of the three short-chain fatty acids (acetate, propionate and butyrate) were given orally, a positive effect on the lipid profile in humans was demonstrated (De Wet, 1999). However, animal studies have shown that SCFA's, associated with a high fibre intake, are not direct responsible for improving glucose metabolism (McBurny *et al.*, 1995). In humans, the effect of the three short-chain fatty acids on glucose levels was also not indicated by any studies.

2.5.2 The effect of SCFAs on insulin

Insulin is a peptide hormone that is synthesized in the B cells of the islets of Langerhans in the pancreas. Insulin is required to facilitate the transport of glucose across cell membranes. The level of insulin after a meal depends on the amount of carbohydrates in the meal, the form of the carbohydrates and the degree of insulin sensitivity (Englyst & Hudson, 2000, p.73). Anderson (1982) reported that diets high in fibre improve glucose metabolism without increasing insulin secretion. The feeding of unabsorbed sugar lactulose to healthy subjects also had no effect on serum insulin or C-peptide levels throughout the day (Jenkins *et al.*, 1990).

The effect of SCFAs was pointed out in a study by Wolever *et al.* (1991) where the rectal infusion of a large bolus of propionate (180mmol) failed to stimulate insulin in humans despite raising blood glucose levels.

Table 2.8 illustrates the relationship between dietary fibre, SCFAs and insulin. The carbohydrates such as NSP, which is found in African plant foods, improve insulin

concentrations in healthy humans (Onyechi *et al.*, 1998). The effect of propionate on insulin levels is, however, not clear. The combination of acetate and propionate, given rectally, did not improve cholesterol levels in humans (Wolever *et al.*, 1991). However, insulin increments were lowered. The effect of a combination of the three SCFAs (acetate, propionate and butyrate), given orally, on insulin levels has not been investigated. The two combinations of acetate & propionate together with the other three combinations supplement (acetate, propionate and butyrate) has also not been investigated in one study, over the same period of time.

Table2. 8 Studies indicating the relationship between dietary fibre, SCFAs and insulin

Reference	Effect of SCFAs on insulin
Leinonen *et al.*, 1999.	Rye bread decreases postprandial insulin response, but does not alter glucose response in healthy Finnish subjects.
Achour *et al.*, 1997.	Pregelatinized corn starch causes a reduction in glycaemic and insulinaemic responses in the absorptive period and in lipolysis in the postabsorptive period.
Venter *et al.*, 1990	Propionate decreased fasting serum glucose and maximum insulin increments during glucose tolerance tests.
Wolever *et al.*, 1991	Propionate alone did not affect insulin levels.
Onyechi *et al.*, 1998	African plant foods rich in non-starch polysaccharides reduced postprandial blood glucose and insulin concentrations in healthy human subjects.
Pick *et al.*, 1996	Oat bran concentrate bread products improved glycaemic and insulinemic responses in men with NIDDM.
Mcburne et al., 1995	Increases in SCFA delivery to the splanchic bed do not directly affect plasma insulin or glucose levels.

2.6 SUMMARY

Fermentation of NSP results in the production of SCFAs of which acetate, propionate and butyrate are the major components. These SCFAs could be responsible for the lowering effect of a high-fibre diet on glucose and insulin levels and therefore on glycometabolic control.

CHAPTER 3

METHODS AND TECHNIQUES

3.1 INTRODUCTION

The main objective of this study is to determine the effect of a combination of short-chain fatty acids on the glycometabolic control in men of the SANDF. To achieve this objective, the study design, selection of subjects, measurements, and statistical analysis as well as limitations of the study will be discussed in this chapter. The study was approved by the Ethics Committee of the Faculty of Health Sciences of the UFS (Ethics number: 227/98). All subjects participating in the study gave their written consent (Appendix 1).

3.2 STUDY DESIGN

The study design was that of a randomised, placebo-controlled, double-blinded clinical trial as represented in Fig 3.1.

Subjects falling within a pre-determined set of inclusion criteria (see 3.3.1 below) were included in the study. Two baseline blood samples were taken (day 0 and day 8) in order to ensure an accurate reflection of the variables and stable baseline. After baseline two subjects were randomised into three groups with 25 subjects in each group. Group 1 (placebo) received a placebo supplement and group 2 (experimental) and 3 (experimental) were supplemented with various combinations of SCFAs. The content of these supplements will be discussed in more detail later in this chapter. All subjects received equal amounts of the placebo for a period of one week prior to the intervention period of the study in order to obtain a stable baseline (day 8). Supplementation with the various combinations of short-chain fatty acids and placebo was then sustained for 4 weeks (day 8 – 36). Baseline measurements were repeated after four weeks (day 36). A washout

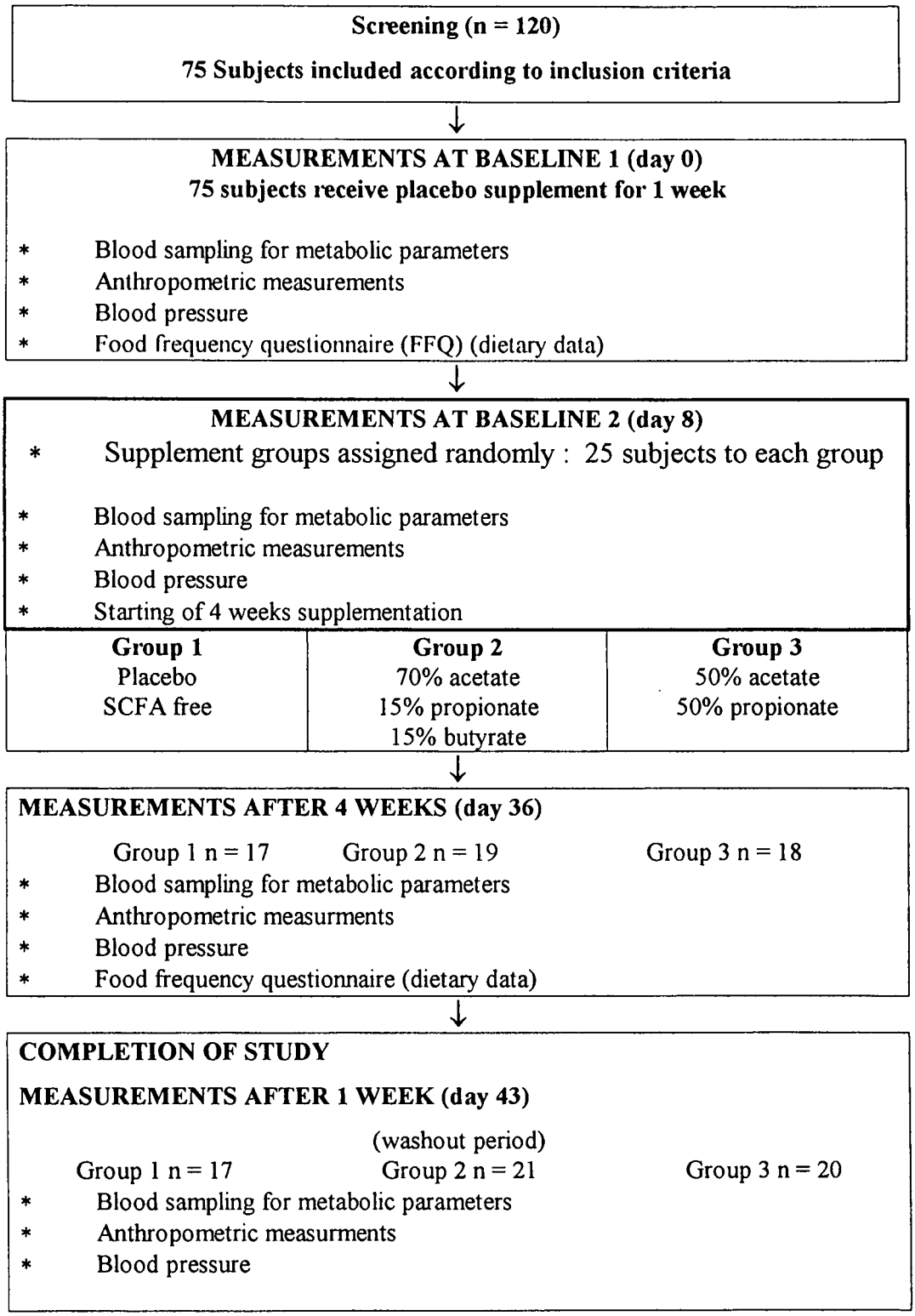

Figure 3.1 Schematic representation of the study design

period followed the intervention period, where all three groups received a placebo supplement for one week to examine any changes in variables at the end of the study. The baseline results were also repeated at the end of the study (day 43).

Blood sampling to determine metabolic parameters as well as anthropometric measurments and blood pressure were taken at baseline 1 (day 0), baseline 2 (day 8) and after the intervention period (day 36), as well as at the end of the study (day 43). Food frequency questionnaires (FFQ) were used to determine dietary intake at baseline 1 (day 0) and after the intervention period (day 36). The study was undertaken at a time specifically designed to minimise seasonal effect on human metabolism.

3.3 SUBJECTS

Approximately one hundred and twenty members of the South African National Defence Force were recruited to voluntarily participate in the study. Subjects were recruited by means of a recruitment/screening questionnaire (Appendix 2) according to the inclusion criteria.

3.3.1 Inclusion criteria

Subjects for the study were chosen according to the following inclusion criteria.

* males aged between 18 and 45
* normal blood glucose levels below 5.8mmol/L
* no alcohol abusers (< 3 drinks / day or <28g alcohol/day)
* no subjects using medication for chronic diseases
* subjects must be permanent staff of the SANDF for at least one year prior to the study

3.3.2 Screening

Screening ensures that participants meet eligibility criteria and are able to comply with the requirements of the study (Dennis & Kris-Etherton, 1991, p.155). Screening took place during an individual interview, using a standardised questionnaire (Appendix 2). The screening data also served as information to describe the subjects. A trained fieldworker was available during the interview to translate any issues, which the subjects might not have understood. Subjects between the age of 18 and 45, with blood glucose levels below 5.8mmol/L, who did not abuse alcohol or use chronic medication or had any chronic diseases and were permanent members for at least 1 year in the SANDF were included in the study.

3.3.3 Sample size

In compliance with the inclusion criteria only 75 volunteers were recruited to voluntarily participate in the study.

Twenty-five subjects were randomly allocated to one of three groups at baseline 2 (day 8) by the Department of Biostatistics, UFS.

3.4 MEASUREMENTS

Once the objectives of the study are defined, variables that may determine the outcome of the results can be determined. This is the first step in the measurement process. Another part of the measurement process is to decide how these variables should be measured in order to select or develop an applicable instrument or techniques that will best perform the task (Compton & Hall, 1972, p. 199).

3.4.1 VARIABLES

Variables defined for the aim of this study included some metabolic parameters, anthropometic measurments, blood pressure and dietary intake.

3.4.1.1 Metabolic parameters

Metabolic parameters for the purpose of this study refer to:

i. Fasting serum glucose and insulin levels as indicators of glycometabolic control (Donelly, 1996, p. 297 ; Franz, 2000, p. 743), and

ii. total serum protein, albumin, total cholesterol, triglycerides and NEFA, were measured as markers for health and nutritional status (Lindsey, 1996, p. 179, 507).

Normal ranges for these variables for black populations, according to the Department of Chemical Pathology and Haematology at the UFS, are summarised in Table 3.1:

Table 3.1 Normal ranges for metabolic indicators used in this study

Metabolic indicators	Normal range
Glucose	3.6 – 5.6 mmol/L
Insulin	5.0 - 25 µIU/mL
Total protein	64 – 84 g/L
Albumin	34 – 52 g/L
Total cholesterol	3.0 – 5.2 mmol/L
Triglycerides	0.3 – 2.0 mmol/L
NEFA	0.1 – 0.9 mmol/L

3.4.1.2 Anthropometric status

For the purpose of this study height, weight, hip- and waist circumferences were measured to determine anthropometric status. Height and weight were used to calculate body mass index (BMI). BMI gives an indication of obesity and an increased risk of develeping health problems (Earl & Borra, 2000, p. 370). The classification of BMI is presented in Table 3.2.

Table 3.2: Classification of BMI (Laquatra, 2000, p. 493).

Underweight	<18.5
Normal	18.5 – 24.9
Overweight	25.0 – 29.9
Obesity, class 1	30.0 - 34.9
Obesity, class 11	35.0 – 39.9
Obesity, class 111	≥ 40

The ratio of the waist or abdominal circumference to the hip or gluteal circumference was measured as an easy way to assess body fat distribution. The waist-to-hip rato (WHR) provides an index of regional body fat distribution, and is an valuable guide in assessing health risk (Lee & Nieman, 1996, p. 245). A WHR $\geq$1.0 in men is indicative of android obesity, and regarded as an increasing risk for obesity-related diseases.

3.4.1.3 Blood pressure

Blood pressure is a dynamic variable and was taken as an indicator of the subjects physical and emotional state at the time of the measurement (De Bono & Boon, 1991, p. 259). The classification of hypertension which refers to high arterial blood pressure, represented by raised systolic and diastolic blood pressure (Seedat *et al.*, 1993), is presented in Table 3.3.

Table 3.3 Classification of hypertension

Normal blood pressure	< 140/90 mm Hg
Borderline hypertension	140/90 mm Hg >< 160/95 mm Hg
Hypertension	> 160/95 mm Hg

3.4.1.4 Dietary intake

The dietary indicators used for this study included the energy, macro- and micronutrient intake of the subjects. The prudent diet guidelines were used as reference for macronutrient intake (Wolmarans *et al.*, 1988). The following recommended guidelines were used to interpret the macronutrient intake of the subjects:

- Total daily energy intake indicated in kilojoules according to the RDA ranges for gender and age to maintain ideal body weight (IBW)
- Total amount of energy represented by carbohydrate in the diet of more than 50%
- Total amount of energy represented by protein in the diet of less than 20 %
- Total amount of energy represented by fat in the diet of less than 30%
- Total daily intake of fibre of 20 to 30g per day

The term Dietary Reference Intake (DRI) is a collective name and refers to a set of at least four nutrient-based reference values where each of these reference values has special use (Earl and Borra, 2000, p. 333). RDA is the average daily dietary intake level that is sufficient to meet nutrient requirements of nearly all (97 to 98%) healthy individuals, a particular life stage, and gender group. When RDA cannot be determined, AI (Adequate Intake) is used. AI refers to a recommended intake value based on observed or experimentally determined approximations of nutrient intake by a group of healthy people, which is assumed to be adequate. For the purpose of this study, the RDA and/or AI were used and will be referred to as RDA/AI.

Alcohol intake refers to the type and amount of drinks consumed per day or per week.

Alcohol abusers, those taking more than 21 drinks per week or more than 28g of alcohol per day, were not included in the study, in accordance with the screening criteria (Gronbaeck & Heitmann, 1996).

3.5 TECHNIQUES

Validity describes the degree to which a dietary method measures what it purports to measure (Gibson, 1990) and reliability is defined as the degree to which a method yields the same results on two different occasions or by two different people (Lee-Han et al., 1988). In order to gather all the necessary information valid techniques were chosen to measure the specific variables.

3.5.1 Blood sampling

Biochemical information was obtained using the appropriate blood samples to determine fasting serum glucose, serum triglycerides, serum albumin, serum insulin, and total protein levels. A full blood count was also performed for each subject during each visit. The analysis was performed within the laboratory of the School of Health Technology at the Technikon Free State, using standardised procedures.

The coefficient of variation (CV) of the methods was determined for each set of measurements for control of accuracy and reproducibility of the methods. The CV for each method was calculated as:

CV = [Standard deviation]/[Mean] x 100

3.5.1.1 Blood sample preparation

The subject's blood samples were taken by a registered nurse with minimum stasis using the bulldog method into evacuated glass tubes.

a. Serum

5 mL of whole blood was left to clot at room temperature. These samples were centrifuged at 2800 x g for 20 minutes in order for the serum to separate. Samples were frozen at –72°C in Eppendorf® vials.

b. EDTA blood

5mL blood was obtained in pre-treated K_3EDTA-tubes (VAC-U-TEST). After using some of the sample for determing full blood counts, the remaining sample was centrifuged for 15 minutes at 2800 x g and stored at –72°C in Eppendorf® vials, and used for biochemical analysis (see Table 3.1).

3.5.1.2 Measurement of metabolic parameters

The methods used to measure the above-mentioned metabolic parameters will be discussed in the next section.

a. Serum glucose

Serum glucose was determined in duplicate by using an enzymatic colorimetric assay (Glucose GDO-PAP; Roche Diagnostics GmbH, Mannheim, Germany; Cat. no. 1448668) on the Roche/Hitachi 902 auto analyzer. The method is based on the oxidation of glucose to gluconolactone in the presence of atmospheric oxygen. The resultant hydrogen peroxide oxidizes 4-aminophenazone and phenol to 4-(p-benzoquinone-monoimino)-phenazone in the presence of peroxidase (POD). The colour intensity of red dye is directly proportional to the glucose concentration and can be measured photometrically. The method was calibrated against the Calibrator for Automated Systems (cat. no. 759 350, Roche Diagnostics GmbH, Mannheim, Germany). Precinorm U /normal values (cat. no. 171 735, Roche Diagnostics GmbH, Mannheim, Germany) and Precipath U /abnormal values (cat. no. 171 760, Roche Diagnostics GmbH, Mannheim, Germany) were used as control serum. The C.V. for the method was 1.41 percent.

b. Serum insulin

Serum insulin was determined in duplicate by using an enzyme immunoassay for the quantative measurement of insulin in human serum (Cat #: EIA-2935), manufactured by DRG Instruments GmbH Germany. The DRG Insulin ELISA is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. During incubation insulin in the sample reacts with biotin-conjugated anti-insulin antibodies and anti-insulin antibodies bound to microtitration well. A simple washing step removes unbound biotin labelled antibody.

During the second incubation step Streptavidin Peroxidase Enzyme Complex binds to the biotin-anti-Insulin antibody. The bound HRP complex is detected by reaction with 3.3.5.5-tetramethylbenzidine. The reaction is stopped by adding acid to the sample and a colorimetric endpoint is read spectrophotometrically. The CV for the method was 6.8 percent.

c. Serum total protein

Total protein was determined using a colorimetric assay supplied by Roche Diagnostics GmbH, Mannheim, Germany (cat. no. 1929917). Divalent copper reacts in alkaline solution with protein peptide bonds to form the characteristic purple-coloured biuret complex. The colour intensity is directly proportional to the protein concentration which can be determined photometrically. The method was calibrated against the Calibrator for Automated Systems (cat. no. 759 350, Roche Diagnostics GmbH, Mannheim, Germany). Precinorm U /normal values (cat. no. 171 735, Roche Diagnostics GmbH, Mannheim, Germany) and Precipath U /abnormal values (cat. no. 171 760, Roche Diagnostics GmbH, Mannheim, Germany) were used as control serum. The C.V. for the method was 1.5 percent.

d. Serum total albumin

Serum albumin was determined in duplicate using the Bromocresol-green (BCG) method supplied by Roche Diagnostics GmbH, Mannheim, Germany (cat. no 197 0909) on the Boehringer Mannheim Hitachi 902 chemistry analyser. Albumin complexates with bromocresol-green at a pH of 4.2. The intensity of the coloured complex is directly proportional to the albumin concentration in the sample. This complex is measured with a spectrophotometer. The method was calibrated against the Calibrator for Automated Systems (cat. no. 759 350, Roche Diagnostics GmbH, Mannheim, Germany). Precinorm U /normal values (cat. no. 171 735, Roche Diagnostics GmbH, Mannheim, Germany) and Precipath U /abnormal values (cat. no. 171 760, Roche Diagnostics GmbH, Mannheim, Germany) were used as control serum. The C.V. for the method was 2.05 percent.

e. Serum total cholesterol

Serum total cholesterol was determined using enzymatic colorimetric test. The method was performed using reagents supplied by Roche Diagnostics GmbH, Mannheim, Germany (cat. no 1491458). Cholesterol is determined enzymatically using cholesterol esterase and cholesterol oxidase. Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. Cholesterol is converted by oxygen with the aid of cholesterol oxidase to choles-4-4-one and hydrogen peroxide. Hydrogen peroxide created forms of red dyestuff by reacting with 4-aminophenazone and phenol under the catalytic action of peroxidase. The colour intensity is directly proportional to the concentration of cholesterol and can be determined photometrically. The method was calibrated against the Calibrator for Automated Systems (cat. no. 759 350, Roche Diagnostics GmbH, Mannheim, Germany). Precinorm U /normal values (cat. no. 171 735, Roche Diagnostics GmbH, Mannheim, Germany) and Precipath U /abnormal values (cat. no. 171 760, Roche Diagnostics GmbH, Mannheim, Germany) were used as control serum. The C.V. for the method was 0.33 percent.

f. HDL cholesterol (HDL-C)

Low-density lipoprotein (LDL) was precipitated qualitatively by the addition of phosphotungstic acid in the presence of magnesium ions (Randox, Crumlin, UK). HDL-C was isolated by centrifugation of the sample. The cholesterol content of the isolate was then determined using exactly the same method as described for the measurement of the TC as described above. The intensity of the produced colour was measured photometrically at 365nm. A special control was used as standard (Boehringer Mannheim-Roche Diagnostic, Mannheim, Germany). Values are expressed in mmol/L. The C.V. for the method was 1.9%.

g. %HDL-C

The %HDL-C was determined using the following calculation:

$$\%\text{HDL-C} = \frac{\text{HDL-C}}{\text{TC}} \times 100$$

h. LDL cholesterol

LDL cholesterol (mmol/L) was determined using the following calculation (Randox, Crumlin, UK):

$$\text{LDL cholesterol} = \text{total cholesterol} - \frac{\text{triglycerides}}{2.2} - \text{HDL cholesterol}$$

Values were expressed in mmol/L. The C.V. for the method was 1.9%.

i. Serum triglycerides

Serum triglyceride concentration was determined using a colometric assay supplied by

Roche, Diagnostics, Germany (cat. no. 1730711). The method is based on the work by Wahlefeld using a lipoprotein lipase from micro-organisms for the rapid and complete hydrolysis of triglycerides to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide thus produced then reacts with 4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form a red dyestuff (Trinder endpoint reaction). The method was calibrated against the Calibrator for Automated Systems (cat. no. 759 350, Roche Diagnostics GmbH, Mannheim, Germany). Precinorm U /normal values (cat. no. 171 735, Roche Diagnostics GmbH, Mannheim, Germany) and Precipath U /abnormal values (cat. no. 171 760, Roche Diagnostics GmbH, Mannheim, Germany) were used as control serum. The C.V. for the method was 1.29 percent.

j. Free fatty acids

The optimised colorimetric assay for the enzymatic determination of free fatty acids (non-esterified fatty acids, NEFA) in duplicate was used to determine free fatty acids (cat. no. FA 115, Randox Laboratories Ltd, Crumlin, United Kingdom). The method involves the formation of Acyl CoA, AMP and PPi in the presence of Acyl CoA Synthetase. The Acyl CoA is converted to 2,3,-trans-Enoyl-CoA and H_2O_2 in the presence of Acyl CoA Oxidase. The H_2O_2 is reacted with a chromogen in the presence of peroxidase, which can be measured photometrically. The intensity of the colour of the chromogen is directly proportional to the concentration of the NEFA in the sample.

3.5.2 Anthropometric measurements

Anthropometry involves obtaining physical measurements of an individual, and relating these measurements to standards that reflect, among others health and nutritional status (Lee & Nieman, 1996, p. 224; Gibson, 1998, p. 427). All the anthropometric measurements were taken by the trained researcher throughout the study.

3.5.2.1 Weight

Body weight was measured according to a standard method described by Lee and Nieman (1996, p.228) with a calibrated Seca digital electronic scale to the nearest 0.1kg. The weight of the subjects wearing light clothing and no shoes was measured before blood samples were collected prior to breakfast and after the subjects went to the bathroom. The weight was measured at the same time of day at baseline and after supplementation for four weeks.

3.5.2.2 Height

Standing height of subjects wearing light clothing and no shoes was measured to the nearest 0.5cm using a stadiometer as done by Lee and Nieman (1996, p.123). The subjects stood with their feet together, heels against the measuring board. They stood erect, neither slumped nor stretching, looking straight ahead, without tipping the head up or down. The top of the ear and outer corner of the eye were in a line parallel to the floor (the "Frankfort plane"). The top of the stadiometer was lowered to rest flat on the top of the head.

3.5.2.3 Body Mass Index (BMI)

BMI was calculated using the standard formula (weight kg/height m^2) (Pressman & Adams, 1990, p.46; Hammond, 2000, p. 370).

3.5.2.4 Waist and hip circumferences

Waist and hip circumferences were measured in duplicate, around the smallest and widest part of the waist and hips, respectively to the nearest 0.1cm. Waist circumference was measured at the most narrow area below the rib cage and above the umbilicus as viewed from the front. Hip circumference was measured at the largest diameter below the

umbilicus or maximum circumference over the buttocks taken perpendicularly on the axial line of the trunk as described by Lee and Nieman (1996, p.134).

3.5.2.5 Waist-to-hip ratio (WHR)

The waist-to-hip ratio was calculated by dividing the waist circumference by the hip circumference (Lee & Nieman, 1996, p. 245).

3.5.3 Blood pressure

Blood pressure was recorded using a sphygmomanometer and a stethoscope before blood samples were collected, according to the methods used by De Bono and Boon (1991, p.259). The subjects were seated with the back supported, the cuff was applied to the right upper arm, with the bag over the brachial artery and connected to a mercury or aneroid manometer. The stethoscope was placed over the brachial artery and the cuff was inflated to a level well above that which abolishes the Korotkov sounds. The pressure in the cuff was then allowed to drop slowly and the point of return of the sounds was taken as the systolic pressure. As the pressure dropped further the sounds became louder and then usually suddenly became muffled and later disappeared, at which stage the diastolic pressure was measured. Three intermittent readings were taken at two-minute intervals and the lowest value was recorded.

3.5.4 Questionnaires

Questionnaires used in this study included a screening questionnaire, a food frequency questionnaire and a tolerance questionnaire.

3.5.4.1 Screening questionnaire

The screening questionnaire was developed to select subjects with specific characteristics according to the purpose of the study. Questions regarding age, activity level, smoking

habits, alcohol intake and medical history were included (Appendix 2).

3.5.4.2 Food Frequency Questionnaire (FFQ)

The purpose of dietary assessment is to estimate food consumption of dietary intake in individuals or groups of people (Nelson, 2000, p. 315). Usual dietary intake is valuable in assessing nutritional status when used in combination with biochemical, anthropometric and clinical data (Lee & Nieman, 1996, p. 91; Dwyer, 1998, p. 937).

According to literature the FFQ is the most accurate method of evaluating usual dietary intake (Gibson, 1990; Dwyer, 1994, p. 847; Goldbohm *et al.*, 1995). According to Bingham *et al.* (1994) a well-designed FFQ, well administrated FFQ provides the same results as a diet history. The FFQ was therefore considered to be a suitable technique to determine the usual dietary intake of the subjects of this study (appendix 3).

A validated questionnaire used for black populations in the Free State region (Hattingh, 2000) which was adopted from the THUSA (Transition and Health during Urbanisation of South Africans) study includes the following:

- The pre-coded FFQ included a list of foods which are grouped according to similarities in type (E.g. cereals, fruits, meats, dairy products etc.).

- Additional sections added to the FFQ included: use of dietary supplements, eating pattern usually followed and the following of any special diet.

The adopted FFQ was further adapted by extending the FFQ according to the menu's, ration scale and standard portion sizes used in the mess.

The subjects were asked to estimate the usual frequency of consumption of range of foods in terms of times per month, week or day. Questions on frequency and type of fat added in cooking or at the table, the skin of the chicken and the fat of the meat were asked.

Questions on frequency of fruit and vegetables eaten and when possible whether fruit was eaten with the skin were also asked. Open-ended questions included which food were eaten more than once a month and not listed in the FFQ. Subjects could add any food eaten, but not asked previously at the end of the FFQ.

The portion sizes were estimated in house measures and converted to grams using the conversion tables in the Food Quantities Manual of the National Research Institute for Nutritional Diseases (NRIND) (Langenhoven *et al.*, 1991a). The dietary data were analysed by computer, using the NRIND Food Composition Tables (Langenhoven *et al.*, 1991b). Nutrient intakes were compared with the RDA/AI.

During the interview the trained fieldworkers concentrated on overcoming the limitations of the FFQ (Dwyer, 1994, p.847) by taking the precautions taken as indicated in Table 3.4.

3.5.4.3 Tolerance questionnaire

The tolerance questionnaire (Appendix 4) was completed after the study to determine whether any of the subjects experienced any side-effects from the intervention. Questions were asked on nausea, constipation, diarrhoea, decrease or increase in appetite and whether the amount of capsules consumed was acceptable.

3.6 SUPPLEMENTS

3.6.1 Capsules

Capsules were filled by Quatro Med, in Bethlehem, Free State. The capsules were enterically coated with a shelac-containing spray, to ensure that the capsules pass the stomach and dissolve in the large gut. The capsules were designed in such a way that they contained equal amounts of the sodium and calcium salts, in order to prevent excess intake of these ions by subjects.

Table 3.4 Limitations of the food frequency questionnaire and precautions taken to overcome the limitations

Limitations of the FFQ	Precautions to overcome the limitations
* Incomplete response may be given	* The living-in members response was monitored by the menu book used in the mess * The portion sizes used in the FFQ were confirmed with the portion sizes used in the mess according to the ration scale of the SANDF.
* Lists compiled for the general population are not useful for obtaining information on groups with different eating patterns.	* The questionnaire was designed considering the cultural eating patterns of the subjects as well as the usual menu used in the mess
* Respondent burden rises as number of food items queried increases	* The subjects were informed on how long the questionnaire would take to fill in. The questionnaire was obtained in the form of an interview. This decreased the burden on the subjects.
Foods differ in the extent to which they are over- and under-reported	* Three-dimensional food models, portion sizes, cups, plates and spoons normally used were used to estimate and confirm the portion sizes. * Open-ended questions were asked for any foods eaten at least once a week that did not appear in the questionnaire.
Translation of food groups to nutrient intake requires that many assumptions be made	* The composition of combined meals as well additions usually made to specific foods were determined beforehand and incorporated in the questionnaire.

3.6.1.1 Supplement 1

Supplement 1 contained a mixture of:

70% acetate, made up of 0.4618 g sodium and 0.2989 g calcium acetate,
15% propionate, made up of 0.0698 g sodium and 0.0677 g calcium propionate, and
15% butyrate, made up of 0.0801 g sodium and 0.0779 g calcium butyrate.

The capsules each weighed 1.0562 g. Each subject was supplied with 8 capsules per day.

3.6.1.2 Supplement 2

Supplement 2 contained a mixture of:

50% acetate, made up of 0.3299 g sodium and 0.2135 g calcium acetate,
50% propionate, made up of 0.2328 g sodium and 0.2257 calcium propionate, and
0% butyrate.

The capsules weighed 1.0019 g each. Each subject was supplied with 8 capsules per day.

3.6.1.3 Placebo

The placebo was filled with sodium chloride, calcium chloride and CMC sodium carmelel DV (S), a non-fermentable form of cellulose. The capsules weighed approximately 1.0288 g each. Each subject had to take 8 capsules per day.

3.7 FIELDWORKERS AND STANDARDISATION OF TECHNIQUES

3.7.1 Fieldworkers

The fieldworkers used in this study included:

- A qualified nurse and a small group of trained medical personnel of the SANDF who took blood samples and measured the subjects' blood pressure;
- four qualified dieticians who were standardised to use the validated FFQ and helped with the completion of a FFQ for each subject;
- a trained primary health care worker of the SANDF who helped with the translation of the questionnaires where necessary;
- anthropometric measurements were solely done by the researcher.

3.7.2 Standardisation of blood sampling

The nurse and the operational medical personnel received special training in the measurement of blood pressure and drawing of blood samples. This was necessary as some research techniques are different from those used for everyday medical purposes. The main difference was that of the quantity of blood taken on each visit. Some samples for specific metabolic parameters have special prerequisites that must be adhered to, i.e. blood tubes that should be taken without stasis, as well as some samples that should be mixed well with the contents of the blood tube in order to prevent clotting of the blood sample within the tube (citrated blood tubes, etc.). The time-limits for blood samples were also adhered to strictly (all samples were taken before 10 am).

3.7.3 Standardisation of the FFQ

The fieldworkers were trained to ask the same questions and to use the FFQ, the food

models and other dietary survey aids in the same way to obtain reliable results.

3.7.4 Standardisation of anthropometrical measurements

Anthropometric measurements were obtained by the researcher and practised beforehand to standardise the technique.

3.8 PILOT STUDY

All the techniques previously mentioned were conducted on 20 members for the pilot study. The adapted FFQ were also tested on five members by the qualified dieticians to apply previously trained methods in order to ensure that all the questions of the FFQ are clear and to identify problems with understanding the information about the FFQ. These questions were stated very clearly during the study.

3.9 MANAGEMENT OF THE STUDY

Daily management is an essential component of quality assurance (Dennis & Kris-Etherton, 1990, p. 151). A strong capable investigative team is the key to avoiding problems that prejudice the study. Good management includes: organisation, communication, clear delineation and coverage of duties and responsibilities, contingency plans, and procedures for dealing with problems (Dennis & Kris-Etherton, 1990, p. 151). The following management measures were taken to ensure that the objectives of the study were met.

3.9.1 The researcher explained the aim of the study, the study design, as well as all the practical arrangements involving the participating subjects to the subjects' superior officers. Superior officers were informed of the progress of the study, in frequent meetings held at regular intervals during the course of the study.

3.9.1.1 The subjects were informed beforehand about the practical execution of the study before the start of the screening phase.

3.9.1.2 A placebo group was included in the study to determine the effect of other factors such as seasonal changes, etc. on the measured metabolic variables.

3.9.1.3 To make sure that fasting blood samples were taken, a late breakfast was arranged at the mess after the blood samples were drawn.

3.9.2 The Department of Biostatistics at the UFS randomly divided the subjects into the experimental and control groups.

3.9.2.1 The capsules were counted beforehand by an outsider participating neither in the project nor in the execution of any aspects of the project.

3.9.2.2 This ensured that both participants and researchers were blinded for the duration of the study.

3.9.2.3 The blind information was made available once all the results were supplied to the statistician in charge.

3.9.2.4 The capsules of the supplements and the placebo looked identical.

3.9.2.5 The section head of the members ensured that the capsules were taken every day. Each subject's capsules were kept in his own bag clearly marked with his number on it.

3.9.3 It was crucial that the subjects be continuously motivated throughout the study. The following measures were taken by the researcher in order to keep the subjects motivated:

3..9.3.1 The researcher encouraged the members with each visit to take their supplements daily.

3.9.3.2 Informal social functions were arranged at regular intervals.

3.9.3.3 The subjects were followed up weekly. This helped with the evaluation of the progress of the study as well as with the participation of the subjects

and also identified any unwanted but inevitable problems.

3.9.3.4 At the end of the study each subject received a gift of acknowledgement for taking part in the study.

3.9.4 Only the researcher and the trained fieldworkers were involved in the collection of data, which ensured the reliability of the data.

3.10 STATISTICAL ANALYSIS

Results were summarised by means, standard deviations (SD), medians, minimums, maximums (numerical variables) and frequencies and percentages (categorical variables). Changes within groups from baseline one (day 0) to baseline two (day 8) and from baseline two (day 8) to the end of the intervention (day 36) were summarised by means and standard deviations, or medians and percentiles as applicable. Blood samples of the participants (n) that were available for day eight (Table 4.2) and day 36 (Table 4.4) were compared (see Table 4.5). The number of blood samples for day eight and day 36 differ because some subjects did not return on day 36 and some blood samples were too small to perform the tests. Only samples of participants that were available for both days were compared in Table 4.5 which was therefore less than indicated for the separate days; day eight (Table 4.2) and day 36 (Table 4.4). The difference in blood samples used to compare (Table 4.5) therefore explains the different results in these tables. Groups were compared by 95% confidence intervals and analysis of variance (Tukey's test) for changes in means. Within groups the student t-test was used to assess the significance of changes. The statistical analysis was performed by the Department of Biostatistics, UFS, using SAS statistical software (SAS, 1990).

3.11. LIMITATIONS OF THE STUDY

A sample of 75 subjects who met the inclusion criteria during the screening phase were initially included in the study. Only 58 subjects (Gr 1: 17 ; Gr 2: 21 ; Gr 3: 20) completed the study.

It was difficult to draw blood from three of the members as it was difficult to detect their veins. The missing values for the biochemical values could explain the difference between the number of subjects at day 36 (Gr1: 17; Gr 2: 19; Gr 3: 18) and subjects completing the study (day 43). The other missing values are ascribed to problems experienced with reagents in certain specimens used in the biochemical analysis and were excluded in the final data.

Although the subjects were motivated to take the supplements daily, it could not be completely ascertained whether the subject who ate their evening meals at home, did take the supplements.

CHAPTER 4

RESULTS

4.1 INTRODUCTION

In this chapter the results of the research are presented in tables, preceded by a short description of each. The aim is to describe the glycometabolic parameters (serum glucose and insulin levels) and background information (other metabolic parameters, anthropometric status and blood pressure and dietary intake) of the three groups from baseline to completion of the supplementation. Group one represented the placebo group, whereas group two (experimental) was supplemented with acetate, propionate and butyrate and group three (experimental) was supplemented with acetate and propionate.

Two baselines of glycometabolic parameters and background information were taken in order to obtain a more accurate reflection of these parameters. A double baseline excludes some problems associated with normal variance, especially within metabolic parameters. Baseline two (day 8) will be used as reference to indicate possible changes in the glycometabolic parameters and background information regarding other metabolic parameters, anthropometric status and blood pressure from baseline two to the end of the intervention period.

The mean differences in glycometabolic parameters and other metabolic parameters, anthropometric variables as well as blood pressure measurements between baseline two (day 8) and day 36 will be compared within each of the experimental groups, as well as the placebo group. This time period represents the period extending from baseline two to completion of the supplementation. The mean difference in dietary intake measured by FFQ was applied only during baseline 1 (day 0) and at the end of the supplementation phase (day 36). Dietary intake is less variable than other measurements. This cancelled the need for a repeat measurement on day 8. A wash-out period (day 36 to 43) during which all subjects consumed placebo-containing supplements was implemented to evaluate metabolic parameters after one week.

4.2 BASELINE RESULTS

The characteristics of the subjects and the two baseline results regarding the metabolic indicators, anthropometric status, blood pressure and dietary intake will be described.

4.2.1 Characteristics of the study group

According to the inclusion criteria, the subjects were members of the SANDF Tempe base in Bloemfontein for at least one year and were male, between the ages of 18 and 45 years and with an average of 27 years. The subjects did not use any chronic medication, had no history of previous diseases such as coronary heart disease, diabetes, etc. and none had physical disabilities. Subjects with physical disabilities were excluded from the study.

4.2.2 Metabolic indicators

The baseline results of the glycometabolic as well as other metabolic parameters for baseline one and two are summarised in Tables 4.1 and 4.2, respectively.

Both baseline mean fasting serum glucose concentrations fell within the normal range for healthy adult males (3.6 – 5.6mmol/L). However, group one, baseline one, had a baseline mean fasting serum glucose concentration of 5.7mmol/L $\pm$1.2, which is just above normal. Groups two and three also had a serum glucose concentration in the higher normal range (5.1 – 5.4mmol/L). Both baseline mean serum insulin concentrations (6.7 – 11.95µIU) fell within the normal range for healthy adult males (5.0 – 25µIU/mL).

Other metabolic indicators of the subject group also fell within normal range of the population (serum albumin: 34 – 52g/L; serum total protein: 64 – 84g/L; serum triglycerides: 0.3 – 2.0mmol/L and NEFA: 0.1 – 0.9mmol/L). The variance between the measured metabolic indicators between baseline one and two was very small (no significant differences were measured between day zero and day eight within any of the

Table 4.1 Metabolic indicators, anthropometry and blood pressure at baseline one (day 0)

	Normal range	GROUP 1 (Placebo)						GROUP 2 (acetate, propionate & butyrate)						GROUP 3 (acetate & propionate)					
		N	MEAN	SD	MIN	MED	MAX	N	MEAN	SD	MIN	MED	MAX	N	MEAN	SD	MIN	MED	MAX
Metabolic indicators																			
Glucose(mmol/L)	3.6 - 5.6[a]	16	5.1	0.6	4.0	5.0	6.5	20	5.4	1.1	2.9	5.4	7.4	18	5.4	0.6	4.4	5.4	6.4
Insulin (µIU/mL)	5.0 - 25[e]	16	8.3	8.9	1.0	5.8	32.0	15	6.7	3.6	1.0	6.4	12.0	17	7.9	4.6	0.5	9.0	14.0
Total protein (g/L)	64 - 84[a]	16	76.9	4.7	71.0	76.5	88.0	20	81.9	6.5	73.0	83.0	93.0	18	82.1	6.3	69.0	82.5	92.0
Albumin (g/L)	34 - 52[a]	16	43.9	2.1	39.8	43.5	47.6	20	46.8	4.7	41.2	45.8	63.7	18	44.8	3.2	37.7	44.8	50.4
Total cholesterol (mmol/L)	3.0 - 5.2[b]	16	4.2	1.0	2.7	4.1	6.2	20	4.5	0.7	2.9	4.4	5.5	18	4.4	1.0	2.3	4.2	6.3
HDL-C(mmol/L)	0.9 - 1.6[b]	16	1.3	0.2	0.8	1.3	1.7	18	1.6	0.6	0.9	1.4	3.6	17	1.4	0.4	0.7	1.4	2.4
%HDL-C* (%)	>20%	16	32.2	6.3	25.5	30.2	46.9	18	34.9	11.9	16.4	32.6	65.5	17	33.6	9.4	17.5	36.6	50.0
LDL-C(mmol/L)	2.0 - 3.4[b]	15	2.2	0.7	1.0	2.2	3.6	18	2.4	0.9	1.0	2.5	3.9	17	2.4	0.9	0.8	2.3	4.5
Triglycerides (mmol/L)	0.3 - 2.0[b]	16	1.3	0.4	0.7	1.3	2.2	19	1.0	0.4	0.6	1.0	1.8	18	1.1	0.4	0.5	0.9	2.0
NEFA(mmol/L)	0.1 - 0.9[b]	16	0.3	0.2	0.2	0.4	0.9	19	0.4	0.2	0.1	0.3	0.8	18	0.5	0.2	0.2	0.5	0.9
Anthropometry																			
Weight(kg)		17	68.6	10.9	56.1	65.6	87.5	19	65.0	7.4	53.4	65.8	78.0	20	65.7	8.4	51.4	65.1	78.7
BMI(kg/m²)	18.5 - 24.9[c]	17	23.2	2.9	18.4	22.0	28.1	19	22.5	1.7	19.2	22.3	27.1	20	22.7	2.3	17.3	22.5	29.0
Waist(cm)		17	78.9	8.6	66.0	78.0	94.0	19	75.5	5.2	66.0	76.0	85.0	20	77.5	6.6	67.0	77.0	91.0
Hip(cm)		17	95.9	8.8	74.0	96.0	110.0	19	95.5	4.2	89.0	97.0	102.0	20	95.0	4.3	85.0	96.0	102.0
WHR	< 1.0[d]	17	0.8	0.1	0.7	0.8	1.3	19	0.8	0.0	0.7	0.8	0.9	20	0.8	0.0	0.7	0.8	0.9
Blood Pressure																			
SBP(mmHg)	< 160e	17	119	15	90	120	160	19	120	10	110	120	140	20	115	7	100	117	130
DBP (mmHg)	< 90e	17	85	15	60	80	130	19	81	9	70	80	100	20	81	8	70	80	100

a Haematology Department, UFS, 2002
b Institute for Pathology, University of Pretoria, 1999
c Laquatra, 2000
d Hammond, 2000
e Steyn *et al*., 1991

Table 4.2 Metabolic indicators, anthropometry and blood pressure at baseline two (day8) and difference between the two baselines

	Normal range	GROUP 1 (Placebo)						GROUP 2 (acetate, propionate & butyrate)						GROUP 3 (acetate & propionate)						
		N	MEAN	SD	MIN	MED	MAX	P-value between baselines	N	MEAN	SD	MIN	MED	MAX	P-value between baselines	N	MEAN	SD	MIN	MED	MAX	P-value between baselines
Metabolic indicators																						
Glucose(mmol/L)	3.6 - 5.6[a]	13	5.7	1.2	3.7	5.4	7.8	0.08	19	5.4	0.5	4.7	5.3	6.5	0.75	18	5.4	1.0	3.8	5.2	7.8	0 51
Insulin (µIU/mL)	5.0 - 25[a]	10	10.1	15.0	0.5	5.5	50.0	0.72	18	12.0	15.2	0.5	7.4	64.0	0 34	16	7.7	7.0	0.5	7 9	24.5	0.51
Total protein (g/L)	64 - 84[a]	13	79.8	3.4	75.0	80.0	85.0	0.06	19	81.1	6.2	70.0	80.0	94.0	0.77	18	78.9	6.1	69.0	77.0	91.0	0.05
Albumin (g/L)	34 - 52[a]	13	45.1	2.8	38.9	46.4	48.1	0.06	19	45.3	4.4	41.7	44.9	51.0	0 27	18	43.9	2.5	39.8	43.0	49.1	0.05
Total cholesterol (mmol/L)	3.0 - 5.2[b]	13	4.4	0.7	3.2	4.4	5.4	0.47	19	4.8	0.8	3.8	4.8	7.2	0 09	18	4.1	0.8	2.6	3.9	64.0	0.14
HDL-C (mmol/L)	0.9 - 1.6[b]	10	1.2	0.2	0.9	1.2	1.6	0.44	19	1.3	0.3	0.8	1.2	1.9	0.16	15	1.3	0.3	0.7	1.3	2.0	0 05
%HDL-C(%)	>20%	10	28.3	8.0	17.0	28.3	47.1	0.72	18	26.3	6.5	14.8	25.8	45.2	0.05	15	31.3	7.3	20.6	33.3	46.5	0.17
LDL-C(mmol/L)	2.0 - 3.4[b]	10	2.6	0.8	1.2	2.5	3.6	0.43	18	3.1	0.8	1.9	3.0	5.3	0.05	15	2.3	0.6	1.2	2.2	3.3	0.63
Triglycerides (mmol/L)	0.3 - 2.0[b]	13	1.2	0.7	0.5	1.2	2.7	0.51	19	1.2	0.5	0.6	1.0	2.7	0.48	18	1.0	0.4	0.6	0.9	2.3	0.94
NEFA(mmol/L)	0.1 - 0.9[b]	13	0.4	0.2	0.2	0.3	0.7	0.08	20	0.4	0.2	0.1	0.3	0.9	0.06	17	0.5	0.2	0.2	0.4	0.9	0.01
Anthropometry																						
Weight(kg)		16	69.6	10.5	56.4	68.0	86.9	0.47	20	66.1	8.2	3.5	67.0	83.7	0.01	20	66.3	8.5	52.0	66.0	80.0	0.06
BMI(kg/m²)	18.5 - 24.9[c]	16	23.4	2.8	18.4	22.8	27.6	0.48	20	22.9	2.1	19.2	23.0	27.8	0.00	20	22.9	2.3	18.0	23.0	29.5	0.07
Waist(cm)		16	80.2	8.7	66.0	79.0	94.0	0.06	20	76.4	5.1	69.0	75.0	87.0	0.19	20	77.0	7.1	66.0	75.0	90.0	0.24
Hip(cm)		16	96.9	9.2	74.0	98.0	110.0	0.01	20	96.5	5.1	85.0	98.0	102.0	0.22	20	94.4	4.0	87.0	94.5	102.0	0.25
WHR	< 1.0[d]	16	0.8	0.1	0.7	0.8	1.2	0.87	20	0.8	0.0	0.7	0.8	0.9	0.92	20	0.8	0.0	0.7	0.8	0.9	0.88
Blood Pressure																						
SBP(mmHg)	< 160[e]	16	124	15	110	120	162	0	20	116	12	91	113	145	0	20	115	8	100	120	130	1
DBP (mmHg)	< 90[e]	16	87	16	70	81	130	1	20	81	9	70	80	110	1	20	81	8	60	80	90	1

a Haematology Department, UFS, 2002
b Institute for Pathology, Universit
c Laquatra, 2000
d Hammond, 2000
e Steyn *et al*., 1991

variables).

At baseline one and two the mean serum total cholesterol (TC) concentrations fell within the moderate risk category for adult males (4.10 – 5.50mmol/L). However, the lipid profiles of this group of subjects were very interesting. The HDL-C levels were relatively high compared to the TC levels (in westernised societies it is recommended that the fraction of HDL-C expressed as a percentage of TC should fall above 20% or should be more than 35mg/dL (1.45mmol/L) (Warnick *et al.*, 1996, p. 329). This group of subjects had a high %HDL-C ratio (28.3 – 32.3%). However, in contrast to these high HDL-C levels, LDL-C levels were also raised when expressed as percentage of TC (making up 54% of the TC). Consequently, lipid profiles of the subject group seem healthy. However, the concomitant higher normal LDL-C levels could place these subjects at high risk for development of cardiovascular conditions. There was no major difference in any of the measured lipid variables between the various subject groups.

4.2.3 Anthropometric status

Tables 4.1 and 4.2 give the baseline one and two results of the anthropometric parameters.

BMI of all three groups at baseline one and two were in the normal range for healthy adult males (18.5 – 24.9kg/m^2). All three groups revealed a slight tendency towards an increase in weight from baseline one. The difference in weight between baseline one and two was only statistically significant in group two ($p = 0.01$), however this difference was not clinically significant. The mean height of the subjects in the three groups at baseline one was more or less identical.

The WHR for all three groups from baseline one to baseline two was identical and fell within the normal range for healthy adult males (<1.0). The tendency towards an increase in the mean waist circumference observed in groups one and two from baseline one to baseline two were not statistically significant. A tendency towards a slight increase in mean hip circumference was observed from baseline one to baseline two for groups one

and two. The difference in group one was statistically significant ($p = 0.01$), however not clinically significant.

4.2.4 Blood pressure

The blood pressure of the subjects in all the three groups fell within the normal range (<140/90mmHg) at baseline one (Table 4.1) and baseline two (Table 4.2).

4.2.5 Dietary intake

The dietary intake of the members was determined by means of a FFQ at the start of the study at baseline one (day 0) and after the intervention period of four weeks of supplementation (day 36) to determine whether any changes in the dietary intake of the subjects occurred during the study period.

The majority of the members in group one (65%) ate their meals at the dining facilities of the SANDF, whereas members in group two (57%) and group 3 (65%) ate all meals at home. Members in group one indicated that they ate three meals a day, whereas members in groups two and three only ate two meals daily. The consumption of two meals a day is characteristic of the traditional eating pattern of the black population in South Africa (Bruwer, 1963). Two members followed a low salt diet, where salt intake was reduced in their normal diet. The mean energy, macronutrient and micronutrient intakes of the three groups, obtained from the FFQ at baseline one, will be described and compared to the prudent guidelines and with the RDA/AI.

4.2.5.1 Macronutrient intakes

The mean energy intakes for the three groups compared well, but were above recommended mean intake for the specific age group (12180kJ) (Table 4.3). The total mean carbohydrate intake was below the prudent guidelines of 50% of total energy intake

Table 4.3 Dietary intake at baseline one (day0) (FFQ1)

	Prudent guidelines	GROUP 1 (PLACEBO)						GROUP 2 (acetate, propionate, butyrate)						GROUP 3 (acetate, & propionate)					
	/ RDA	N	Mean	SD	Min	Med	Max	N	Mean	SD	Min	Med	Max	N	Mean	SD	Min	Med	Max
Macronutrients																			
Total kilojoule (kJ)	12180.0	17	16873.6	8124.1	2573.6	16682.4	35323.3	21	17013.0	6445.6	6383.6	15669.5	31029.2	20	16229.2	5894.2	8020.8	15577.6	28081.6
Total carbohydrates (g)		17	492.6	267.2	91.6	518.1	1209.8	21	468.5	173.1	196.2	447.9	965.3	20	414.2	115.9	229.3	397.7	662.4
Total carbohydrates (%TE)	>50%TE	17	49.4	8.0	35.0	49.0	66.0	21	47.7	7.3	34.0	50.0	59.0	20	46.1	11.7	29.0	43.5	65.0
Added sugar (g)		17	125.7	68.1	17.2	116.4	298.7	21	129.1	44.4	56.6	135.4	205.7	20	127.4	63.6	28.6	124.3	256.4
Added sugar (%E)	<15%TE	17	13.4	5.5	5.0	14.0	23.0	21	13.9	4.6	6.0	13.0	24.0	20	14.9	9.9	4.0	12.5	45.0
Total protein (g)		17	144.5	69.2	26.2	143.3	341.6	21	148.5	61.4	56.0	139.6	282..69	20	155.2	85.2	50.8	134.6	374.9
Total protein (%E))	12 - 20%TE	17	15.0	2.2	10.0	15.0	18.0	21	14.9	3.2	10.0	15.0	25.0	20	15.6	4.7	9.0	14.5	25.0
Total fat (g)		17	145.2	77.7	10.2	142.1	377.7	21	156.0	77.0	50.2	140.5	357.6	20	156.3	88.6	38.1	135.5	383.4
Total fat (%E)	<30%TE	17	32.3	7.2	15.0	31.0	44.0	21	33.8	5.7	23.0	34.0	44.0	20	34.6	9.3	18.0	35.5	52.0
Total dietary fibre (g)	20 - 30	17	37.5	19.6	11.4	34.1	90.7	21	34.7	14.5	14.0	30.5	63.3	20	31.7	13.2	12.6	31.8	59.5
Total NSP(g)		17	16.7	12.9	1.1	13.2	45.0	21	17.9	7.3	4.1	18.7	30.7	20	16.2	10.2	6.2	10.3	43.4
Alcohol (g)	<28g	17	1.1	2.2	0.0	0.2	7.6	21	5.5	7.8	0.0	3.5	30.9	20	7.8	14.4	0.0	1.7	57.9
Micronutrients	RDA/AI*																		
Vitamin A(µg RE)[a]	900.0	17	1862.2	1222.3	60.8	1706.3	4264.7	21	2166.8	1951.9	427.9	1641.6	8840.4	20	1195.1	545.1	277.0	1313.6	2265.1
Vitamin C (mg)	90.0	17	320.9	582.9	10.4	102.9	2432.0	21	189.7	131.9	44.9	172.9	477.0	20	157.1	137.1	40.7	98.5	584.9
Vitamin D (µg)	5.0	17	8.9	6.0	0.2	7.8	26.8	21	10.2	6.9	2.4	7.4	27.2	20	11.4	12.4	1.8	8.1	57.9
Tiamine (mg)	1.2	17	2.1	1.0	0.4	2.3	4.6	21	2.4	1.2	0.8	2.2	5.6	20	2.1	0.9	0.5	2.0	4.0
Riboflavin (mg)	1.3	17	2.9	1.4	0.2	2.0	5.7	21	2.9	1.4	1.1	2.5	5.1	20	3.2	2.0	0.7	3.1	8.1
Niacin (mg)	16.0	17	34.3	17.0	8.2	34.2	85.7	21	36.7	16.1	12.4	35.8	67.9	20	37.5	19.6	14.3	35.5	89.5
Vitamin B6 (mg)	1.3	17	2.6	1.2	0.6	2.6	5.2	21	3.1	1.4	1.0	2.6	6.4	20	2.7	1.3	1.0	2.5	6.6
Vitamin B12 (µg	2.4	17	9.1	4.9	0.6	8.1	18.7	21	8.1	4.9	2.4	6.6	21.2	20	9.8	7.8	0.8	8.2	28.8
Calcium (mg)	1000*	17	1273.1	551.9	134.0	1197.8	2413.7	21	1227.8	617.9	494.7	1088.8	2707.9	20	1020.0	548.0	191.2	936.2	1759.6
Magnesium (mg)	400.0	17	483.1	269.2	116.0	496.0	1345.0	21	468.4	193.5	225.4	436.7	884.8	20	475.8	174.5	228.0	464.7	776.6
Phosphorus(mg)	700.0	17	2111.2	949.7	368.9	2026.6	4704.7	21	2142.4	879.1	874.2	1970.3	4134.4	20	2090.4	939.0	611.5	1973.5	3795.9
Iron (mg)	8.0	17	25.6	16.7	3.5	22.6	70.9	21	23.4	10.9	6.3	21.6	45.5	20	22.0	10.7	7.1	18.7	43.7

RDA Recommended Daily Allowances

AI Adequate intake

%TE Values espressed as percentage of total energy intake

a RE: Retinol equivalents: 1 retinol equavalent = 1µg retinol

and more or less similar in the three groups. The reported added sugar intake was similar for all three groups and within the recommended 15% of total energy intake. The mean fibre intake of all three groups was somewhat similar, but higher (34.6 – 36.3g) than the recommended 20 to 30 g per day. The total mean fat intake of the three groups was also similar, but higher than the recommended intake of less than 30% of total energy. Dietary protein intake compared well between the three groups and contributed to approximately 15% of total energy intake, which fell within the recommended prudent guideline of 12 to 20%. A tendency was observed towards a high fat (>30%TE) and low carbohydrate intake (<50%TE), which is characteristic of an atherogenic westernised diet.

The alcohol intake of all three groups fell within the recommended intake of less than 28g alcohol per day. This could be expected as to the inclusion criteria, excluded subjects who consumed more than 21 drinks per week or above 28g alcohol per day.

4.2.5.2 Micronutrient intakes

The micronutrient intakes compared well between the three groups (Table 4.3) and tended to meet the RDA/AI.

4.3 INTERVENTION RESULTS

To indicate the intervention results the mean difference between day 8 (baseline 2) and day 36 (end of four week supplementation) will be compared. Table 4.4 and Table 4.8 give the results of day 36 and day 43 of the wash-out period respectively. These will not be discussed. As mentioned in chapter three (p.54) only blood samples that were available on day eight and day 36 were compared (Table 4.5) which explains the difference in the data.

4.3.1 Metabolic indicators

Table 4.5 gives the mean difference between the metabolic parameters from baseline two

Table 4.4 Metabolic indicators, anthropometry and blood pressure at the end of intervention (day 36)

	Normal range	GROUP 1 (Placebo)						GROUP 2 (acetate, propionate & butyrate)						GROUP 3 (acetate & propionate)					
		N	MEAN	SD	MIN	MED	MAX	N	MEAN	SD	MIN	MED	MAX	N	MEAN	SD	MIN	MED	MAX
Metabolic indicators																			
Glucose(mmol/L)	3.6 - 5.6[a]	17	5.2	1.2	2.3	5.1	6.9	19	5.2	0.9	4.1	5.2	6.9	18	5.3	0.4	4.6	5.3	6.5
Insulin (µIU/mL)	5.0 - 25[a]	15	9.1	15.3	0.5	7.4	62.5	17	14.2	18.9	1.0	6.4	72.0	15	6.6	6.2	0.5	5.6	26 0
Total protein (g/L)	64 - 84[a]	17	77.0	4.4	71.0	77.0	86.0	19	75.9	8.4	55.0	75.0	91.0	18	77.8	9.5	64.0	78.0	96.0
Albumin (g/L)	34 - 52[a]	17	43.9	2.3	37.8	43.8	48.0	19	43.8	3.1	39.0	43.6	49.2	18	44.5	3.3	38.7	44.9	51.8
Total cholesterol (mmol/L)	3.0 - 5.2[b]	17	4.3	0.5	3.5	4.3	54.0	19	4.6	0.8	2.7	4.6	6.0	18	4.3	0.8	3.2	4.5	5.7
HDL-C(mmol/L)	0.9 - 1.6[b]	17	1.3	0.2	1.0	1.3	1.7	19	1.5	0.7	0.8	1.2	3.3	18	1.3	0.3	0.9	1.4	2.1
%HDL-C(%)	>20%	17	30.5	6.1	21.7	30.2	42.1	19	32.3	12.4	18.0	30.8	66.0	18	30.6	7.2	20.0	30.8	41.2
LDL-C(mmol/L)	2.0 - 3.4[b]	17	2.4	0.5	1.1	2.5	3.0	17	2.6	0.9	1.2	2.4	4.5	18	2.5	0.7	1.3	2.8	3.8
Triglycerides (mmol/L)	0.3 - 2.0[b]	17	1.3	0.4	0.6	1.2	2.1	19	1.3	156.0	0.2	1.3	2.2	18	1.1	0.4	0.6	1.0	2.0
NEFA(mmol/L)	0.1 - 0.9[b]	17	0.5	0.2	0.1	0.3	0.9	19	0.2	0.2	0.1	0.3	0.8	18	0.4	0.3	0.1	0.3	1.5
Anthropometry																			
Weight(kg)		17	69.6	102.5	57.8	66.5	92.2	20	65.7	7.8	54.2	63.0	81.9	20	66.5	8.8	52.3	74.7	80.5
BMI(kg/m²)	18.5 - 24.9[c]	17	23.6	2.7	19.1	22.5	28.8	20	22.8	2.1	19.5	22.7	27.8	20	22.9	2.3	18.8	22.6	29.7
Waist(cm)		17	78.4	8.1	68.0	77.0	95.0	20	76.5	4.9	69.0	75.5	86.0	20	76.4	7.2	63.0	76.0	91.0
Hip(cm)		17	97.5	6.5	86.0	96.0	108.0	20	96.4	4.7	87.0	97.0	102.0	20	94.4	4.3	86.0	95.5	101.0
WHR	< 1.0[d]	17	0.8	0.0	0.7	0.8	0.9	20	0.8	0.0	0.7	0.8	0.9	20	0.8	0.0	0.7	0.8	0.9
Blood Pressure																			
SBP(mmHg)	< 160[e]	17	116	11	95	120	130	20	116	8	100	120	130	20	116	8	100	115	130
DBP (mmHg)	< 90[e]	17	83	10	70	80	110	20	79	8	60	80	90	20	82	6	70	80	90

a Haematology Department, UFS, 2002
b Institute for Pathology, University of Pretoria, 1999
c Laquatra, 2000
d Hammond, 2000
e Steyn *et al*., 1991

Table 4.5 Mean difference between baseline two(day 8) and end of intervention (day 36)

	GROUP 1 (Placebo)							GROUP 2 (acetate, propionate & butyrate)							GROUP 3 (acetate & propionate)							
	N	MEAN	SD	MIN	MED	MAX	P-value in group	N	MEAN	SD	MIN	MED	MAX	P- value in group	N	MEAN	SD	MIN	MED	MAX	P-value in group	P-value between groups
Metabolic Indicators																						
Glucose(mmol/L)	13	-0.27	0.80	-0.92	-0.44	1.48	0.23	17	-0.21	0.89	-2.18	-0.16	1.70	0.32	16	-0.19	0.87	-2.25	-0.24	1.46	0.40	0.96
Insulin (µIU/mL)	9	0.68	8.40	-17.00	0.80	12.50	0.81	15	-0.11	9.80	-20.00	-2.20	24.80	0.96	14	0.81	7.34	-13.00	0.75	18.80	0.69	0.95
Total protein (g/L)	13	-2.67	5.18	-12.00	-3.00	6.00	0.07	17	-6.70	6.90	-23.00	-5.00	2.00	0.00	16	-0.75	8.99	-22.00	-0.50	18.00	0.74	0.07
Albumin (g/L)	13	-1.06	2.74	-6.30	-0.80	3.20	0.18	17	-1.90	3.05	-7.90	-1.60	5.60	0.02	16	0.83	4.04	-8.50	0.65	10.10	0.43	0.07
Total cholesterol (mmol/L)	13	-0.10	0.58	-1.70	0.10	0.40	0.52	17	-0.37	0.88	-2.20	-0.40	2.10	0.10	16	0.16	0.55	-0.70	0.00	1.30	0.55	0.10
HDL-C (mmol/L)	10	0.07	0.11	-0.10	0.10	0.20	0.06	17	-0.02	0.29	-1.10	0.00	0.20	0.68	13	0.09	0.27	-0.60	0.10	0.50	0.25	0.40
%HDL-C(%)	10	1.76	4.60	-5.55	2.16	10.21	0.25	16	2.70	9.20	-26.63	3.21	19.19	0.25	13	0.80	5.24	-13.33	1.75	9.17	0.59	0.77
LDL-C(mmol/L)	10	-0.19	0.60	-1.58	-0.05	0.64	0.33	16	-0.57	0.55	-1.75	-0.45	0.58	0.0008	13	0.01	0 44	-0.52	-0.13	0.84	0.91	0.02
Triglycerides (mmol/L)	13	0.06	0.63	-1.41	0.18	1.02	0.70	17	0.13	0.62	-1.14	0.18	1.36	0.38	16	0.15	0.44	-0.58	0.08	1.15	0.17	0.91
NEFA(mmol/L)	13	-0.47	1.27	-0.23	-0.60	1.80	1.59	18	-0.68	1.78	-5.20	-0.45	3.00	-0.12	15	0.38	2.73	-3.90	-0.40	6.20	0.59	-0.88
Anthropometry																						
Weight(kg)	16	0.61	1.70	-1.90	0.34	5.30	0.17	20	-0.41	2.12	-6.20	0.00	2.80	0.41	20	0.14	1.76	-3.60	0.00	3.20	0.73	0.28
BMI(kg/m²)	16	0.20	0.56	0.64	0.11	1.65	0.15	20	-0.12	0.72	-2.04	0.00	0.96	0.45	20	0.03	0.61	-1.28	0.00	1.20	0.79	0.31
Waist(cm)	16	-1.43	6.90	-22.00	0.00	10.00	0.42	20	0.10	3.53	-7.00	0.50	5.00	0.90	20	-0.55	4.54	-14.00	-0.50	8.00	0.59	0.67
Hip(cm)	16	0.87	5.30	-3.00	-1.00	19.00	0.52	20	-0.07	3.27	-4.00	-1.00	12.00	0.91	20	0.03	4.22	-12.00	0.00	7.00	0.98	0.77
WHR	16	-0.03	0.14	-0.50	0.02	0.10	0.39	20	0.00	0.04	-0.07	0.00	0.06	0.91	20	-0.01	0.03	-0.04	-0.01	0.04	0.35	0.47
Blood Pressure																						
SBP(mmHg)	16	-8.58	15.00	-40.00	-10.00	15.00	0.04	20	0.45	14.35	-25.00	0.00	39.00	0.89	20	1 05	8.73	-10.00	0.00	18.00	0.59	0.06
DBP (mmHg)	16	-4.12	16.50	-50.00	-3.50	20.00	0.33	20	-2.25	13.32	-30 00	0.00	20.00	0.45	20	1.00	11 19	-20.00	0.00	30.00	0.69	0.52

(D8) to the end of supplementation (D36).

The mean fasting serum glucose concentrations indicated a tendency towards a decrease for groups two and three (D8 – D36). The observed differences within each group (D8 - D36) were not statistically significant. The mean differences between the three groups were also not statistically significant ($p = 0.96$).

The mean serum insulin concentrations indicated a slight tendency towards a increase in groups one and three, whereas a slight tendency towards an decrease was observed in group two ($p=0.96$). The differences between the groups were also not statistically significant ($p = 0.95$).

The mean total protein concentrations indicated a statistically significant decrease (D8 – D36) for group two ($p = 0.00$). The difference between the three groups was however, statistically not significant ($p = 0.07$).

The mean serum albumin concentrations indicated a decrease (D8 - D36) in groups one (-1.06) and two (-1.90), whereas a tendency towards an increase was observed in group three (0.83). The decrease in group two was statistically significant ($p = 0.02$), while the differences between the groups were not statistically significant ($p = .07$).

A tendency towards an increase in mean total serum cholesterol concentrations was observed in group three (D8 – D36). In groups one and two a slight decrease was observed. These differences were however, not statistically significant. The mean differences between the groups were also not statistically significant ($p = 0.10$). A slight tendency was observed towards an increase in HDL-C in group one and a decrease in LDL-C in groups one and two (D8 – D36). The differences were not statistically significant. Except the decrease in LDL-C levels in group two (D36) ($p = 0.0008$). The differences between the groups were also not statistically significant.

The mean serum triglycerides concentrations increased slightly in all three groups (D8 –

D36). The mean differences observed in the groups and between the groups were not statistically significant (p=0.91).

A slight decrease in NEFA was observed in groups one and two (D8 – D36), but there was no statistically significant difference. The mean differences between the groups were not statistically significant.

4.3.2 Anthropometric status

Table 4.5 gives the mean difference between anthropometry from baseline two to the end of supplementation.

The mean BMI in all three groups remained somewhat similar (D8 – D36). The tendency towards a decrease in group two and an increase in group one, as well as the differences between the three groups were not statistically significant. The slight decrease in mean weight observed in group two and the slight increase observed in group three, were not statistically significant. The mean difference between the groups was not statistically significant ($p = 0.28$).

The mean WHR (D8 – D36) remained unchanged in all three groups. The mean difference between the groups was not statistically significant ($p= 0.47$). A tendency towards an increase in waist circumference in group two and a decrease in waist circumference in groups one and three (D8 – D36) was statistically not significant. The mean difference between the groups was not statistically significant ($p = 0.67$). The slight increase in mean hip circumference in group one and a slight decrease in mean hip circumference in group two (D8 – D36), as well as the mean differences between the groups were not statistically significant ($p = 0.77$).

4.3.3 Blood pressure

The blood pressure fell within the normal ranges (<140/90mmHg) for all three groups at the completion of supplementation (D36). However, a statistically significant decrease in

systolic blood pressure (SBP) was observed in group two ($p = 0.04$) after supplementation. The mean difference in SBP between the groups however, was not statistically significant.

4.3.4 Dietary intake

The mean dietary intake at day 36 (end of supplementation) is presented in Table 4.6, whereas the mean difference in dietary intake from baseline one (D0) to the end of intervention (D36) is presented in Table 4.7 and will now be described.

4.3.4.1 Macronutrient intake

The mean energy intake did not alter (D0 – D36) and remained higher than the recommended mean intake for the specific age group (12180kJ) (Table 4.6). A slight decrease in mean carbohydrate intake was observed in all three groups at day 36 and the mean carbohydrate intake remained lower than the prudent guidelines of more than 50% of TE. The amount of added sugar intake reported decreased slightly in all three groups after supplementation (D36), and remained under the recommended intake of less than 15% of TE. The mean fibre intake also decreased after supplementation (D36) in all three groups, but was still above the recommended intake (20 – 30g/day). All these differences were, however, not statistically significant.

A tendency towards a slight increase in the mean total fat intake was observed in group one and two, whereas a tendency towards a slight decrease was observed after the intervention period (D36) in group three. The mean total protein intake indicated a slight tendency towards an increase in all the three groups after supplementation (D36). These differences were not statistically significant.

Table 4. 6 Dietary intake at end of intervention (day 36) (FFQ2)

	Prudent guidelines	GROUP 1 (PLACEBO)						GROUP 2 (acetate, propionate, butyrate)						GROUP 3 (acetate, & propionate)					
	/ RDA	N	Mean	SD	Min	Med	Max	N	Mean	SD	Min	Med	Max	N	Mean	SD	Min	Med	Max
Macronutrients																			
Total kilojoule (kJ)	12180.0	17	17717.4	6392.7	3119.1	17702.7	27937.3	21	17039.5	5845.2	7788.7	16649.1	29707.8	20	15013.8	6967 9	5699.4	13897.6	34525.9
Total carbohydrates (g)		17	472.4	177.6	117.4	486.5	776.5	21	449.6	161.1	230.1	437 9	959.4	20	400.4	190 4	153.1	349.6	976.2
Total carbohydrates (%TE)	>50%TE	17	46.1	8.5	34.0	47.0	64.0	21	45.6	7.4	32.0	45.0	60.0	20	46.5	7.8	33.0	46.5	61.0
Added sugar (g)		17	119.7	44.4	18.0	125.0	199.8	21	127.9	47.7	45.5	115.9	221.3	20	104.6	64 3	26.7	95.2	301.2
Added sugar (%TE)	<15%TE	17	11.8	3.8	6.0	11.0	22.0	21	13.2	4.4	6.0	12.0	22.0	20	12.1	5.7	3.0	12.0	25.0
Total protein (g)		17	159.1	53.6	29.2	165.4	270.6	21	156.6	65.5	60.9	149.1	337.8	20	144.8	83.9	39.7	128.9	338.0
Total protein (%TE)	12 - 20%TE	17	15.6	2.2	10.0	16.0	20.0	21	15.5	3.3	10.0	15.0	24.0	20	15.9	4.2	10.0	14.5	28.0
Total fat (g)		17	163.4	71.6	11.5	146.1	318.9	21	162.2	66.7	46.2	155.7	300.3	20	140.0	73.0	35 9	137.2	326.1
Total fat (%TE)	<30%TE	17	34.1	8.8	14.0	34.0	47.0	21	35.5	6.1	23.0	36.0	49.0	20	34.6	7.0	17.0	35.5	49.0
Total dietary fibre (g)	20 - 30	17	36.3	12.9	11.9	39.1	63.6	21	34.6	15.5	15.6	30.1	67.5	20	28.9	10.9	12.3	20.0	49.6
Total NSP(g)		17	16.2	7.4	1.1	17.6	29.7	21	17.1	8.1	2.8	17.5	31.8	20	13.4	6.3	3.1	11.5	27.2
Alcohol (g)	<28g	17	10.9	34.3	0.0	0.0	142.6	21	4.9	9.6	0.0	0.0	31.5	20	2.5	3.8	0.0	0.0	10.5
Micronutrients	RDA/AI[a]																		
Vitamin A(µg RE)	900.0	17	1882.5	1369.3	89.1	1514.6	4954.2	21	1813.5	1115.3	425.1	1613.1	3753.9	20	1545.6	1293.5	282.2	1131.8	54738.0
Vitamin C (mg)	90.0	17	206.7	203.6	16.7	139.0	728.0	21	162.5	82.7	61.3	165.6	450.6	20	137.8	103.5	31.5	107.0	494.0
Vitamin D (µg)	5.0	17	11.4	5.3	0.8	11.4	20.4	21	10.5	6.1	2.0	9.4	23.9	20	9.0	6.7	1.3	8.4	31.7
Tiamine (mg)	1.2	17	2.7	1.2	0.8	2.4	6.4	21	2.3	0.8	1.1	2.1	3.9	20	2.8	3.5	0.5	1.9	17.1
Riboflavin (mg)	1.3	17	3.2	1.7	0.7	2.6	8.4	21	2.9	1.4	0.9	2.2	6.8	20	3.9	4.6	0.5	2.5	21.2
Niacin (mg)	16.0	17	39.0	18.5	12.5	35.7	95.8	21	37.8	16.1	13.2	33.6	72.6	20	38.8	37.8	10.2	33.6	188.5
Vitamin B6 (mg)	1.3	17	3.1	1.8	1.0	2.5	8.3	21	3.0	1.1	1.3	2.6	5.4	20	2.7	1.7	0.7	2.5	8.8
VitaminB12 (µg)	2.4	17	10.0	6.3	1.1	8.7	29.1	21	9.6	5.1	3.3	8.7	23.7	20	9.4	6.2	0.6	8.4	20.8
Calcium (mg)	1000*	17	1268.7	455.3	168.8	1223.7	2028.5	21	1258.4	589.7	458.1	1015.5	2460.4	20	1194.9	846.2	197.9	961.0	3004.7
Magnesium (mg)	400.0	17	489.9	158.9	122.1	464.7	751.2	21	477.2	180.8	206.0	423.8	837.5	20	478.3	296.8	187.8	418.7	1442.3
Phosphorus(mg)	700.0	17	2235.4	417.4	2307.0	2306.9	3350.8	21	2203.6	846.1	852.5	2076.7	4114.9	20	2371.9	2272.5	609.3	1814.8	11030.8
Iron (mg)	8.0	17	26.2	12.8	6.4	25.5	65.0	21	24.0	12.6	8.8	22.0	64.0	20	20.8	10 5	6.2	18.6	37.1

RDA Recommended Daily Allowances
AI Adequate intake
%TE Values espressed as percentage of total energy intake
a RE: Retinol equivalents: 1 retinol equavalent = 1µg retinol

Table 4.7 Change in dietary intake from baseline 2 (day 8) to the end of supplementation (day 36)

	GROUP 1 (Placebo)							GROUP 2 (acetate, propionate, butyrate)							GROUP 3 (acetate, & propionate)							
	N	Mean	SD	Min	Med	Max	P-value within group	N	Mean	SD	Min	Med	Max	P-value within group	N	Mean	SD	Min	Med	Max	P-value within group	P-value between groups
Macronutrients																						
Total kilojoule (KJ)	17	843.75	5258.68	-9750.47	545.52	13365.82	0.52	21	26.50	3737.20	-91188.16	122.99	8053.92	0.97	20	-1215.37	4055.35	-11774.17	-638.87	6444.34	0.20	0.35
Total carbohydrates (g)	17	-20.15	182.92	-592.37	-0.42	287.40	-66.00	21	-18.91	118.95	-353.73	-6.20	193.39	0.47	20	-13.72	161.71	-317.10	-20.66	469.89	0.71	0.99
Total carbohydrates (%TE)	17	-3.40	6.00	-17.00	-2.00	5.00	0.03	21	-2.10	6.60	-23.00	-2.00	8.00	0.20	20	0.40	8.30	-19.00	-1.00	17.00	0.80	0.94
Added sugar (g)	17	-3.48	57.39	-179.40	2.90	105.72	0.81	21	-8.87	63.02	-252.10	-1.11	66.98	0.53	20	-11.04	76.91	-185.31	2.84	121.48	0.53	
Added sugar (%E)	17	-1.50	5.40	-17.00	-1.00	7.00	0.30	21	-0.60	2.90	-7.00	-1.00	6.00	0.30	20	-2.80	8.70	-31.00	-1.00	6.00	0.20	
Total protein (g)	17	14.61	43.66	-117.82	7.55	80.98	0.18	21	104.00	29.67	-41.01	2.19	70.37	0.23	20	-10.33	69.72	-206.34	-3.28	185.08	0.52	0.29
Total protein (%E)	17	0.60	2.50	-5.00	1.00	5.00	0.30	21	0.60	2.20	-2.00	0.00	7.00	0.20	20	0.40	4.50	-11.00	0.00	12.00	0.70	
Fat (g)	17	18.20	44.59	-58.84	6.73	96.08	0.11	21	6.22	47.50	-110.42	5.80	100.00	0.56	20	-16.37	39.99	-156.00	-2.50	21.25	0.08	0.06
Total fat (%E)	17	1.80	5.00	-9.00	1.00	11.00	0.20	21	1.80	6.20	-7.00	1.00	18.00	0.20	20	-0.10	6.50	-16.00	0.00	13.00	1.00	
Total dietary fibre (g)	17	-1.20	11.90	-27.10	-0.80	17.60	0.70	21	-0.10	7.00	-10.70	-0.50	19.00	1.00	20	-2.90	9.00	-25.80	0.00	11.00	0.20	
Total NSP(g)	17	-0.50	10.96	-25.65	0.48	16.25	0.85	21	-0.83	4.18	-8.55	-1.42	7.09	0.37	20	-2.80	7.40	-23.14	-0.76	5.34	0.11	0.61
Alcohol (g)	17	9.82	33.22	-1.62	2.00	137.71	33.28	21	-0.65	10.62	-30.85	0.00	26.24	0.78	20	-5.28	14.74	-56.54	0.00	8.17	0.13	0.09
Micronutrients																						
Vitamin A(µg RE)	17	20.30	957.10	-1718.10	44.20	2690.80	0.90	21	-353.30	1667.20	-7287.50	-16.90	1253.90	0.30	20	350.50	1062.40	-888.20	1.10	3208.70	0.20	0.20
Vitamin C (mg)	17	-114.20	426.20	-1704.00	5.60	115.10	0.30	21	-27.20	108.30	-307.80	-20.50	142.10	0.30	20	-19.20	139.00	-441.40	-7.80	338.90	0.50	0.50
Vitamin D (µg)	17	2.50	6.10	-15.20	1.80	10.50	0.12	21	0.30	4.60	-9.60	0.20	11.20	0.80	20	-2.30	7.00	-26.30	-1.00	5.20	0.20	0.06
Tiamine (mg)	17	0.50	1.10	-0.70	0.20	3.80	0.08	21	-0.20	0.90	-3.40	-0.04	1.40	0.40	20	0.60	3.50	-1.90	0.10	15.20	0.40	0.50
Riboflavin (mg)	17	0.30	0.80	-1.10	0.30	2.70	0.10	21	0.10	0.80	-1.90	0.10	1.70	0.50	20	0.70	4.20	-2.60	-0.02	17.90	0.50	0.80
Niacin (mg)	17	4.70	15.90	-26.80	3.00	53.40	0.20	21	1.10	9.70	-26.00	0.15	20.40	0.60	20	1.34	38.40	-43.00	-1.40	153.40	0.90	0.90
Vitamin B6 (mg)	17	0.60	1.40	-0.80	0.20	5.60	0.10	21	-0.10	1.00	-3.10	0.03	2.30	0.70	20	0.00	1.70	-3.60	-0.02	6.40	0.90	0.30
Vitamin B 12 (µg)	17	0.80	3.30	-5.90	0.40	10.30	0.30	21	1.50	3.00	-2.50	0.90	8.90	0.03	20	-0.80	5.80	-18.80	-0.60	12.70	0.50	0.10
Calcium (mg)	17	-4.40	414.00	-1190.00	58.60	529.10	0.10	21	30.70	277.60	-389.90	8.50	801.00	0.60	20	174.90	472.90	-281.90	8.60	1544.10	0.10	0.30
Magnesium (mg)	17	6.80	16.40	-593.90	41.90	155.40	0.90	21	8.70	76.70	-123.40	-1.70	194.60	0.60	20	2.40	263.50	-385.30	-23.80	968.90	0.90	1.00
Phosphorus(mg)	17	124.20	562.20	-1819.20	126.60	740.40	0.40	21	61.20	368.20	-480.50	8.10	897.50	0.40	20	381.40	2034.40	-1925.00	-3.50	8599.90	0.50	0.80
Iron (mg)	17	0.60	7.40	-14.00	1.10	12.60	0.70	21	0.60	6.80	-10.00	0.10	19.20	0.70	20	-1.20	7.70	-24.90	-0.50	20.10	0.50	0.70

%TE Values espressed as percentage of total energy intake
a RE: Retinol equivalents: 1 retinol equevalent = 1µg retinol

Table 4.8 Metabolic indicators, anthropometry and blood pressure at end of study (day 43)

	Normal range	GROUP 1 (Placebo)						GROUP 2 (acetate, propionate & butyrate)						GROUP 3 (acetate & propionate)					
		N	MEAN	SD	MIN	MED	MAX	N	MEAN	SD	MIN	MED	MAX	N	MEAN	SD	MIN	MED	MAX
Metabolic indicators																			
Glucose(mmol/L)	3.6 - 5.6[e]	14	5.5	1.1	3.7	5.5	8.1	18	5.7	0.7	4.2	5.6	6.9	10	5.0	0.5	4.0	5.2	5.8
Insulin (µIU/mL)	5.0 - 25[a]	14	14.5	9.7	5.0	13.8	46.0	16	15.8	9.9	6.8	14.7	48.0	10	13.8	9.7	6.0	9.8	37.0
Total protein (g/L)	64 - 84[a]	14	79.7	5.0	72.0	82.0	87.0	18	78.2	5.2	71.0	77.0	88.0	10	77.4	8.1	66.0	76.0	95.0
Albumin (g/L)	34 - 52[a]	14	45.8	1.7	43.3	45.4	48.4	18	45.4	2.4	41.1	44.9	49.8	10	43.5	2.4	38.9	44.3	46.8
Total cholesterol (mmol/L)	3.0 - 5.2[b]	14	4.4	1.0	2.9	4.7	5.4	18	4.7	0.9	3.4	4.6	6.3	10	3.9	0.8	3.0	3.8	5.5
HDL-C(mmol/L)	0.9 - 1.6[b]	13	1.4	0.3	1.0	1.5	2.0	17	1.7	0.8	1.1	1.3	3.7	10	1.4	0.3	1.0	1.4	1.9
%HDL-C* (%)	>20%	13	0.3	0.1	0.2	0.3	0.6	17	0.3	0.1	1.8	0.3	0.7	10	0.4	0.1	0.2	0.4	0.5
LDL-C(mmol/L)	2.0 - 3.4[b]	13	2.5	0.8	0.8	2.6	3.9	17	2.6	0.9	1.2	2.4	4.5	10	2.1	0.7	1.3	2.0	3.5
Triglycerides (mmol/L)	0.3 - 2.0[b]	13	1.2	0.8	0.5	1.0	3.4	18	1.0	0.5	0.5	1.0	2.7	10	1.0	0.3	0.5	1.0	1.5
NEFA(mmol/L)	0.1 - 0.9[b]	14	0.4	0.2	0.2	0.3	0.9	18	0.3	0.2	0.0	0.3	0.9	10	0.2	0.1	0.1	0.2	0.4
Anthropometry																			
Weight(kg)		17	69.7	10.8	56.3	67.7	92.0	19	66.6	7.4	55.0	66.1	80.5	19	67.6	7.7	52.7	65.2	80.0
BMI(kg/m²)	18.5 - 24.9[c]	17	23.6	2.8	18.8	22.6	28.7	19	23.0	1.9	19.8	22.8	27.8	19	23.1	2.1	19.5	23.2	29.5
Waist(cm)		17	78.1	7.4	68.0	77.0	92.0	19	75.6	4.8	69.0	74.0	86.0	19	78.5	7 2	68.0	78.0	95.0
Hip(cm)		17	98.0	6.8	86.0	96.0	109.0	19	96.0	4.8	87.0	97.0	104.0	19	95.7	4.8	86.0	96.0	105.0
WHR	< 1.0[d]	17	0.8	0.0	0.7	0.8	0.9	19	0.8	0.0	0.7	0.8	0.9	19	0.8	0.0	0.7	0.8	0.9
Blood Pressure																			
SBP(mmHg)	< 160[e]	17	115	11	90	120	130	19	118	8	100	120	130	19	116	6	100	120	120
DBP (mmHg)	< 90[e]	17	83	12	60	80	110	19	83	9	60	80	100	19	81	6	70	80	90

a Haematology Department, UFS, 2002
b Institute for Pathology, University of Pretoria, 1999
c Laquatra, 2000
d Hammond, 2000
e Steyn *et al*., 1991

The mean alcohol intake indicated a tendency towards an increase in group one after supplementation (D36), whereas a tendency towards a decrease was observed in groups two and three. However, these differences were not statistically significant The results indicate that the usual dietary intake of the subjects in all three groups did not differ significantly and remained somewhat similar from baseline one (D0) to the end of supplementation (D36). Thus, it can be assumed that the changes observed in the metabolic parameters could not have been influenced by the dietary intake of the subjects.

4.3.4.2 Micronutrient intake

The micronutrient intake remained somewhat similar in all three groups (D0 – D36) except for the increased Vitamin B_{12} intake observed in group two. This difference was statistically significant.

Thus, except for the vitamin B_{12} intake in group two, the micronutrient intake did not alter in the three groups from baseline one (D0) to the end of supplementation (D36).

4.3.5 Tolerance questionnaire

All three groups (Gr 1: 64%; Gr 2: 76%; Gr 3: 95%) complained about the amount of capsules they had to consume. Members in group three complained about an increase in appetite, while only five percent in group 2 complained of experiencing stomach cramps and no one in group three had any side-effects while consuming the capsules.

4.4 SUMMARY

At baseline one and two the subjects fell within the normal ranges for all the parameters which indicated a healthy study group with no metabolic abnormalities. From baseline one (D0) to baseline two (D8) differences in the metabolic indicators (serum glucose, serum insulin, serum albumin, serum total protein, serum triglycerides, NEFA), anthropometric status (weight, BMI, WHR, waist & hip circumferences) and blood pressure observed in

the three groups were not of statistical significance. Except for weight and BMI in group two and hip circumference in group one, but not clinically significant. This indicates an accurate reflection of the parameters. The dietary intake at baseline one (D0) reflected a study group presenting a tendency towards the adoption of a high-fat (>30% TE), low-carbohydrate (<50% TE) westernised diet. The difference between the groups was not statistically significant.

The dietary intake remained the same after supplementation (D36) as at baseline one (D0), still revealing a tendency towards the adoption of a high-fat (>30% TE), low-carbohydrate (<50% TE) westernised diet, but with a higher fibre intake (>30g). The BMI and WHR remained somewhat similar during the study. The blood pressure fell within the normal range for all three groups, whereas a statistically significant decrease in systolic blood pressure (SBP) was observed in group two ($p = 0.04$). The observed differences in metabolic indicators within each subject group measured between day eight and day 36 were not significant, regarding both clinical and statistical guidelines. A tendency towards a decrease in fasting serum blood glucose concentrations was however, observed in group two (acetate, propionate & butyrate) and group 3 (acetate & propionate). The serum blood glucose concentrations remained in the high normal ranges (5.2 – 5.3mmol/L) in all three groups. Serum insulin concentrations indicated a tendency to decrease after supplementation in group three, but a tendency to increase was observed in group 2 after intervention (D36). In group two (acetate, propionate & butyrate) the serum protein concentrations as well as the serum albumin concentrations decreased significantly ($p=0.00$; $p = 0.00$ respectively), and the serum LDL-C also decreased significantly ($p = 0.0008$). The total serum cholesterol concentrations remained within the high normal ranges (4.3 – 4.6mmol/L) for all three groups. This TC concentration together with the high LDL-C concentration and the high dietary fat intake (>30%TE) could place the subjects at high risk for the development of cardiovascular conditions.

CHAPTER 5

DISCUSSION

5.1 INTRODUCTION

The aim of the study was to determine the effect of a combination of SCFAs on glycometabolic control in men. The effects of two supplements were measured against a placebo group in order to determine whether these SCFAs have any effect on glycometabolic control and background information which include other metabolic parameters, anthropometric status, blood pressure as well as the usual dietary intake.

In this chapter changes in results from baseline two (D8) to the end of intervention (D36), in terms of metabolic indicators, anthropometric status, blood pressure and dietary intake, will be discussed and interpreted in the light of findings from other relevant studies.

5.2 METABOLIC INDICATORS

5.2.1 Glycometabolic control

The mean fasting serum blood glucose and insulin concentrations for all subjects fell within the normal range (3.6 – 5.6mmol/L and 5.0 – 25μIU, respectively) at baseline one and two with no significant differences between the two baselines (D0 – D8). The mean blood glucose concentrations were, however, higher (5.1 – 5.7mmol/L) than expected of a physically active group as well as the fact that they were fasting. High glucose levels were found in urban black populations in Durban, where a tendency towards higher blood glucose (7.9% >7.9mmol/L) levels was observed (Seedat *et al.*, 1992). In a previous study conducted on males of the SANDF in Bloemfontein, similar glucose levels were found ranging from 4.6mmol/L to 4.7mmol/L (De Wet, 1999).

In the study by De Wet (1999) the combination of three SCFAs (acetate, propionate & butyrate) significantly decreased glucose levels. Alamowitch *et al.* (1996), on the other hand, found that the acute ileal perfusion of the combination of acetate, propionate and butyrate did not significantly reduce glucose levels in healthy men. Glucose levels and insulin sensitivity improved following propionate supplementation only as indicated by Venter *et al.* (1990). However, Jenkins *et al.* (1995) found no evidence of decreased insulin concentrations, or improved glucose tolerance after raising serum acetate concentrations. In the present study the supplementation of acetate, propionate and butyrate as well as acetate and propionate caused a tendency towards a decrease in fasting serum glucose levels. A tendency towards a decrease in serum insulin concentration was only observed in group two (acetate, propionate & butyrate). Furthermore, the insulin levels indicated a large, but not significant increase in circulating concentration following the supplementation of group three (acetate and butyrate), which could be ascribed to the large standard deviation within the group. In the present study the supplementation of acetate, propionate and butyrate as well as acetate and propionate caused no significant change in serum glucose and insulin concentrations of the subjects.

5.2.2 Other metabolic parameters

Albumin is acknowledged as an important indicator of nutritional status and longevity (Carlson, 2000). The mean total protein (76.9 – 82.1g/L) and serum albumin (43.9 – 46.8) levels also fell within the normal ranges at baseline one (D0) and two (D8), confirming the apparently healthy status of the subject group. This healthy status of the subject group was evident throughout the study, because of the decrease in the ratio of TP to albumin following the supplementation of acetate, propionate and butyrate, which indicates that the circulating pool of proteins contains more albumin.

The study group also presented a favorable lipid profile at baseline one and two. However, the total serum cholesterol levels (4.2mmol/L to 4.5mmol/L) indicated a tendency towards the higher normal range (3.0mmol/L to 5.2mmol/L) which was also found in urban black populations in the Free State (4.7 – 4.9mmol/L) (Mollentze *et al.*, 1995) as well in urban black men on the UFS campus of the UFS (Slabber *et al.*, 1997)

following a westernised (high-fat, low-carbohydrate) diet. De Wet (1999) also reported high normal ranges for TC (4.5mmol/L ±0.7) in her study on soldiers in the SANDF in the Free State. Urban black South African populations in the Cape Peninsula, however, had lower TC (3.57 – 4.02mmol/L) levels (Oelofse *et al.*, 1996). The normal triglyceride levels at both baselines (1.0 – 1.3mmol/L) correlate with other studies reported for urban black people in South Africa (Mollentze *et al.*, 1995; Oelofse *et al.*, 1996) (1.3 – 1.7mmol/L ; 0.94 – 0.99mmol/L, respectively).

In this study no significant changes were observed in the TC levels of the subjects following the supplementation of three (acetate, propionate & butyrate) and two (acetate & propionate) SCFA's. De Wet (1999) also found that a combination of three (acetate, propionate and butyrate) SCFAs did not significantly change TC levels. A study by Wolever *et al.* (2002) could explain this lack of effect on TC. They found that changes in serum acetate were significantly related to changes in serum concentrations of cholesterol only after three and six months. In contrast with these findings, Veldman *et al.* (1999) found that acetate supplementation alone decreases TC and triglyceride concentrations in hypercholesterolaemic men after several weeks. According to Wolever *et al.* (1989), acetate could reduce serum cholesterol levels by reducing FFA concentration, but Wolever *et al.* (1995) concluded that propionate inhibits the utilisation of acetate for cholesterol synthesis in men. This study indicated that acetate, propionate and butyrate could probably favour higher HDL-C – and lower LDL-C concentrations.

A decrease in NEFA was observed in both SCFA supplementation groups. Although the change was not significant, it is ascribed to increased SCFA circulation (Wolever, Spadafora & Eshuis, 1991), indicating that the capsules were consumed.

5.3 ANTHROPOMETRY

The mean BMI at baseline one and two fell within the normal ranges (18.5 – 24.9) (Laquatra, 2000), indicating that this study group was apparently healthy. Other urban black populations in the Cape Peninsula (Steyn *et al.*, 1991),and Durban region (Seedat *et al.*, 1992) also reported normal BMI in black men in the same age group (18 – 45yrs).

Similar results were reported in the study by De Wet (1999). The WHR for the study group at both baselines indicated that this group was not at risk of android obesity, which according to Mollentze (1995) is an indicator of IHD. The reason for the difference in hip circumference from baseline one and baseline two in group one is unclear, it could be ascribe to a measuring fault.

5.4 BLOOD PRESSURE

The blood pressure of all subjects also fell within the normal ranges for systolic (<140mmHg) and diastolic blood (<90Hg) pressure at baseline one and two and remained within these normal ranges, emphasizing that the subject group was healthy. After intervention, systolic blood pressure decreased statistically significantly in group two (acetate, propionate & butyrate) ($p = 0.04$). This difference was, however, not clinically significant.

5.5 DIETARY INTAKE

The dietary intake of the subjects was within or on the borderline of the recommended prudent guidelines and included higher intakes of total fat (>30% TE), fibre (20-30g/day); while the total protein intake were in the recommended range (12 – 20%TE) and total carbohydrate intake (<50%TE) fell in the lower range. Although the total grams of protein consumed were above the normal recommendations for males 18 – 45 years (52 – 56g/day) (Trumbo *et al.*, 2000), it still fell in the recommended range of 12 – 20% of total energy because of their high energy intake. Other studies conducted on urban black men in the Free State (De Wet, 1999; Silvis & Mollentze, 1995; Slabber *et al.*, 1997) and the Cape Peninsula Study (Bourne *et al.*, 1993) also demonstrated a trend towards the adoption of a Westernised diet (>30% fat TE & <50%CHO TE). The mean intakes for micronutrients, however, met the RDA and AI components of the DRI's, and indicate adequate micronutrient intakes. The adequate vitamin and mineral intake of the subjects could be ascribed to the fruits and vegetables available daily at the mess and eaten daily by the members not eating at the mess. The dietary intake did not change throughout the study.

5.6 TOLERANCE QUESTIONNAIRE

Only group two complained about some side effects experienced during the consumption of the capsules, while all three groups indicated that the amount of capsules were too much. Thus, the capsules were well tolerated.

5.7 SUMMARY

The background information with regard to anthropometric status, blood pressure and dietary intake, reflected a study group that remained healthy throughout the study and that indicated a tendency towards adopting a high-fat, low-carbohydrate atherogenic westernised diet.

Fasting serum blood glucose and serum TC concentrations fell within the high normal range for healthy adult males. The combination of SCFAs (acetate, propionate & butyrate and acetate & propionate) indicated a tendency to lower serum glucose levels following supplementation, but is of no statistical significance. The decrease in LDL-C and systolic blood pressure in group two (acetate, propionate & butyrate) was statistically significant. The clinical significance of these changes is however, very small.

The change in NEFA following supplementation also indicated that the SCFAs were absorbed, and probably caused these changes in the glycometabolic and other metabolic indicators.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 INTRODUCTION

Interest in the effects of short-chain fatty acids on carbohydrate metabolism in humans was prompted by the suggestion that these acids may play a role in mediating the effects of dietary fibre in glucose control (Anderson & Bridges, 1984). Wolever *et al.* (1989) suggested that acetate could reduce blood glucose levels. Venter *et al.* (1990) found that propionate improves glucose tolerance and insulin sensitivity. However Alamowitch et al (1996) and Jenkins *et al.* (1991) did not found that acetate decreased blood and insulin concentrations following raised serum acetate concentrations. No human studies were found in which the effect of a combination of SCFAs on glycometabolic control were determine.

In this study two combinations of SCFA were given. One group was supplemented with acetate, propionate and butyrate, and the other group was supplemented with acetate and propionate. Each subject received eight capsules daily. The standardised techniques used for the execution of the study, as described in chapter three, ensured that the results of this study are deemed valid and reliable. A placebo group was included in the study to measure the effect of the two supplements.

The study group was of a homogeneous nature, with no metabolic abnormalities present at the beginning and during the execution of the study. Although the subjects were normoglycaemic, serum glucose concentrations were within the higher normal range. The usual dietary intake derived from the FFQ indicated a tendency towards a Westernised (>30%fat; <50% CHO) diet among the subjects. SCFA supplementation was sustained for four weeks and was tolerated by the subjects with no sign of side effects. The usual dietary pattern of the subjects did not alter and remained somewhat similar until completion supplementation as at the start of the study. It is therefore suspected that the

results of the study were no0t affected by the diet and could be ascribed to SCFA supplementation. The reduction in NEFA concentrations was probably due to increased SCFA circulation, indicating that the SCFAs were absorbed. The following conclusions can be drawn from the findings of the present study.

6.2 CONCLUSIONS

The study indicated that the SCFA supplements had no statistically or clinically significant effect on the metabolic indicators (serum glucose, serum insulin, serum albumin, total protein, TC, HDL-C, LDL-C, NEFA), anthropometric status and blood pressure of normoglycaemic subjects. However, the LDL-C showed a significant decrease in group two supplemented with acetate, propionate and butyrate. Systolic blood pressure also decreased significantly in this group. However, these changes are not clinically significant.

Both SCFA supplements, however, indicated a tendency towards a decrease in blood glucose concentrations, which were not statistically significant. Although the combinations of SCFA had no effect on the blood glucose levels in this study, a decrease in blood glucose levels was found from propionate (Todesco *et al.*, 1991; Venter *et al.*, 1990) and acetate (Jenkins *et al.*, 1991) as well as a combination of acetate, propionate and butyrate (De Wet, 1999). However, the findings by Alamowitch *et al.* (1996) support the findings in this study that SCFAs (acetate, propionate & butyrate) do not significantly alter glucose metabolism.

The acetate and propionate supplement revealed a decrease in insulin levels of the subjects. These findings were, however, not statistically significant. Jenkins *et al.* (1991) also reported that elevated serum acetate levels had no effect on insulin concentrations. However, Venter *et al.* (1990) found that propionate may improve insulin sensitivity following propionate supplementation. The two SCFAs supplements did not significantly alter the TC levels of the subjects. This is consistent with the hypothesis that propionate resulting from colonic fermentation inhibits the utilisation of acetate for cholesterol synthesis (Anderson, 1995, p. 519).

The metabolic effects of acetate and propionate in humans have been carefully studied in humans. Acetate has been administrated orally, intravenously and rectally. Propionate has been administrated orally and rectally. Acetate, propionate and butyrate have been administrated orally. Most studies were of short duration (a few hours), but the long-term administration of propionate as well as acetate, propionate and butyrate was examined. The effect of one SCFA or a combination of two was studied on glucose and lipid metabolism, while the effect of the three SCFA was examined on plasma fibrinogen concentrations. It is therefore difficult to compare the results in this study, but it is clear that both combinations of SCFAs seem to have an effect on glycometabolic control.

6.3 RECOMMENDATIONS

The findings of the present study indicate that further research is needed to evaluate the effect of the combinations of SCFAs (acetetate, propionate & butyrate and acetate & propionate) that could be derived from the fermentation of carbohydrates on hyperglycaemic subjects.

The effect of the combination of SCFA (acetate, propionate & butyrate) on lipid metabolism (Wolever *et al.*, 2002) and haemostasis (De Wet, 1999) was investigated, but no evidence on glucose metabolism was found. Further research is therefore needed on the effect of different combinations of SCFAs on glucose metabolism.

The positive effect of acetate, propionate and butyrate on LDL-C and systolic blood pressure should be investigated further, to confirm possible health benefits of these SCFAs on the lipid profile and blood pressure.

The amount of capsules consumed by the subjects in the present study will be impractical over a long period of time. Dietary fibre will probably be a better choice than consuming a large amount of capsules. The amount of SCFAs needed to indicate a clinical effect on glucose metabolism sho: 'd therefore be investigated further.

It is also recommended that other metabolic parameters such as fructosamine be used as a variable to determine whether SCFAs have any effect on glucose metabolism.
The subjects fall in a high risk group for metabolic related diseases, and should therefore be consulted and followed up.

The study clearly emphasise the need for further investigation. Another study using more subjects over a long period of time, would be difficult because of practical implications to ensure that the capsules are consumed.

The findings in the study indicate that the combination of the SCFA may play a role in glycometabolic control in men. Although the results were not statistically significant, it was pointed out that these combinations of SCFAs may be beneficial in the treatment of hyperglycaemic subjects.

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APPENDIX 1

CONSENT FORM

THE EFFECTS OF SHORT-CHAIN FATTY ACIDS ON GLYCOMETABOLIC CONTROL IN MEN

DECLARATION BY OR ON BEHALF OF THE PARTICIPANT

I,..... undersigned,
of.....
..... (address)
Identity number: .....

A I confirm that:

1. I have been asked to participate in the above-mentioned research project, carried out by the Fibrinogen Unit, Technikon Free State and University of the Orange Free State.

2. The information including the purpose of the study, advantages and disadvantages have been completely explained to me.

3. I give my permission for the use of the results obtained in this research project for publication purpose, thus making other scientists aware of the findings as long as my anonymity is protected at all times. The information obtained will be confidential.

4. It was clearly explained to me that I can refuse to participate in this study or I can withdraw my permission to participate at any time. If I refuse or withdraw, I will not be disadvantaged in any way and it will not be held against me.

5. The information was explained to me by..... (name of interviewer) in ..... (language) and I confirm that I have good command of this language and understood the explanations. I was also given the opportunity to ask questions on things I did not understand, and I can also ask further questions at any time during the project.

6. No pressure was applied on me to take part in this research project.

B I hereby agree voluntarily to take part in this research project.

Signed/confirmed at ..... on 2001

.....(signature) .....
Participant Witness

DECLARATION BY OR ON BEHALF OF RESEARCHER

I, ..., declare that:

1. I have explained the information in this document and about the project to .... (name of participant).

2 I asked the participant to ask any questions if something was not clear.

3 That the interview was conducted in English, Afrikaans, or ..... (language) OR that the interview was conducted in ..... (language) with the help of a translator, ... (name of translator).

Signed/confirmed at ..... on 2001

..... (signature) .....
Researcher or his representative Witness

DECLARATION BY TRANSLATOR

I, ..., declare that:

1 I have translated the content of this document from English/Afrikaans into ..... (language) to ..... (name of participant) and that I have translated the participant's questions to the researcher or his/her representative as well as the answers of the latter to the participant.

2 That the information that I have so translated was a correct interpretation of what I was asked to translate

Signed/confirmed at ..... on 2000

..... (signature) .....
Translator Witness

Appendix 2

TECHNIKON FREE STATE FIBRINOGEN UNIT

SCFA PROJECT: SCREENING QUESTIONNAIRE

DATE: _____/_____/_____

INTERVIEWER: _____

SURNAME **AND** **INITIALS**:

_____

HOUSE **DOCTOR**:

_____

PATIENT:
NAME:_____

ADDRESS: _____

_____

_____

_

SECTION/DEPARTMENT:_____

TEL:_____

AGE: _____years_____months

SMOKING HABITS:

YES

NO

BODY MASS: _____kg

LENGTH: _____cm

BLOOD PRESSURE: _____/_____

ACTIVITY LEVEL:

INACTIVE

MEDIUM ACTIVE

ACTIVE

FAMILY HISTORY:

CORONARY HEART DISEASE_____
DIABETES MELLITUS_____
HYPERCHOLESTEROLAEMIA_____
OTHER (specify):

_____

____

MEDICAL HISTORY:

ANGINA/CORONARY HEART DIASEASE _____

MYOCARDIAL INFARCTION_____
STROKE_____
BYPASS_____
BLOOD CLOTS_____
HIGH BLOOD PRESSURE_____
DIABETES TYPE I/II_____
FAMILIAL CHOLESTEROL_____

ANY CHRONIC DISEASES MEDICATION:

YES

NO

SPECIFY:_____

HOW OFTEN AND HOW MANY DAYS IN A WEEK OR WEEKEND DO YOU USE ALCOHOL:

DAILY

WEEKLY

MONTHLY

OTHER: _____

On a weekday when you drink alcohol how many drinks do you usually have?

	Per day
Beer (how many bottles/cans	
Brandy/whisky (how many tots)	
Vodka/gin (how many tots)	
Sjerrie/sweetwine (how many glasses)	
Table wine (how many glases)	
Other, specify:	

On a weekend day when you drink alcohol how many drinks do you usually have?

	Per day
Beer (how many bottles/cans	
Brandy/whisky (how many tots)	
Vodka/gin (how many tots)	
Sjerrie/sweetwine (how many glasses)	
Table wine (how many glases)	
Other, specify:	

BLOOD TESTS

BLOOD CHOLESTEROL: _____

FASTING GLUCOSE: _____

PLASMA FIBRINOGEN: _____

FOR OFFICE USE ONLY:

SUBJECT: APPROVED REJECTED

ASSIGNED SUBJECT NO:

APPENDIX 3

THE EFFECT OF SHORT-CHAIN FATTY ACIDS ON GLYCOMETABOLIC CONTROL IN MEN

Name: _____ Respondent number: [ ][ ] 1-2

Force number : _____

Interviewer: _____ [ ][ ] 3-4

QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE

Thank you for giving your time to participate in this survey. We would like to find out if short-chain fatty acids, which is a fermentation product of dietary fiber, have a effect on blood sugar and insulin levels in men. The short-chain fatty acids will be given to you in the form of a capsule. The information about your eating habits is important as it will tell us if this supplement has any effect on the eating habits of people taking it.

Please think carefully about the food and drinks you have consumed during the past 6 months. I will now go through a list of foods and drinks with you and I would like you to tell me

- if you eat these particular foods.
- how the food is prepared (by the mess or elsewhere)
- how much of the food you eat at a time, and
- how many times a day you eat it and if you do not eat it every day, how many times a week or a month it is eaten?

To help you describe the amount of food, I will show you models of different amounts of food. Please say which model is the closest to the amount eaten, or if it is smaller, between sizes or bigger than the models. Amounts must be reported as cups (c), tablespoons (T), serving spoons (SP) or teaspoons (t)

- THERE ARE NO RIGHT OR WRONG ANSWERS.
- EVERYTHING YOU TELL ME IS CONFIDENTIAL.
- IS THERE ANYTHING YOU WANT TO ASK NOW?
- ARE YOU WILLING TO GO ON WITH THE QUESTIONS?

- ENCIRCLE APPROPRIATE ANSWER

Do you follow any special diet? Yes (1) No (2) [ ] 5

If yes, please specify (encircle appropriate answer) [ ] 6

1. Diabetic diet
2. Slimming diet
3. Cholesterol diet
4. Allergies
5. Other, (specify) _____

- Do you use salt in your food? Yes (1) NO(2) Don't know (3) [ ] 7

Do you use any dietary supplements? Yes (1) NO(2) Don't know (3) [ ] 8

If yes, please specify the type (name), how often , and how much:

Vitamins: _____				9-11
Minerals: _____				12-14
Protein: _____				15-17
Energy: _____				18-20
Other: _____				21-23

TING PATTERNS: (FREQUENCY OF EATING)

EASE INDICATE WHICH OF THE FOLLOWING BEST DESCRIBES THE EATING PATTERN YOU UALLY FOLLOW (MARK ONLY ONE):

[ ] 24

1. More than three meals with eating between meals
2. Three meals with eating between meals
3. Three meals with no eating between meals
4. Two meals with eating between meals
5. Two meals with no eating between meals
6. One meal with eating between meals
7. One meal with no eating between meals
8. Nibble the whole day, no specific meals
9. Others (please specify) _____

YOU EAT BREAKFAST?

[ ] 25

1. Regularly (> 4 times a week)
2. Sometimes (1 - 3 times a week)
3. Never

OW OFTEN DO YOU EAT AT THE FOLLOWING PLACES AWAY FROM HOME?

mily	1. Never	2. > once/week	3. Weekly	4. Monthly	5. > once/month	[ ] 26
ends	1. Never	2. > once/week	3. Weekly	4. Monthly	5. > once/month	[ ] 27
fé	1. Never	2. > once/week	3. Weekly	4. Monthly	5. > once/month	[ ] 28
staurant, Fast food	1. Never	2. > once/week	3. Weekly	4. Monthly	5. > once/month	[ ] 29
her specify _____	1. Never	2. > once/week	3. Weekly	4. Monthly	5. > once/month	[ ] 30

you drink coffee with your meals?

[ ] 31

1. Yes
2. No

yes, at which meals

eakfast	1. Yes	2. No	[ ] 32
nch	1. Yes	2. No	[ ] 33
pper	1. Yes	2. No	[ ] 34
acks	1. Yes	2. No	[ ] 35

o you drink tea (except Rooibos) with your meals?

1. Yes
2. No

[ ] 36

yes, at which meals

reakfast	1. Yes	2. No	[ ] 37
unch	1. Yes	2. No	[ ] 38
upper	1. Yes	2. No	[ ] 39
nacks	1. Yes	2. No	[ ] 40

Vith how many meals per day do you eat meat fish, or poultry?

1. One meal
2. Two meals
3. All meals
4. None

[ ] 41

o you eat fresh fruit and/or vegetables with the following meals?

Breakfast	1. Yes	2. No	[ ] 42
Lunch	1. Yes	2 No	[ ] 43
Supper	1. Yes	2. No	[ ] 44
Snacks	1. Yes	2. No	[ ] 45

How often do you usually drink alcohol?

[ ] 46

1. Every day
2. 5-6 days / week
3. 3-4 days / week
4. 1-2 days / week
5. Weekends
6. Less than once a week
7. Never

On a weekday when you do drink alcohol how many drinks do you usually have ?

	per day	
Beer (how many bottles/cans)		[ ][ ] 47-48
Brandy/whisky (how many tots)		[ ][ ] 49-50
Vodka/gin (how many tots)		[ ][ ] 51-52
Sjerrie/ sweetwine (how many glasses)		[ ][ ] 53-54
Table wine (how many glases)		[ ][ ] 55-56
Other, specify:		[ ][ ] 57-58

On a weekend day when you do drink alcohol how many drinks do you usually have ?

	per day		
Beer (how many bottles/cans)			59-60
Brandy/whisky (how many tots)			61-62
Vodka/gin (how many tots)			63-64
Sjerrie/ sweetwine (how many glasses)			65-66
Table wine (how many glases)			67-68
Other, specify:			69-70

Do you smoke? | | 71

1. Never smoked
2. Smoked previously, but not currently
3. Currently smoking

If currently smoking, how many sigarettes do you smoke per day ? _____ | | 72 - 73

If you smoked previously, how many years did you smoke? _____ | | 74 - 75

How many cicarettes did you smoke a day? _____ | | 76 - 77

Are you a living-in member ? 1. Yes 2. No | 78

SUMMARY OF FOOD FREQUENCY QUESTIONNAIRE

FOOD	CALCULATIONS	CODE				AMOUNT/DAY (g)				
										1-8
										9-16
										17-24
										25-32
										33-40
										41-48
										49-56
										57-64
										65-72
										73-80
										1-8
										9-16
										17-24
										25-32
										33-40
										41-48
										49-56
										57-64
										65-72
										73-80
										1-8
										9-16
										17-24
										25-32
										33-40
										41-48
										49-56
										57-64
										65-72
										73-80
										1-8
										9-16
										17-24
										25-32
										33-40
										41-48
										49-56
										57-64
										65-72
										73-80
										1-8
										9-16
										17-24
										25-32
										33-40
										41-48
										49-56
										57-64
										65-72
										73-80

SUMMARY OF FOOD FREQUENCY QUESTIONNAIRE

FOOD	CALCULATIONS	CODE				AMOUNT/DAY (g)				
										1-8
										9-16
										17-24
										25-32
										33-40
										41-48
										49-56
										57-64
										65-72
										73-80
										1-8
										9-16
										17-24
										25-32
										33-40
										41-48
										49-56
										57-64
										65-72
										73-80
										1-8
										9-16
										17-24
										25-32
										33-40
										41-48
										49-56
										57-64
										65-72
										73-80
										1-8
										9-16
										17-24
										25-32
										33-40
										41-48
										49-56
										57-64
										65-72
										73-80
										1-8
										9-16
										17-24
										25-32
										33-40
										41-48
										49-56
										57-64
										65-72
										73-80

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/never		
e-meal porridge	Stiff (pap)						3400	
e-meal porridge	Soft (slappap)						3399	
e-meal porridge	Crumbly (phutu)						3401	
porridge	Specify ratio Mabella/Maize						3399	
lla porridge	Stiff, coarse, fine						3437	
porridge	Brand name:						3239	
kfast cereals	Puffed Wheat, plain						3325	
	Puffed wheat, sweet							
	Corn Flakes, plain						3243	
	Weet Bix						3244	
	Puffed Rice, sweet						3372	
	Rice crispies						3252	
	Pronutro, plain						3245	
	Pronutro, High fibre						3436	
	Muesli						3303	
	Specify types usually eaten _____ _____ Brand names of cereals available at home now _____							
on porridge or	None							
al:	Whole/fresh						2718	
le type usually	sour						2787	
d:	25 fat						2772	
	Fat free/ skimmed						2775	
	Milk blend						2771	
	Soy milk						2737	
	Condensed (whole, sweet)						2714	
	Condensed (skim, sweet)						2744	
	Evaporated whole						2715	
	Evaporated low fat						2827	
	Non-dairy creamer						2751	
ugar added to	None							
ridge or cereal?	White						3989	
k box)	Brown						4005	
	syrup						3988	
	Honey						3984	
	Sweetner: type _____							
at added to	None							
ridge or cereal?	Animal fat (butter)						3479	
k box)	Hard margarine						3484	
	Soft margarine						3496	
	Oil						3507	
	Peanut butter						3485	
mp/ Maize rice	Bought						3250	
	Self ground						3725	
mp and beans	Specify ratio (1:1)						3402	
mp and peanuts	specify ratio							

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/n ever		
ice: specify brands	White						3247	
ames:	Brown						3315	
	Sorghum rice						3437	
amped wheat							3249	
astas	Macaroni						3262	
	spaghetti						3262	
	Spaghetti in tomato sauce						3258	
	Other:							
read/Bread rolls	White						3210	
read slices: thin	Brown						3211	
edium, thick	whole wheat						3212	
ther breads	Specify types, e.g.							
	Raisin						3214	
	Maize Meal						3278	
	Sweetcorn						3379	
	Rye						3213	
	Other							
zza (specify ppings)	Cheese, tomato & onion						3353	
ot dogs (specify								
usage)								
amburger (specify								
eat)								
re any of the	Butter						3479	
llowing spreads	Butro						3523	
sed on bread?	Animal fat (beef tallow)						3494	
ick box)	Lard						3495	
	Hard margarine (brick)						3484	
	Soft margarine (light)						3496	
	Cooking fat						3516	
eanut butter							3485	
weet spreads	Jam						3985	
	Syrup						3988	
	honey						3984	
Marmite/							4030	
OXO/							4029	
Bovril							4029	
ish paste							3109	
Meat paste							2917	
Cheese	Specify types:							
	Cottage low-fat cheese						2760	
	Cream cheese						2725	
	Gouda						2723	
	Cheddar						2722	
	Other:							
Cheese spreads	Low fat						4310	
	Full fat						2730	
	Specify types:							

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/never		
r							3117	
spreads: ify types)								
ling							3210	
ek							3257	
a							3235	
ers	Refined						3331	
	Whole wheat						3391	
s - *Commercial*	Bran						3330	
	Buttermilk						3329	
	White						3364	
	Boerebeskuit, white						3364	
-made	All-bran						3380	
	Raisins						3380	
	Buttermilk, white						3215	
	Buttermilk, whole wheat						3255	
	Other, specify							
es							3237	
ns	Plain						3408	
	Bran						3407	

V MANY TIMES A DAY DO YOU EAT BREAD?

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/never		
ken	Boiled with skin						2926	
	Without skin						2963	
ou eat the	Fried: in batter/crumbs						3018	
ken with the	Fried but not coated						2925	
?	Roasted/grilled - with skin						2925	
☐ No ☐	Roasted/grilled - without skin						2950	
ken bones stew							A003	
ken heads, raw							2999	
ken stew, veg & skin							3005	
ken offal	Giblets						2998	
ken pie	Commercial						2954	
	Home-made						2954	
meat: Beef	Fried/grilled: with fat						2908	
	without fat						2959	
	Stewed/boiled: with fat						3006	
	without fat						2909	
	Mince with tomato & onion						2987	
	Mince - curry						3015	
	Meatball regular						2966	
	lean						3034	
meat: Mutton	Fried/grilled: with fat						2927	
	without fat						2934	
	Stewed/boiled: with fat						3040	

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/never		
	without fat						2916	
meat: Pork	Fried/grilled: with fat						2930	
	without fat						2977	
	Stewed/boiled: with fat						3046	
	without fat						3045	
	Crumbed/Schnitzel						2992	
meat: Goat	Fried/grilled: with fat						4281	
	without fat							
	Stewed/boiled: plain						4281	
	with veg						4282	
:	Intestines: boiled, nothing added						3003	
ify type:	"Vetderm" fried						3003	
	Stewed with vegetables							
	Liver						2955	
	Kidney						2956	
	Tripe "pens" trotters, head						3003	
	Pluck (lungs, heart, gullet)						3019	
ify vegetables in meat stews if not tioned where)								
s/sausage	Fried						2931	
on							2906	
meats	Polony						2919	
	Ham						2967	
	Vienna's canned						2936	
	Russian						2948	
	Frankfutter						2937	
	Other (specify)							
ned meat	Bully beef						2940	
	Other (specify)							
t pie	Bought						2939	
ong	Beef with fat						2911	
	fat trimmed						3021	
	"Droë wors"						2949	
umes:	Stews & curries (specify)							
cify dried beans/	Soups						3157	
s/lentils	Salads						3174	
ed beans							3176	
a products e.g.	Brands at home now Don't know							
ppers	Show examples						3196	
na								
d fish (fresh or	With batter/crumbs						3072	
en fried in sun	Without batter/crumbs						3060	
sh water fish	Specify cooking method						3094	
ecify type	Medium fat, batter, fried							

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/n ever		
ed fish:								
rds	In brine						3055	
	In tomato sauce						3102	
	Mashed with fried onion						A005	
nes	In oil						3087	
	In tomato sauce						3087	
	In oil						3093	
	In brine						3054	
erel							3113	
on							3101	
ed fish/curried							3076	
cakes	Fried: oil/butter/margarine,						3080	
ify canned or	commercial							
d dried fish							2867	
	Boiled/poached						2876	
	Scrambled: in oil						2889	
	in butter						2886	
	in margarine						2887	
	Fried: in oil						2869	
	in butter						2868	
	in margarine						2877	
	in bacon fat						2870	
	Curried						2902	

MANY TIMES A WEEK DO YOU EAT:

MEAT _____
BEANS _____
CHICKEN _____
FISH _____ AND
EGGS _____ ?

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/n ever		
BAGE	Boiled, nothing added						3756	
	Boiled with potato, onion, fat						3813	
	Fried, in margarine (nothing added)						3810	
	Fried, in oil (nothing added)						3912	
	Boiled, then fried with potato and onion						A006	
	Other:							
nach/morogo/	Boiled, nothing added						3913	
no/ other greem	Boiled fat added (margarine)						3898	
y vegetables:	Boiled with onion/tomato, fat						A011	
names:	- onion & potato (margarine)						3901	
	- onion, tomato & potato							
	- with peanuts							
	Other:							
nato and onion	Home made with fat						3910	
vy'/relish/chow	without fat						3925	

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/never		
	Canned						4129	
n	Cooked in fat and sugar						3893	
y Type:	Boiled, little sugar and fat							
	Boiled						4164	
	Other:							
s	Boiled sugar & fat						3819	
	Boiled nothing added						3757	
	Boiled, potato, onion, no fat						3934	
	Boiled, potato, onion, margarine						3822	
	Boiled, with sugar						3818	
	Raw, salad (orange juice)						3711	
	Chakalaka							
	Other							
s/	On cob						3725	
corn	Off cob - creamed sweetcorn						3726	
	Off cob - whole kernel						3942	
ot	Cooked						3698	
	Salad (bought or home made)						3699	
es	Boiled with skin						4155	
	without skin						3737	
	Baked in skin (flesh and skin)						3736	
	Baked in skin (flesh only)						3970	
	Mashed - skim milk, margarine						3875	
	Mashed - whole milk, margarine						3876	
	Roasted in beef fat						3878	
	French fries/potato chips (oil)						3740	
	Salad (mayonnaise and egg)						3928	
	Other:							
potatoes	Boiled with skin						3748	
	without skin						3903	
	Baked with skin (flesh only)						3748	
	without skin						3903	
	Mashed						3903	
	Mashed with fat & sugar						3749	
	Other:							
	Green, frozen						4146	
	Green, frozen with sugar						3720	
	With sugar and butter						3859	
	Tinned peas						4149	
n peppers	Raw						3733	
	Cooked (stew with oil)						3865	
al/egg plant	Cooked						3700	
	Fried in oil						3802	
	Stew (oil, tomato, onion)						3798	
rooms	Raw						3842	
	Sautéed in brick margarine						3839	
	Sautéed in oil						3841	
ns	Sautéed in sun oil						3730	
	Sautéed in margarine						3844	
d vegetables	Raw tomato						3750	
	Lettuce						3723	

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/never		
	Cucumber						3718	
	Avocado's						3656	
Beans	Boiled nothing added						3696	
	Cooked, potato, onion, margarine						3792	
	Cooked, potato, onion, no fat						3933	
flower	Boiled						3716	
vegetables								
fy								
fry veg or add	Butter						3479	
ecify type of	Butro						3523	
ually used	Animal fat (beef tallow)						3494	
box)	Lard						3495	
	Hard margarine (brick)						3484	
	Soft margarine (tub)						3496	
	Soft margarine (light)						3524	
	Sunflower oil						3507	

MANY TIMES A WEEK DO YOU EAT VEGETABLES? _____

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/never		
nnaise/	Mayonnaise: Bought						3488	
	home-made						3506	
d dressing	Cooked sald dressing						3503	
	Salad dressing low-oil						3505	
	Salad dressing French						3487	
	Oil: Olive						3509	
	Oil: Sunflower						3507	
	Oil: Canola						4280	
les	Fresh						3532	
	Canned, unsweetened						4216	
s	Fresh						3582	
	Canned, in syrup						3583	
anas							3540	
nges							3560	
rtjies							3558	
pes							3550	
ches	Fresh						3565	
	Canned, in syrup						3567	
icots	Fresh						3534	
	Canned, in syrup						3535	
ngoes	Fresh						3556	
ypaw	Raw						3563	
apple	Raw						3581	
	Canned, in syrup						3648	
avas	Fresh						3551	
	Canned, in syrup						3553	
termelon							3576	

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/n ever		
ek	Orange flesh						3541	
	Green flesh						3575	
it/berries y types)								
uit (also as	Raisins						3552	
	Prunes (raw)						3596	
	Prunes (cooked with sugar)						3564	
	Peaches (raw)						3568	
	Peaches (cooked with sugar)						3569	
	Apples (raw)						3600	
	Dried fruit sweets						3995	
	Other _____							
ruit								

ANY TIMES A WEEK DO YOU EAT FRUITS? _____

W WILL ASK YOU QUESTIONS ABOUT WHAT YOU USUALLY DRINK

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/n ever		
							4042	
	Ceylon						4038	
	Rooibos						4054	
							4037	
per cup of	White						3989	
coffee	Brown						4005	
er cup of tea	Fresh/Longlife						2718	
fee	Fresh Longlife 2%						2772	
ype of milk	Goat						2738	
u put in tea	Fresh/Longlife/Fat free (skimmed milk)						2775	
	Whole milk powder						2831	
	Reconstituted Specify brand							

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/never		
	Skimmed milk powder Reconstituted Specify brand _____						2719	
	Milk blend, reconstituted Specify brand						2771	
	Whitener/non-dairy creamer Specify brand _____						2751	
	Condensed milk (whole)						2714	
	Condensed milk (skim)						2744	
	Evaporated milk (whole)						2715	
	Evaporated milk (low fat)						2827	
	None							
uch:	Fresh/long life/ whole						2718	
pe of milk do	Fresh/long life/ 2%						2772	
nk as such?	Fresh/longlife/fat free(skimmed)						2775	
	Goat						2738	
	Sour / Maas						2787	
	Buttermilk						2713	
nks	Nestlé - nesquik _____						4287	
brands,	Milo						2735	
ng milk	Flavoured milk _____						2774	
ments and								
milk used	Other							
t	Drinking yoghurt						2756	
	Thick yoghurt, plain, fruit						2732	
	SixO						3990	
	Oros						3982	
	Lecol with sugar						3982	
	Lecol with artificial sweetner						3990	
	Kool Aid						3982	
	Other: _____							
ice	Fresh/ Liquifruit/Ceres/						2866	
	"Tropica"/mixtures with milk						2791	
yrups	Average						2865	
	Guava syrup						2864	
drink	Sweetened						3981	
Fanta	Diet _____						3990	
/Motogo	Sorghum beer						4056	
olic beverages	Specify: _____						4039	
s sorghum								
specify:	Beer average						4031	
	Wine						4033	
	Cider						4057	

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/never		
	Spiritus, e.g brandy, whisky, gin, vodka						4035	
	Liqueur						4055	
	Other: _____							

INDICATE WHAT TYPES AND AMOUNTS OF SNACKS, PUDDINGS AND SWEETS YOU EAT:

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/never		
risps/chips							3417	
	Roasted, unsalted						3452	
	Roasted, salted						3458	
curls:	Average						3267	
, etc.	Savoury						3418	
n	Plain (no salt and butter)						3332	
	Plain (salt and butter added)							
	Sugar coated						3359	
(seeds)							4231	
ɔtes	Milk						3987	
	Kit Kat						4024	
	Peppermint crisp						3997	
	Specify types and names.							
s	Sugus, gums, hard sweets (specify)						3986	
	Peppermint						4004	
	Toffees						3991	
	Hard boiled						3986	
	Fudge, caramels (specify)						3991	
s/cookies	Specify type: _____							
	Home made plain						3233	
	shortbread, butter						3296	
	Commercial, plain						3216	
	Commercial, with filling						3217	
and tarts	Chocolate, plain						3419	
kes/crumpets							3344	
sters							3231	
ries	Sausage rolls						2939	
	Samousas - vegetable						3414	
	Samousas - mutton						3355	
	Biscuits e.g. bacon kips						3331	
	Other: _____							
ng: jelly							3983	

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/n ever		
d pudding	Plain batter						3429	
nt pudding	Skim milk						3314	
	Whole milk						3266	
eam	Commercial regular						3483	
	Commercial rich						3519	
	Soft serve						3518	
	Sorbet						3491	
	Ice lollies						3982	
	Chocolate coated individual ice creams (e.g. Magnum)							
rd	Home made, whole milk						2716	
	Ultramel						2716	
n	Fresh						3520/ 3480	
puddings								
ify)								

MANY TIMES A WEEK DO YOU EAT SNACK FOODS? _____

ES / GRAVIES / CONDIMENTS

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/n ever		
to sauce							3139	
ester sauce							4309	
ey	Fruit						3168	
	Tomato						3114	
s							3866	
t soups							3158	
stock							4029	
en stock							4029	
s:								

BIRDS, ANIMALS, INSECTS OR FRUITS AND BERRIES (hunted or collected in rural areas or on farm, specify)

SE MENTION ANY OTHER FOODS YOU EAT MORE THAN ONCE EVERY TWO WEEKS WHICH WE HAVE NOT TALKED T AND OR FOODS EATEN IN OTHER HOMES OR PLACES DURING THE PAST WEEK

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/n ever		

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/never		

ERE ANY FOODS THAT YOU EAT WHICH WE HAVEN'T TALKED ABOUT? PLEASE LIST THEM.

FOOD	DESCRIPTION	AMOUNT USUALLY EATEN	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/never		

THANK YOU FOR YOUR CO-OPERATION AND PATIENCE

GOOD BYE !

APPENDIX 4

TOLERANCE QUESTIONNAIRE FOR SUBJECTS WHO COMPLETED THE STUDY

Please answer the following questions regarding your participation in the research study:

Respondent number: _____

Surname and Initials: _____

Employee Number: _____

Date of Birth: _____

---

1. Did you experience any vomiting during the study?

1. Yes 2. No

If yes, how many times?

1. After each meal 2. Once a day 3. Once a week

4. Other

2. Did you experience any flatulence during the study?

1. Yes 2. No

If yes, how many times?

1. After each meal 2. Once a day 3. Once a week

4. Other

3. Did you experience any stomach cramps during the study?

1. Yes 2. No

If yes, how many times?

1. After each meal 2. Once a day 3. Once a week

4. Other

4. Did you experience any changes in your lifestyle during the study?

1. Yes 2. No

If yes, describe the changes:

_____

_____

_____

5. Did you experience any constipation during the study?

1. Yes 2. No

If yes, how frequent?

1. After each meal 2. Once a day 3. Once a week

4. Other

6. Did you experience an increased appetite during the study?

1. Yes 2. No

If yes, explain:

_____

_____

7. Did you experience any changes in your alcohol consumption during the study?

1. Yes 2. No

If yes, to what extent?

_____

_____

8. Did you use any medication/supplements during the study?

1. Yes 2. No

If yes, what is the name of the medication/supplement you used?

_____

_____

If yes, how many times did you use this medication/supplement?

1. After each meal 2. Once a day 3. Once a week

4. Other

and for how long (days)?

9. Did you consume all of the experimental capsules every day?

1. Yes 2. No

10. Was the amount of capsules consumed acceptable?

1. Yes 2. No

11. Would you be willing to consume these capsules daily if they are considered as healthy?

1. Yes 2. No

12. Did you experience any other side-effects of the supplement during the study?

1. Yes 2. No

If yes, please specify?

_____

_____

If yes, how frequent?

1. After each meal 2. Once a day 3. Once a week

4. Other

13. Did you experience any positive effects on your health during the study?

1. Yes 2. No

If yes, explain.

_____

_____

14. Do you have any other comments you would like to make regarding the study?

_____

_____

The research team would like to thank you for your co-operation during the study. The project is very important for gaining new scientific knowledge.