



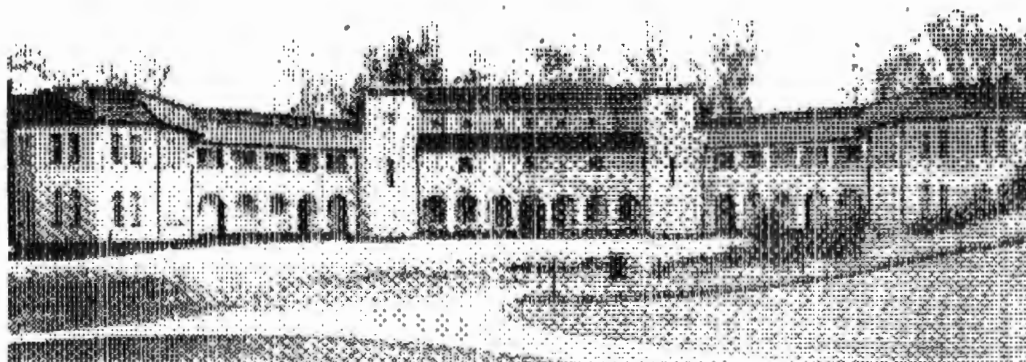
**THE CONTRIBUTION OF PROPIONIC ACID TO SOME
PHYSIOLOGICAL
EFFECTS OF DIETARY FIBRE**

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SUMMARY

Increased intake of dietary fibre (DF) is associated with several beneficial effects on carbohydrate and lipid metabolism, and possibly also on haemostasis. Various mechanisms have been proposed to explain these effects. Dietary fibre influences the secretion, digestion, absorption, and fermentation functions, as well as the morphologic structure of the gastrointestinal tract, and the transit time of food through the gut. This may lead to changes in blood nutrient dynamics and endocrine responses, which may influence cellular substrate availability and hormonal regulation of enzyme activities, with resultant changes in metabolism. Recently, questions have arisen about the physiological importance of short chain fatty acid metabolites of certain plant fibres. Some investigators have speculated that the short chain fatty acids (SCFAs) have metabolic effects that may explain some of the physiologic responses attributed to dietary fibre.

To test the hypothesis that propionate, one of the SCFAs derived from the fermentation of DF and α -amylase resistant starch, contributes to the well-known effects of DF on carbohydrate and lipid metabolism (increased insulin sensitivity and decreased serum cholesterol), the diet of healthy human volunteers was supplemented with sodium propionate. The effect of dietary propionate on carbohydrate and lipid metabolism, as observed from changes in glucose tolerance, insulin response and serum lipid profiles after seven weeks, were compared with the known effects of fermentable DF on these variables.

The study of the effects of propionate on metabolism in human subjects was, of necessity, confined to measurements of metabolites in blood. The literature survey indicated that the metabolism of the liver may be modified by propionate. Consequently, the contribution of propionate to the effects of DF on metabolism was investigated in animal models. The long-term effects of the soluble dietary fibre concentrate konjac-glucomannan (K-GM) and of sodium propionate on serum and liver lipids, glucose tolerance, serum insulin response and sensitivity, liver glycogen content, and plasma fibrinogen were compared in baboons (*Papio ursinus*) fed a semi-synthetic Western diet (SSW-diet). The Zucker "fatty" rat was further used as an insulin resistant, hyperfibrinogenaemic model to compare the therapeutic effects of K-GM and sodium propionate against a background SSW-diet.

Resistant starch and other types of starch which escape digestion in the small intestine may quantitatively be more important as substrates for fermentation and propionate production than DF. The acute (short-term) effects of maize porridge with suspected varying amounts of digestible starch on blood glucose and serum insulin response in healthy volunteers were investigated to determine the physiological importance of resistant starch.

The results of the various studies, which are discussed in detail in each separately presented study, and as a whole in chapter 9, show, *inter alia*, that:

- Supplementation of a Westernised diet with K-GM as well as propionate lower fasting circulating free fatty acid (FFA) levels significantly. This observation has far-reaching implications. It is known that high serum concentrations of FFA are associated with insensitivity to insulin and inhibition of cellular glucose uptake ('Randle effect'), increased plasma fibrinogen levels and also with atherosclerosis. Lower plasma fibrinogen concentrations were observed in experimental animals fed K-GM- or propionate-supplemented SSW-diets. Both supplements resulted in lower fasting serum insulin levels, as well as smaller glucose increments during glucose tolerance tests. These observations may indicate improved insulin sensitivity. An increase in insulin sensitivity may further explain the consistently higher percentage high-density lipoprotein cholesterol observed with both supplements in all the studies.
- In the animal models supplementation of a Westernised diet with K-GM as well as propionate lowered liver glycogen stores. The lower glycogen stores were associated with higher blood glucose levels in the animals fed propionate. These observations indicate that in addition to the documented effect of propionate on gluconeogenesis, propionate also stimulates glycogenolysis and glucose utilisation in the fasting state. It seems as if lipolysis (mobilisation of FFA) is inhibited at the same time. However, the raised blood glucose levels were not observed in the humans.
- Liver cholesterol concentrations were significantly lower in baboons and Zucker rats fed the propionate- and K-GM-supplemented SSW-diets than in animals fed the unsupplemented SSW-diet. This observation is supported by results reported in the literature, indicating inhibition of HMG-CoA reductase, a rate-limiting enzyme in cholesterol synthesis. Changes in the ratios of serum : liver cholesterol indicated that, in combination with inhibition of cholesterol synthesis, a shift in cholesterol from liver to serum occurred.
- Calculations to quantify the contribution of propionate to the biochemical effects of DF in the various models, clearly revealed that the magnitude varies in different models because of species-differences and probably also as a result of an interaction with other specific effects of the DF in a particular model.

The conclusion was reached that propionate contributes substantially to the ability of DF to increase insulin sensitivity and decrease liver cholesterol, circulating FFA and plasma fibrinogen concentrations. Hyperfibrinogenaemia, hypercholesterolaemia, raised FFA levels and glucose intolerance are risk factors for the pathogenesis of atherosclerosis. The results of this investigation reveal basic information on the mechanism of action of DF and also on the physiological importance of propionate, derived from bacterial fermentation of DF. A recommendation is made that subjects at risk for the development of degenerative Western

diseases should increase their intake of fermentable dietary carbohydrate (substrates for propionate).

OPSOMMING

Verhoogde inname van dieetvesel en ensiemweerstandbiedende stysel word met verskeie voordelige effekte op koolhidraat- en lipiedmetabolisme en moontlik ook op hemostase geassosieer. Die inname van dieetvesel veroorsaak deur kort- en langtermynmeganismes sekere veranderinge in die funksie van die spysverteringskanaal. Hierdie veranderinge mag lei tot 'n veranderde nutriëtdinamika in die bloed, en 'n veranderde endokriene respons, byvoorbeeld insuliensekresies, na voedselinname. Dit mag die substraatbeskikbaarheid en die endokriene beheer van metaboliese prosesse in selle oor die hele liggaam beïnvloed. Een van die gevolge van dieetveselinname wat die afgelope paar jaar besondere belangstelling, uitgelok het, is die vorming van kortkettingvetsure (KKVS) in die dikderm wanneer dieetvesel (nie- styselpolisaggariede), asook stysel wat alpha-amilase-aktiwiteit in die dunderm weerstaan, deur mikroflora gefermenteer word. Al drie die KKVS, asynsuur, propioonsuur en bottersuur, word in die poortvene aangetref, maar slegs asynsuur bereik die perifere sirkulasie. Bottersuur word hoofsaaklik deur die kolonselle gemetaboliseer en propioonsuur word deur die lewerselle opgeneem.

Die metaboliese gevolge van KKVS-absorpsie is nog grootliks onbekend en is nog glad nie in mense bestudeer nie. Om die hipotese dat propioonsuur afkomstig van fermentering van dieetvesel en ensiemweerstandbiedende stysel bydra tot die bekende effek van dieetvesel op koolhidraat- en lipiedmetabolisme (verhoogde insuliensensitiwiteit en verlaagde serumcholesterolvlakke) eksperimenteel te toets, is die dieet van gesonde vrywilligers gesupplementeer met natriumpropionaat. Die effekte van propionaat, soos waargeneem kon word uit veranderinge in glukosetoleransie, sirkulerende insulienkonsentrasies en serumlipiede, is na 'n periode van sewe weke bestudeer, en vergelyk met bekende effekte van fermenteerbare dieetvesel.

Die effek van propionaatsupplementering in proefpersone kon vanselfsprekend slegs in veranderinge in metaboliete in die bloed waargeneem word. Uit 'n oorsig van die toepaslike literatuur wil dit voorkom asof metabolisme in die lewer deur propionaat beïnvloed word. Vervolgens is die bydrae van propionaat tot vesel se effekte op metabolisme dus in dierlike modelle bestudeer. Omdat primate as die mees geskikte dierlike model vir studies in verband met kronêre hartvatsiekte sowel as veselfermentering beskou word, is besluit om die dieet van bobbejane met 'n dieetveselisolaat, konjak-glukomannan (K-GM), en met propionaat te supplementeer, ten einde die bydrae van propionaat tot die langtermyn effek van vesel op koolhidraat- en lipiedmetabolisme, sowel as plasmafibrinogeenvlakke te kan kwantifiseer. Die Zucker vetrot is verder as 'n insulienweerstandbiedende, hiperfibrinogenemiese model gebruik om die terapeutiese effekte van konjak-glukomannan en propioonsuur te vergelyk.

Stysel wat gedeeltelik of geheel en al weerstand bied teen hidrolise deur α -amilase in die dunderm, mag kwantitatief belangriker wees as nie-styselpolisaggariëde as substrate vir fermentering en die produksie van propionsuur. Ten einde die fisiologiese belang van ensiemweerstandbiedende stysel te ondersoek, is die akute effekte van mieliepap met verskillende hoeveelhede ensiemweerstandbiedende stysel op bloedglukose en seruminsulienrespons in vrywilligers ondersoek.

Die resultate van die onderskeie studies, wat in die afsonderlike hoofstukke gerapporteer word, en in hoofstuk 9 gesamentlik bespreek word, het onder andere aangetoon dat:

- Supplementering van 'n Westerse dieet met K-GM sowel as propionaat, verlaag die konsentrasie vrye vetsure (VVS) in serum in die vastende toestand betekenisvol. Hierdie bevinding het verreikende implikasies. Verhoogde sirkulerende VVS-konsentrasies word in die literatuur geassosieer met verlaagde insuliensensitiwiteit, verhoogde plasmafibrinogeenkonsentrasies en aterosklerose. Laer plasmafibrinogeenkonsentrasies is dan ook met propionaat- en K-GM-supplementering van Westerse diëte in proefdiermodelle waargeneem. Propionaat- en K-GM-supplemente het ook laer sirkulerende vlakke van insulien, in die vastende toestand sowel as tydens glukosetoleransietoetse, en kleiner inkremente in bloedglukose tydens glukosetoleransietoetse tot gevolg gehad. Hierdie bevindings mag dui op verbeterde insuliensensitiwiteit. 'n Verbetering in insuliensensitiwiteit mag weer die verhoging in hoëdigheidslipoproteïencholesterol, wat in al die studies met beide supplemente waargeneem is, verklaar.
- Supplementering van 'n Westerse dieet met K-GM sowel as propionaat, verlaag die hoeveelheid glikogeen opgeberg in die lewer. Hieruit kan afgelei word dat propionaat glukoseverbruik in die vastende toestand stimuleer, terwyl lipolise en die vrystelling van VVS geïnhibeer word.
- Aansienlik minder cholesterol het in die lewer van proefdiere wat propionaat- of K-GM-supplemente ontvang het, voorgekom. Hierdie bevinding word uit die literatuur ondersteun deur resultate wat daarop dui dat propionaat HMG-CoA-reduktase, 'n snelheidsbeperkende ensiem in die biosintese van cholesterol, in die lewer inhibeer.

Die gevolgtrekkings wat hieruit gemaak kan word, is dat propionaat 'n aansienlike bydrae lewer tot die vermoë van dieetvesel en ensiemweerstandbiedende stysel om insuliensensitiwiteit te verhoog en plasmacholesterol- en -fibrinogeenvlakke te verlaag. Verhoogde aktiwiteit of konsentrasie van fibrinogeen, verhoogde serumcholesterolkonsentrasies en versteurde glukoseverdraagsaamheid is almal bekende risikofaktore vir koronêre hartvatsiektes. Die resultate van hierdie ondersoek dra grootliks by tot kennis in verband met die meganisme waarvolgens dieetvesel die mens hierteen mag beskerm.

ABBREVIATIONS

BI	Behring Institute
BM	Boehringer Mannheim
BMI	body mass index
cAMP	cyclic adenosine monophosphate
cat. no.	catalogue number
CHD	coronary heart disease
dL	desiliter
DF	dietary fibre
ELISA	enzyme linked immunosorbent assay
FDP	fructose 1,6-diphosphate
FFA	free fatty acids
g	gram
GI	glycaemic index
GTT	glucose tolerance test
HDLC	high-density lipoprotein cholesterol
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
HMW	high-molecular weight kininogen
IRI	immunoreactive insulin
IV	intravenous
IU	international units
kg	kilogram
K-GM	konjac-glucomannan
kJ	kilojoule
L	litre
LDLC	low-density lipoprotein cholesterol
mg	milligram
min	minute
MJ	megajoule
mL	millilitre

mmol/L	millimole per litre
NADP	nicotinamide adenine dinucleotide phosphate (oxidised)
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
PA	propionic acid
PEP	phosphoenolpyruvate
Pk	Prekallikrein
POD	peroxidase
PPi	inorganic pyrophosphate
RDS	readily-digestible starch
RS	resistant starch
SCFA	short-chain fatty acids
SD	standard deviation
SEM	standard error of the mean
SSW-diet	semi-synthetic Western diet
TC	total cholesterol
TG	triglycerides
μg	microgram
$\mu\text{U/mL}$	micro units per millilitre
VLDL	very-low-density lipoprotein

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CHAPTER 1

INTRODUCTION

A decade ago, Anderson & Chen (1979) reviewed the effects of dietary fibre (DF) on carbohydrate and lipid metabolism. They proposed a number of mechanisms for the action of DF, *inter alia* a possible effect of 'short chain fatty acid metabolites of certain fibres' on cholesterol synthesis. The literature survey (chapter 2) reveals that in the past few years interest in the breakdown of dietary non-starch polysaccharides (NSP) by anaerobic bacteria (fermentation) to short-chain fatty acids (SCFAs) has grown substantially. Evidence has been presented that 75-80% of dietary NSP is fermented in the gut (Stephen & Cummings, 1980), and that the main SCFAs, acetate, propionate and butyrate could be absorbed rapidly from the colonic lumen (McNeil *et al.*, 1978). Butyrate is largely taken up by colonic epithelial cells, which utilise it to synthesise ketones (Roediger, 1980, 1982). Remaining butyrate, propionate and some acetate are cleared by the liver (Cummings *et al.*, 1988). Acetate passes to peripheral tissues where it is metabolised by muscle (Lindeneg, 1964; Lundquist *et al.*, 1973; Skutches *et al.*, 1979). Propionate metabolism has been studied extensively in ruminants, where it is a major glucose precursor (Bergman *et al.*, 1966). In monogastric species however, much less is known.

The principal energy source for colonic bacterial fermentation is carbohydrate. At first non-starch polysaccharides (NSP) was believed to be the only carbohydrate of significance to the flora. In order to quantify the amount of NSP fermented, Englyst *et al.* (1982) developed a new analytic procedure to separate NSP into cellulose and water-soluble and insoluble non-cellulosic polysaccharides. During the development of the method a 'fraction of starch made resistant to alpha-amylase by food processing was identified. This starch was largely resistant to digestion in ileostomists (Englyst & Cummings, 1985). Further studies on the digestion of the carbohydrates of banana (Englyst & Cummings, 1986) and potato (Englyst & Cummings, 1987a) in the human small intestine, revealed that the amount of starch reaching the large bowel exceeds by up to eight times the amount of NSP. Starch which escapes digestion in the small intestine is therefore a major substrate for fermentation in man. The physiological importance of starch, fermented in the colon to SCFAs which are then absorbed, remains to be established.

From a survey of the literature (chapter 2) it appears that metabolism in the liver may be modified by propionate, the SCFA which is largely cleared by the liver after absorption from the colon. It has been demonstrated *inter alia* that propionate increases hepatic glucose utilisation in rats (Anderson & Bridges, 1984; Veech *et al.*, 1986, see section 2.3), acts as an important substrate for gluconeogenesis in starved rats (Demigné *et al.*, 1986) and decreases liver cholesterol concentration in rats (Chen *et al.*, 1984, see section 2.4). From these

observations, a hypothesis (chapter 3) was formulated that propionate contributes to the well known effects of fermentable NSP on carbohydrate and lipid metabolism (increased insulin sensitivity and decreased serum cholesterol). It was reasoned that if propionate facilitates glucose utilisation as energy substrate in fasting conditions to such an extent as to postpone a rise in circulating free fatty acid (FFA) levels, insulin sensitivity may improve. This reasoning is supported from the literature by the suggestion of Jenkins *et al.* (1980) that lower circulating FFA and ketone levels, induced by a guar-containing first meal, could be associated with the observed improved glucose tolerance four hours later, an effect designated as the 'metabolic' effect of dietary fibre (Jenkins & Wolever, 1981). It is known that high serum concentrations of FFA are associated with hepatic (Strömblad & Björntrop, 1986) as well as peripheral (Randle *et al.*, 1963) insulin insensitivity and inhibition of glucose uptake by cells. High serum FFA levels are also associated with increased fibrinogen synthesis in the liver (Pilgeram & Pickart, 1968).

The hypothesis was tested in two animal models and also in man (chapters 5-8). Standardised methods used to measure the metabolic changes in the different studies, are discussed in detail in chapter 4. In man, the hypothesis was tested experimentally by supplementing the diet of healthy human volunteers with sodium propionate (chapter 7). The effects of dietary propionate on carbohydrate and lipid metabolism, as observed from changes in glucose tolerance, insulin response, fasting serum FFA and other lipid levels, were compared with known effects of DF on these variables.

In an attempt to quantify the contribution of propionate to the effects of DF on serum and liver variables, the long-term effects of propionate supplementation were compared to those of konjac-glucomannan (K-GM), a soluble DF concentrate in animals under controlled conditions. The baboon (*Papio ursinus*), regarded as one of the most suitable models for studies concerning the association of fibre-depleted diets and Western lifestyle diseases (Kritchevsky, 1970), was used as an animal model in which the effects of propionate supplementation could be compared to that of K-GM supplementation on glucose tolerance, immunoreactive insulin response, insulin sensitivity, serum lipid profiles, liver lipid and glycogen content and plasma fibrinogen concentration (chapter 5). The diet fed to the animals was similar in comparison to the diet of industrially developed populations ("Western" diet). The Zucker fatty rat, characterised by insulin resistance and hyperfibrinogenaemia, served as a model in which therapeutic effects of K-GM and propionate on fasting insulin, glucose, serum and liver lipids, liver glycogen and plasma fibrinogen could be examined (chapter 6). The contribution of propionate to the effects of K-GM, as emerged from the results of these studies, is discussed and quantified in chapter 9. The calculations to quantify the contribution of propionate to the biochemical ('metabolic') effects of DF in the various models, clearly revealed that the magnitude varies because of interspecies-differences and because of other confounding effects, such as decreased weight gain caused by K-GM in Zucker rats, but not in baboons.

The conclusion was reached that propionate contributes substantially to most of the beneficial effects of DF on insulin sensitivity, cholesterol homeostasis and lowered plasma fibrinogen concentrations. It is recommended that the dietary intake of substrates for gut fermentation be increased, i.e. NSP as well as those starch fractions which resist hydrolysis. These starch fractions appeared to improve insulin sensitivity from the results of the study reported in chapter 8. It is also recommended that dietary measures should be taken to decrease the concentration of FFA between meals, i.e. increased intake of NSP and resistant starch and increased frequency of meals. This should contribute largely to the improvement of glucose intolerance, hypercholesterolaemia and hyperfibrinogenaemia.

CHAPTER 2

LITERATURE SURVEY: EFFECTS OF DIETARY FIBRE AND RESISTANT STARCH

2.1 DEFINITIONS

The concept of dietary fibre (DF) lacks a precise definition. The definition most widely accepted describes DF as "the skeletal remains of plant cells that are resistant to digestion by enzymes of man" (Trowell, 1974). This physiological definition disregards the chemical characteristics of DF. Chemically, the principal constituents of DF are cellulose, hemicellulose and pectic substances, conveniently designated non-starch polysaccharides (NSP) (Trowell, 1985). The DF component lignin, which is a complex polymer of phenyl propane, is present in negligible amounts in the human diet (Southgate & Englyst, 1985; Englyst *et al.*, 1987c). Details of the chemistry of DF have been described elsewhere (Southgate, 1976; Cummings, 1976; Selvendran, 1984; Southgate & Englyst, 1985; Southgate, 1986). Dietary fibre defined as NSP consists of plant polysaccharides sharing the common feature that they do not contain α -glucosidic linkages (Englyst *et al.*, 1987a). This includes the cell-wall and related plant polysaccharides, but does not include starch (α -glucans) in any form. On further analysis NSP can be divided into cellulose and non-cellulosic polysaccharides (NCP) (Southgate *et al.*, 1978). Cellulose fibres consist of many glucose polymers held together by hydrogen bonds, making for a crystalline X-ray diffraction pattern. Non-cellulosic polysaccharides are a diverse group of substances including hemi-celluloses, pectic substances and similar molecules from plant tissues other than the cell wall, such as gums (e.g. guar gum) and seed mucilages (e.g. ispaghula) (Cummings & Branch, 1986). With the development of analytical methods it became possible to classify NSP into water-soluble and water-insoluble components (Furda, 1977; Schweizer & Wursch, 1979; Asp & Johansson, 1981; Englyst & Hudson, 1987). Insoluble fibres include cellulose, lignin, and many hemicelluloses (Anderson, 1986a). Soluble fibres include the pectic substances, gums, mucilages, and some hemicelluloses (Fleming & Floch, 1986). Soluble fibre may be combined in nature with insoluble fibre substances in complex arrangements which render them insoluble. Vegetables, wheat, and most grain fibres contain more insoluble fibre, while fruits, oats, barley, and legumes contain more soluble fibre (Anderson, 1986a).

It has generally been assumed that starch, the other main class of polysaccharides in the diet of man, is more or less completely digested and absorbed in the small intestine. Recently, however, studies in man indicated that a considerable amount of dietary starch may escape digestion in the small intestine and pass on to the large bowel where it is readily fermented by microbial flora (Anderson *et al.*, 1981; Stephen *et al.*, 1983; Chapman *et al.*, 1985; Englyst & Cummings, 1985, 1986, 1987a). These studies led to a proposed new nutritional classification of starch based on its physical form and susceptibility to pancreatic amylase (Englyst & Cummings, 1987b). The main classes of starch proposed are readily digestible

starch (RDS 1 to 2), three types of partly resistant starch (PRS 1 to 3), and one type (RS) totally resistant to digestion in the small intestine of man (Table 2.1).

TABLE 2.1 NUTRITIONAL CLASSIFICATION OF STARCH*

Type of starch	Physical form	Present in	Predicted fate in small intestine
Readily digest- ible starch (RDS)			
RDS(1)	Dispersed and amor- phous starch	Freshly-cooked food	Completed digested
RDS(2)	Crystalline starch with X-ray diffraction pattern A	Most uncooked cereals and gelatinised starch dried at high tempera- ture	Slowly but almost com- pletely digested
Partially resist- ant starch (PRS)			
PRS(1)	Physically inaccessible starch	Whole or partly milled grains and legumes	Partly digested
PRS(2)	Starch granules with X-ray diffraction pattern B or C	Raw potato and banana	Partly digested
PRS(3)	Mainly retrograded amylopectin	Cooled cooked potato	Partly digested
Resistant starch (RS)			
RS	Mainly retrograded amylose	Cooled cooked potato, white bread and corn- flakes	Resists digestion totally

*From Englyst & Cummings, 1987b.

Readily-digestible starch (RDS) 1

This is dispersed and amorphous starch which is highly susceptible to digestion by pancreatic amylase, and includes most freshly-cooked starchy foods. It is completely hydrolysed in, and absorbed from the small intestine, but may on cooling convert to less digestible types.

Readily-digestible starch (RDS) 2

This is crystalline starch found in most uncooked cereals and in gelatinised starch dried at high temperature. It is degraded slowly but virtually completely in the small intestine.

Partially resistant starch (PRS) 1

This includes starch to which digestive enzymes cannot gain access because of physical barriers in the plant tissue e.g. whole or partly milled grains and legumes.

Partially resistant starch (PRS) 2

This consists of starch granules showing X-ray diffraction pattern type B or C as described by Katz (1937) and is e.g. found in raw potato and banana.

Partially resistant starch (PRS) 3

This consists mainly of retrograded amylopectin. It is formed on cooling moist-heated starchy foods such as potato.

Resistant starch (RS)

RS totally escapes digestion in the small intestine and consists mainly of retrograded amylose which is formed on cooling moist-heated starchy foods, for example cornflakes, bread and potato products. The actual amount of RS in food products is small, ranging from less than 1% in bread to 3.1% in cornflakes (Englyst & Cummings, 1987b) but can be increased to 20% or more during processing. The formation of RS during processing of starchy foods is controlled by water content, pH, heating temperature and time, number of heating/cooling cycles, freezing and drying (Englyst & Cummings, 1987b). Whether starch, which resists digestion in the small intestine and passes into the large intestine where it is fermented, should be included in the DF concept, is currently not clear (Asp, 1987; Englyst & Cummings, 1987b). However, before it has been shown that RS has the same physical and physiological effects as DF in the upper gut, inclusion of RS in any DF definition should be made with caution.

2.2 GASTROENTEROLOGICAL EFFECTS

2.2.1 INTRODUCTION

The different constituents of DF have different effects on and fates in the gut. Other factors which influence the effects of DF on the gastrointestinal tract are summarised in figure 2.1, and include, for example, residence time or transit, maturity of the plant source, the extent of preparation, the overall diet and the length of period over which a particular fibre was eaten (Eastwood & Brydon, 1985). The physical properties of DF that influence function along the gut include water holding, cation exchange, organic adsorption, gel filtration and particle size distribution (Eastwood & Mitchell, 1976). The effect of DF in the digestive tract has been reviewed extensively recently by Vahouny (1987) and Kritchevsky (1988). Effects contributing to eventual changes in metabolism will be discussed briefly.

2.2.2 EFFECTS IN THE MOUTH

Fibre-rich foods have a lower energy density, generally require a longer chewing time (McCance *et al.*, 1953; Duncan *et al.*, 1983), stimulate the flow of saliva (Heaton, 1983) and dilute oral contents (Vahouny, 1987). The prolonged chewing time may promote satiety and reduce food intake (Duncan *et al.*, 1983).

2.2.3 EFFECTS IN THE STOMACH

In the stomach dietary fibre, in general, may delay emptying of nutrients because of the more solid nature of the digested food (MacGregor *et al.*, 1977). The increased volume of gastric juices and the greater distention of the stomach due to the fibre bulk may contribute to a sense of satiation (Davis & Collins, 1978). It appears that the effect of DF on gastric emptying may vary depending on the chemical characteristics of the fibre source (Tadesse, 1986). In general, gastric emptying is slowed in response to the viscous fibre derivatives such as guar gum and pectin (Holt *et al.*, 1979; Wilmshurst & Crawley, 1980; Leeds *et al.*, 1981). Whether the delay in delivery of a viscous mixture from the stomach into the small intestine contributes to the blunting of glucose tolerance curves observed with viscous fibre derivatives, is not clear (Rosenthal & Nasset, 1958; Forman & Schneeman, 1982; Leeds, 1982; Blackburn *et al.*, 1984).

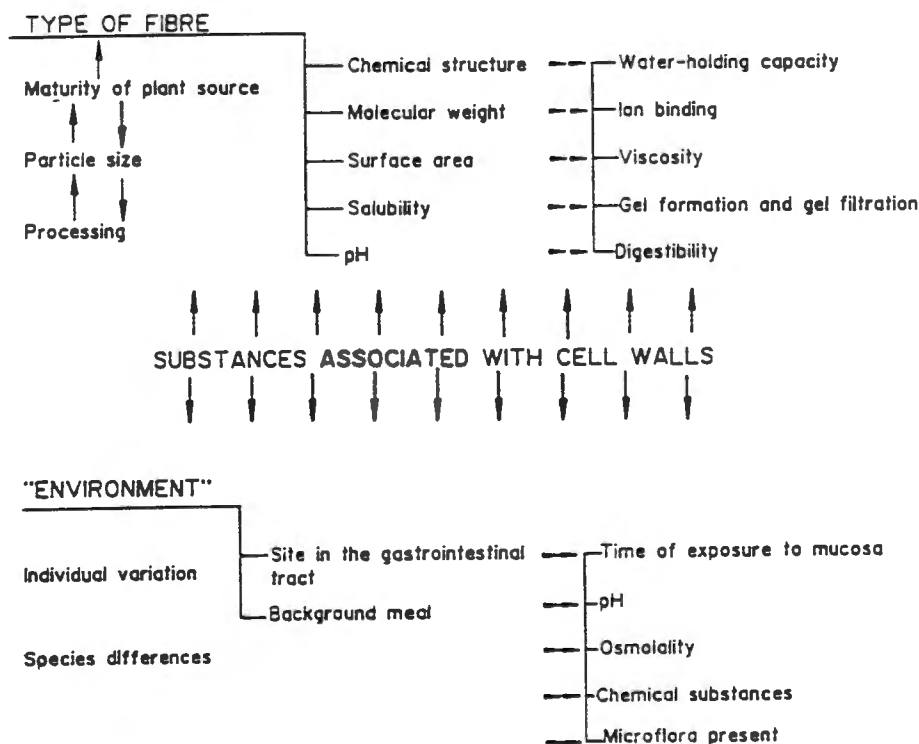


FIGURE 2.1 SCHEMATIC PRESENTATION OF FACTORS WHICH INFLUENCE THE PHYSIOLOGICAL EFFECTS OF DIETARY FIBRE*

* From Vorster (1987)

2.2.4 EFFECTS IN THE SMALL INTESTINE

Dietary fibre has short- as well as long-term effects in the small intestine. The short-term effects are acute responses, for example the decrease in post-prandial glycaemic response to mixed meals or oral glucose loads taken with water-soluble fibres (Jenkins *et al.*, 1978). The effects of prolonged high fibre intake would include adaptation in the morphological structure and function of the small intestine as well as modulation of certain aspects of extraintestinal metabolism

There seems to be a relationship between dietary fibre intake and the morphology of the intestinal wall. The jejunal villi in adults in Western cultures are usually finger-like and regular in contrast to vegetarians (Owen & Brandborg, 1977) and individuals in developing countries (Chacko *et al.*, 1969) who have broad, leaf-shaped villi with many ridges and convolutions. Rats fed fibre-free diets show a finger-shaped villus pattern, whilst rats fed diets containing fibre develop mucosal ridges and a decreased number of jejunal villi (Tasman-Jones *et al.*, 1978). Certain soluble fibre derivatives change the ultrastructural appearance of the intestinal wall (Cassidy *et al.*, 1981) and increase the weight (Farness & Schneeman, 1982) and length (Johnson *et al.*, 1984, Johnson & Gee, 1986) of the small intestine of rats. Dietary fibre may also affect the activities of mucosal enzymes (Brown *et al.*, 1979; Thomsen & Tasman-Jones, 1982) and that of the goblet cells on the villus column (Vahouny *et al.*, 1985). These intestinal adaptations after prolonged intake of specific DF derivatives may alter the absorption of nutrients. In rats, cholesterol absorption is depressed by soluble fibre supplements and oleic acid absorption is delayed (Vahouny *et al.*, 1988).

Some fibres increase the bulk, volume and viscosity of the contents of the intestine and this may lead to slower digestion and absorption. Viscous fibres, such as guar gum and pectin, delay the rate of intestinal glucose absorption by increasing the thickness of the unstirred water layer (Flourie *et al.*, 1984), decreasing the rate of diffusion in the upper small intestine (Blackburn *et al.*, 1984) or resisting the convective effects of intestinal contractions (Edwards *et al.*, 1987, 1988). Delay in glucose absorption may result in the exposure of glucose to a greater length of the small intestine and to more chemical receptors which trigger intestinal feedback inhibition of gastric emptying (Meyer & Fink, 1984). Alternatively, the glucose could reach the ileum before being absorbed (Read *et al.*, 1984) and could trigger other mechanisms that slow gastrointestinal transit ("ileal brake"). Whatever the mechanism, prolonged absorption of glucose results in flattened glycaemic and insulin responses (Jenkins *et al.*, 1977; Miranda & Horwitz, 1978). Insoluble fibres delay glucose absorption to a lesser extent (Anderson *et al.*, 1981).

Diets high in fibre or supplemented with viscous fibre derivatives may affect the secretion of gastrointestinal hormones. A decrease in plasma gastric inhibitory peptide (GIP) (Morgan *et al.*, 1979) and in glucagon (Miranda & Horwitz, 1978; Jenkins *et al.*, 1980) and an increase in somatostatin (Hagander *et al.*, 1986) has been reported. A change in gastrointestinal hormone

secretions could either be because of a physical stimulus of DF on the intestinal mucosal cells or a secondary effect of a changed absorption of nutrients. These gastrointestinal hormones are involved in the regulation of insulin secretion (Marks & Turner, 1977), and their responses to diet modifications may contribute to the overall influence of dietary fibre on nutrient metabolism.

In vitro bile acid adsorption by dietary fibre has been reported for many fibre types (Story & Kritchevsky, 1976). The viscous fibres and the lignins appear to have the greatest bile acid sequestering ability (Leveille & Sauberlich, 1966; Vahouny *et al.*, 1980, 1981). The binding of cholesterol from bile acid micelles by cereal fibres and alfalfa is roughly proportional to bile acid binding (Balmer & Zilversmit, 1974; Eastwood & Mowbray, 1976; Vahouny *et al.*, 1980). The studies of Vahouny *et al.* (1980, 1981) have shown that the sequestration of lipid micelles by fibre also results in reduced availability of fatty acids and monoglycerides for absorption in the upper intestine. This may decrease the rate of absorption or even increase faecal fat. The ability of soluble fibres to adsorb bile acids and increase faecal bile acid and steroid excretion contributes to their plasma cholesterol lowering effect, which is discussed in section 2.4.

In addition to the above-mentioned effects of gel-forming DF on nutrient absorption (delayed gastric emptying of liquid meals, impaired intestinal diffusion of small molecules like glucose and entrapment of fat micelles) the viscosity of guar also promotes the transit to mid-intestine of large, poorly digested pieces of food with markedly reduced absorption of [^{14}C] triolein in the mid-intestine of dogs (Meyer & Doty, 1988).

2.2.5 EFFECTS IN THE LARGE INTESTINE

The functions of the large intestine which are measurably affected by DF and RS are the following: bacterial metabolism/fermentation; adsorption of bacterial metabolites (including secondary bile acids); transit time; intracolonic pressure; flatus production; faecal composition and water content; faecal bulk and consistency, and defaecation (Royal College of Physicians of London, 1980). The subject has been reviewed recently by Eastwood & Brydon (1985), Roth & Leitzman (1985), Read (1986), and by Ornstein & McLean Baird (1987).

Plant fibre components and other nutrients that have escaped digestion in the small intestine, as well as endogenous carbohydrate and protein, are broken down by anaerobic bacteria in the colon, a process usually referred to as fermentation, closely resembling that occurring in the rumen. Cummings (1981a, b, 1983, 1984a, b, c, 1985), Cummings & Branch (1986), Fleming & Floch (1986), and Cummings & Englyst (1987) have reviewed the literature and discussed at length the evidence for fermentation in the human large intestine; the site and biochemistry of fermentation; the microflora involved in the process; substrates; end-products and implications for health. The latter three aspects will be summarised briefly. The possible effects of propionate on carbohydrate and lipid metabolism will further be discussed in sections 2.3 and 2.4 respectively.

Substrates for fermentation

Cummings *et al.* (1986) and Cummings & Englyst (1987) have tabulated the potential substrates for fermentation (table 2.2).

TABLE 2.2 POTENTIAL SUBSTRATES FOR FERMENTATION*

A. Carbohydrate

1. Starch
2. Nonstarch polysaccharides (dietary fibre)
3. Non-absorbed sugars (lactose, lactulose, sorbitol, xylitol)
4. Oligosaccharides (raffinose and stachyose)
5. Modified cellulose

B. Protein

1. Unabsorbed dietary residues
2. Endogenous secretions (eg. pancreatic enzymes)

C. Other substances

1. Mucus (glycoprotein)
 2. Dead cells (epithelial and microbial)
-

*From Cummings *et al.* (1986) and Cummings & Englyst (1987).

Resistant starch (RS) and other types of starch which escape digestion in the small intestine (see section 2.1) may quantitatively be more important as substrates for fermentation than NSP (Cummings & Englyst, 1987). Measuring the amount of starch that actually reaches the colon has proved to be extremely difficult. In some studies breath hydrogen excretion has been used as a marker for fermentation (Anderson *et al.*, 1981; Wolever *et al.*, 1986). Other methods used include direct measurements in ileostomy patients (Chapman *et al.*, 1985; Englyst & Cummings, 1985, 1986, 1987) and intubation of healthy subjects to measure the amount of carbohydrate passing through the ileum (Stephen *et al.*, 1983). Amounts of carbohydrate, malabsorbed from various dietary starch sources, measured by the different techniques, ranged from 2.6% from wheat and potato starch (Chapman *et al.*, 1985) up to 69% from banana starch (Englyst & Cummings, 1986). From the results of studies reported at that time, Cummings & Branch (1986) concluded that 5-10% (15-20g per day) of the starch in Western type diets is available for fermentation. Where starchy foods are the main part of the diet, such as maize in the rural African diet, and especially when it is eaten cooled, much more carbohydrate may escape digestion and absorption in the small intestine. Depending on the type of food and its processing, as much as eight times more starch than NSP may be available for fermentation (Cummings *et al.*, 1986).

The amount of NSP reaching the colon varies from between 15 and 30 g/day in the UK, Scandinavia, the Netherlands and Japan (Bingham *et al.*, 1979; Englyst *et al.*, 1982; Van Staveren *et al.*, 1982; Minowa *et al.*, 1983) to more than 100 g/day in parts of Africa (Lubbe, 1971). The extent of NSP fermentation depends on its chemical structure e.g. lignification and

water solubility (Keys *et al.*, 1969, 1970; Van Soest, 1973; Cummings *et al.*, 1979), its particle size (Heller *et al.*, 1980), residence time in the colon (Stephen, 1980), and other factors such as the microflora of the host (Salysers, 1979). Digestibility may also be greatly influenced by the physical characteristics of the bolus that enters the colon; a complex gel filtration system may exclude bacteria and decrease digestion (Eastwood & Mitchell, 1976). In general, NSP from foods are virtually completely degraded except for the highly lignified sources such as bran (Cummings & Englyst, 1987).

Other substrates for fermentation include malabsorbed lactose (Wolin & Miller, 1983; Scrimshaw & Murray, 1988), and some low molecular weight oligosaccharides, such as raffinose and stachyose, found mainly in beans (Calloway & Murphy, 1968; Tadesse *et al.*, 1980). Modified celluloses, which are nowadays incorporated into food to improve texture and properties, are probably partly fermented (Figdor & Bianchine, 1983; Fritz *et al.*, 1985). Excluding modified celluloses, the amount of dietary carbohydrate other than starch and NSP is probably not more than 10g per day, unless the diet contains unusual amounts of beans or undigested lactose (Cummings & Englyst, 1987). Endogenous carbohydrate sources for fermentation include intestinal mucous (Salysers, *et al.*, 1977; Hoskins & Boulding, 1981) and chondroitin sulfate (Salysers *et al.*, 1982) of which 3-5g may daily be degraded by colonic bacteria (Cummings & Englyst, 1987).

The total amount of carbohydrate entering the large intestine in those living on Western diets may be 40-50g/day (Cummings & Englyst, 1987). Unabsorbed dietary protein residues and endogenous protein, mainly pancreatic enzymes, add a further estimated 12g daily to the substrates available for fermentation (Cummings & Englyst, 1987).

End products

The final products of fermentation are hydrogen, carbon dioxide, methane in a third of the European and North American populations, and short-chain fatty acids (Bond *et al.*, 1971; Pitt *et al.*, 1980; Bjorneklett & Jenssen, 1982). The gases are excreted per rectum or are absorbed, circulated and excreted through the lungs. The short-chain fatty acids (SCFAs) in mixed faecal culture include acetate, propionate and butyrate, with acetate as the predominant anion (Rubinstein *et al.*, 1969). Small amounts of branched chain fatty acids isobutyrate, valerate and isovalerate are derived from polypeptide fermentation (Thomsen *et al.*, 1982; Rasmussen *et al.*, 1988). Other products of fermentation include ammonia, various carboxylic and phenolic acids and amines (Cummings & Englyst, 1987). Ammonia may be incorporated into bacterial protein or absorbed depending on the amount of carbohydrate being fermented, whilst the phenols may be excreted in urine (Cummings & Englyst, 1987). During the process of fermentation materials trapped by fibre, e.g. metal ions may be liberated either for use by host or bacteria (Jenkins *et al.*, 1986a). The SCFAs produced in the colon lower the pH (Cummings, 1983; Robblee *et al.*, 1986) and, as a result, 7- α -dehydroxylation of bile acids is reduced (Midtvedt & Norman, 1968).

One of the approaches used to estimate the amount of SCFAs produced in the human colon is to measure the supply of substrate for fermentation and then calculate the theoretical production rates by using equations derived from rumen metabolism (Hungate, 1966) or that proposed for man (Miller & Wolin, 1979). By using these equations it can be predicted that 40-50g carbohydrate will yield theoretically 400-500 mmol SCFAs (Cummings & Branch, 1986). The rate of fermentation may be affected by the nature of the carbohydrate. *In vitro* studies have shown that soluble polysaccharides such as pectin are fermented more rapidly than relatively insoluble ones like xylan (Englyst *et al.*, 1987b). Furthermore, fermentation of polysaccharides varies markedly from person to person (Stephen, 1980).

Different types of substrate also produce different proportions of SCFAs. All substrates produce acetate as the major end product but the relative amounts of propionate and butyrate vary. Starch yields large amounts of butyrate in an *in vitro* faecal incubation system whereas xylan and pectin lead to very little butyrate and propionate (Englyst *et al.*, 1987b). In the same system arabinogalactan produces more propionate. Differences in the relative proportions of SCFA produced and/or absorbed with different substrates have also been found *in vivo* in animal studies. In rats, fermentation of guar gum raises propionate concentrations (Tulung *et al.*, 1987); pectin raises acetate and lowers butyrate (Thomsen *et al.*, 1984) and gum arabic produces large amounts of butyrate (Topping *et al.*, 1987b; Tulung *et al.*, 1987). Wheat bran and porridge oats produce mainly acetate and propionate in pigs, with butyrate contributing less than 8% of the total SCFAs (Topping *et al.*, 1985a). From the observations of Cheng *et al.* (1987) in rats it appears that the aleuron fraction of wheat bran is responsible for the high yield of propionate. The relative proportion of butyrate is raised by feeding fibre fractions as mixtures rather than as individual components (Topping *et al.*, 1985b). When feeding individual components, the SCFA profile seems to be related to the monosaccharide composition of the substrate. For example, lactulose is converted to acetate only, as is the case with its monosaccharide components D-galactose and D-fructose (Mortensen *et al.*, 1988). Differences in fermentation profiles from various substrates have also been ascribed to changes in the microflora of the host (Cheng *et al.*, 1987; Tulung *et al.*, 1987).

Major species differences exist in the absolute and relative amounts of SCFAs produced and/or absorbed (Mitchell *et al.*, 1985). In a comparative study of the *in vitro* SCFA profile of humans, baboons, pigs and rats in response to pectin and cellulose it was found that the SCFA profile of the rat most closely resembles that of the human (Lupton & Villalba, 1988). Nyman *et al.* (1986) also reported a good correlation between man and rat in fibre degradation *in vivo*. In the *in vitro* system used by Lupton & Villalba (1988) the greatest differences were seen with the pig. The only interspecies difference in pectin fermentation was a higher concentration of propionate in pigs relative to humans. Cellulose fermentation varied largely between the different species. Greater amounts of acetate and butyrate were produced in baboon faeces than in human material. Furthermore, comparison between the hepatic portal vein SCFA profile of rats and pigs fed the same fermentable substrates also revealed species

differences (Topping *et al.*, 1985a). These results are in contrast to previously reported similarity in various animals (Hungate, 1966; Argenzio & Southworth, 1974; Rémésy & Demigné, 1976). The reason for this discrepancy is not clear, but may be the result of improvement in analytical methods. Species differences may be of significance in relation to the choice of animal models for fermentation studies in man.

Absorption of SCFAs

The SCFAs are rapidly absorbed from the colonic lumen (McNeil *et al.*, 1978; Hoverstad, 1986; Rechkemmer *et al.*, 1988) in a process that stimulates the uptake of sodium and water (Ruppin *et al.*, 1980; Roediger & Moore, 1981). Bugaut (1987) reviewed the subject extensively. Butyrate is largely taken up by colonic epithelial cells, which utilise it to synthesise ketones (Roediger, 1980, 1982). Butyrate also affects nucleic acid metabolism in colonic cells through its capacity to stabilise chromatin structure during cell division (Kruh, 1982; Smith, 1986). Furthermore, butyrate is also known to inhibit tumour growth (Sakata & Yajima, 1984; Nordenberg *et al.*, 1986). Remaining butyrate, propionate and some acetate are cleared by the liver (Cummings *et al.*, 1988). Acetate passes to peripheral tissues where it is metabolised by muscle (Lindeneg *et al.*, 1964; Lundquist *et al.*, 1973; Skutches *et al.*, 1979). Acetate levels rise and peak 2-4 hours after a lactulose dose (Pomare *et al.*, 1985). Pectin does not produce elevations in acetate until 6 hours after the dose. The acetate remains elevated for 12 hours. Despite the difference in time course, the areas under the curves are the same regardless of the source of carbohydrate, indicating equal total absorption (Pomare *et al.*, 1985).

Absorbed SCFAs represent about 70% of the energy available in the original fermented substrate (McNeil *et al.*, 1978). SCFA metabolism accounts for *ca* 7% of energy maintenance requirement in the rat (Yang *et al.*, 1970), dog (Stevens *et al.*, 1980) and man (Cummings, 1981b); 30% in ponies (Glinsky *et al.*, 1976) and 30-76% in the pig (Stevens *et al.*, 1980). The effect of propionate on metabolism in man is largely unknown. The effect of propionate on carbohydrate and lipid metabolism in rats and pigs (Anderson & Bridges, 1984; Chen *et al.*, 1984; Illman *et al.*, 1988) will be discussed in sections 2.3 and 2.4 respectively.

The unfermented material, along with bacterial cells, salts, and water form the faecal bulk. The effects of dietary fibres on faecal bulk have been reviewed extensively by Cummings *et al.* (1978) and Smith & Eastwood (1980). In general, the insoluble fibres, such as wheat bran, are the least fermentable and have the greatest effects on faecal output and frequency. Soluble fibres, such as guar and pectin however, are virtually completely fermented and have little effect on stool bulk or frequency (Jenkins *et al.*, 1979a; Stephen & Cummings, 1980; Fleming *et al.*, 1983; Nyman *et al.*, 1986). Fermentable plant material may raise faecal bulk through an increase in faecal bacteria (Stephen & Cummings, 1980).

2.2.6 SUMMARY

Dietary fibre influences the secretion, digestion, absorption and fermentation functions, as well as the morphologic structure of the gastrointestinal tract and transit time of food through the gut. The response within the gastrointestinal tract is dependent on the physical and chemical properties of the fibre source, the amount of starch maldigested in the small intestine and on the location within the gut. The amount of substrate available for fermentation in the large intestine can be regulated to a great extent by the composition of the diet and the processing of the food before consumption. The changes in gut function as a result of DF and RS intake, will lead to changes in blood nutrient dynamics (i.e. changes in tempo of concentration changes of nutrients in the blood). Furthermore, changes in endocrine responses after fibre rich meals can be expected. This may influence cellular substrate availability and hormonal regulation of enzyme activities, with eventual resultant changes in metabolism. These changes in metabolism will be discussed in the next section, and are probably partly responsible for the protective effect of dietary fibre against degenerative Western diseases, noted in the early seventies (Burkitt *et al.*, 1974).

2.3 EFFECTS ON CARBOHYDRATE METABOLISM

The acute and long-term effects of DF on carbohydrate metabolism have been evaluated by supplementing the diet with fibre or fibre components (e.g. Jenkins *et al.*, 1979b) or by modifying the diet to provide high fibre foods (e.g. Anderson & Ward, 1978). Studies on the effect of DF intake on glucose and lipid metabolism of diabetic subjects have recently been summarised by Anderson (1986b) and Anderson & Bryant (1986). Reduced postprandial glucose and insulin levels with increased fibre intake have been observed in diabetic subjects (Anderson, 1986b), healthy subjects (Jenkins *et al.*, 1978), and in animal models e.g. rats (Vachon *et al.*, 1988) and baboons (Vorster & De Jager, 1984). The most viscous fibres seem to be the most effective in this respect (Jenkins *et al.*, 1978), although improvement in glucose tolerance after a test meal containing bran (Jeffreys, 1977), and diets rich in insoluble plant fibres such as wheat bran has been reported for healthy subjects (Brodrigg & Humphreys, 1976; Munoz *et al.*, 1978).

In non-diabetic human subjects, Jenkins *et al.* (1980) have shown that guar taken with a test meal can also improve glucose tolerance with a guar-free meal 4 hours later. Addition of guar to the first glucose load resulted in lower plasma free fatty acid (FFA) and blood 3-hydroxybutyrate levels 4 hours later, compared to a fibre-free first meal (Jenkins *et al.*, 1980). The authors suggested that slow absorption of carbohydrate with guar might result in sustained improvement in glucose handling, and might also alter fat metabolism (Jenkins & Wolever, 1981). The uptake and utilisation of glucose in tissues are known to be inhibited by raised circulating levels of FFAs and ketones (Randle *et al.*, 1963). Lower FFA and ketone levels, induced by a fibre-containing first meal, could thus be associated with the lower blood

glucose and serum insulin responses observed with a second meal, compared to the second meal after a fibre-free first meal (Jenkins *et al.*, 1980).

The incorporation of fibre-rich foods into mixed meals results in significantly different postprandial blood glucose response than when taken alone (Coulston *et al.*, 1984). The results of studies regarding the second meal response to mixed-food meals revealed contradictory results. Post-prandial glucose response to a wheat-bread lunch was found to be lower when a breakfast meal containing lentils had been consumed (Jenkins *et al.*, 1982). However, post-prandial glucose response was very similar after a standard whole-wheat-bread meal was consumed 4 hours after three mixed food breakfast meals consisting predominantly of either red kidney beans, bran cereal or white bread (Shaheen & Fleming, 1987). Insulin response following the bean-containing breakfast was consistently, although not statistically significantly, lower than responses following the standard second meal 4 hours after the other breakfasts.

Contradictory results have also been reported in investigations of the relative ability of mixed meals, containing carbohydrate-rich foods, to raise plasma glucose (so-called glycaemic index). The glycaemic index (GI) concept, which was developed by Jenkins *et al.* (1981a), compares blood glucose responses to 50g carbohydrate portions of various test foods to bread or 50g glucose. Large differences in the GIs of common carbohydrate-containing foods, when eaten alone, have been demonstrated. These differences were ascribed to differences in digestibility (Jenkins *et al.*, 1982) and other factors (Jenkins *et al.*, 1986b). When eaten as part of a mixed meal, many other factors may influence the glycaemic response (O'Dea *et al.*, 1980; Thorne *et al.*, 1983; Heaton *et al.*, 1988) such as anti-nutrients, particle size of DF and interaction between other nutrients in the meal. Some authors reported a good correlation between the response observed to mixed meals and that predicted by published glycaemic indices of the component foods in healthy subjects (Wolever *et al.*, 1985; Laine *et al.*, 1987; Chew *et al.*, 1988) as well as diabetic subjects (Collier *et al.*, 1986). However, no significant difference in glycaemic response to mixed meals containing different carbohydrate sources was reported for healthy subjects by Calle-Pascual *et al.* (1986) and for subjects with noninsulin-dependent diabetes by Coulston *et al.* (1984) and Laine *et al.* (1987). Overall, it appears that the glycaemic index approach is reasonably successful in predicting the glucose and insulin responses of healthy subjects, and that the incorporation of legumes in mixed meals results in responses which correspond well with predicted values.

Cooking or processing may alter the glycaemic effects of foods (Thorne *et al.*, 1983; Brand *et al.*, 1985; Wolever *et al.*, 1986). With the exception of pasta (Jenkins *et al.*, 1983) and potato crisps (Brand *et al.*, 1985), processed foods produce a higher glycaemic index than conventionally-cooked foods, possibly due to an increased degree of starch gelatinisation (Wolever *et al.*, 1986; Ross *et al.*, 1987), or increased disruption of the organised granular structure (Brand *et al.*, 1985). In view of the suggestion of Englyst & Cummings (1987a, b) that

the formation of starch, resistant to hydrolysis with pancreatic amylase, can be increased during the processing of starchy foods, an attempt should be made to evaluate the glycaemic response of foods with varying amounts of partially and totally resistant starch.

Studies using xylose, which is not metabolised, have shown that the lower glycaemic response to fibre-enriched meals is due to delayed carbohydrate absorption, rather than to malabsorption (Jenkins *et al.*, 1978). In addition to delayed absorption, increased oxidation of glucose was observed in the first hour after consumption of a ¹⁴C-starch meal in rats adapted to a 5% pectin diet (Forman & Schneeman, 1982). The authors referred to reduced blood glucagon levels, reported by Miranda & Horwitz (1978) and Munoz *et al.* (1979), and speculated that lower blood glucagon might result in decreased glycogenolysis, gluconeogenesis and mobilisation of fatty acids from adipose tissue, and an increase in insulin:glucagon ratio (Forman & Schneeman, 1982). These shifts in metabolism would favour a greater oxidation of glucose in the pectin-fed rats. However, it has been shown repeatedly that high fibre intake lowers insulin levels (Jenkins *et al.*, 1976; Kay *et al.*, 1981; and many others). An increase in insulin:glucagon ratio, as suggested by Forman & Schneeman (1982), thus seems unlikely. Enhanced peripheral insulin sensitivity, observed by Anderson (1986b), seems to be responsible for increased glucose utilisation. Increased insulin receptor numbers on circulating monocytes (Anderson, 1986b) as a result of an upregulation of receptors associated with lowered circulating insulin levels (Kahn, 1985), may contribute to the enhanced insulin sensitivity associated with long-term high fibre intake (Anderson, 1986b). Further confirmation of the effect of DF on insulin sensitivity comes from the results of Ogunwole *et al.* (1987), who showed that DF increases tissue responsiveness to insulin, as measured by stimulation of pyruvate dehydrogenase activity in adipose tissue.

Effects of propionate on hepatic carbohydrate metabolism

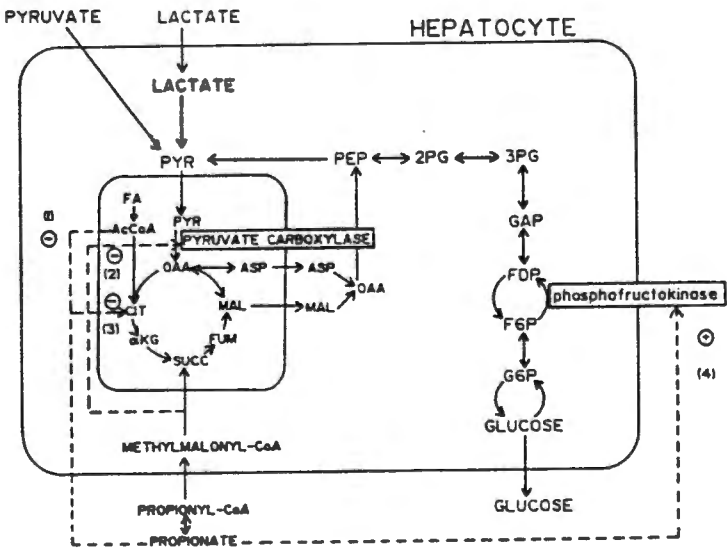
Propionate is potentially glucogenic in ruminants (Bergman *et al.*, 1966; Leng, 1970) and to a lesser extent in other mammals (Marty & Vernay, 1984; Ford & Simmons, 1985). Rats adapted to a high fibre diet use mainly propionate as gluconeogenic substrate during periods of starvation, as opposed to rats on a fibre-free diet who predominantly utilise lactate (Demigné *et al.*, 1986). Gluconeogenesis from lactate is inhibited by propionate *in vivo* (Williams *et al.*, 1971; Demigné *et al.*, 1986), in the perfused rat liver (Williams *et al.*, 1971; Chan & Freedland, 1972; Blair *et al.*, 1973) and in isolated rat hepatocytes (Anderson & Bridges, 1984; Demigné *et al.*, 1986). Furthermore, propionate stimulates glycolysis in isolated rat hepatocytes by 15-24% (Anderson & Bridges, 1984).

The mechanism(s) whereby propionate could stimulate hepatic glycolysis (in the fed state) and hepatic glucose production (in the fasting state) are shown in figure 2.2. Inhibition of conversion of lactate to glucose has been ascribed to inhibition of pyruvate carboxylase resulting from a decrease in acetyl-CoA concentration (Williams *et al.*, 1971). Propionate inhibits pyruvate carboxylase activity further via the production of methylmalonyl-CoA and

succinyl-CoA, specific inhibitors of this enzyme (Scrutton & Utter, 1967). Glycolytic rates are influenced by the activity of phosphofructokinase. Citrate, which appears to be an inhibitor of this enzyme (Pilkes *et al.*, 1978), is reduced by propionate metabolites (Blair *et al.*, 1973). The result is a prevention of inhibition of phosphofructokinase and thus an increased rate of glycolysis.

The role of glucose as a substrate for liver metabolism has been questioned by Katz & McGarry (1984). However, in rats fed high carbohydrate diets, the liver constitutes a significant site of glucose utilisation (30% of absorbed glucose) (Demigné *et al.*, 1986). During starvation propionate contributed to about 25% of produced glucose in rats adapted to a high fibre diet (Demigné *et al.*, 1986). By promoting hepatic glucose utilisation, propionate may contribute to the favourable effects of fibre intake on glucose metabolism. Much further study is necessary to delineate the mechanisms of action of SCFAs on glucose metabolism and the physiological significance thereof.

Increased metabolism (utilisation) of glucose, accompanied by lower circulating levels of glucose and insulin, will affect lipid metabolism. Furthermore, propionate may affect lipid metabolism directly by inhibiting fatty acid oxidation (Glasgow & Chase, 1976) and by other mechanisms not yet delineated.



1. Williams *et al.*, 1971

2. Scrutton & Utter, 1967

3. Blair *et al.*, 1973

4. Anderson & Bridges, 1984

FIGURE 2.2 PROPOSED NETTO EFFECTS OF PROPIONATE ON GLUCONEOGENESIS AND GLYCOLYSIS IN THE LIVER.

Abbreviations: PYR: pyruvate, FA: fatty acid, AcCoA: acetyl-CoA, CIT: citrate, αKG: α-ketoglutarate, SUCC: succinate, FUM: fumarate, MAL: malate, OAA: oxaloacetate, ASP: aspartate, PEP: phosphoenolpyruvate, 2PG: 2-phosphoglycerate, 3-PG: 3-phosphoglycerate, GAP: glyceraldehyde-3-phosphate, FDP: fructose, 1,6-diphosphate, G6P: glucose 6-phosphate.

2.4 EFFECT ON LIPID METABOLISM

The hypocholesterolaemic effects of water-soluble DF have been documented in numerous studies in humans and animals (Chen & Anderson, 1986; Kritchevsky, 1987; Miettinen, 1987). Insoluble NSP fractions have very little influence on total serum cholesterol (TC) levels (Truswell & Kay, 1975). Reductions of serum cholesterol are accompanied by decreases in cholesterol content of the liver in experimental animals such as chickens (Fisher *et al.*, 1966), guinea pigs (Wells & Ershoff, 1962) and rats (Wells & Ershoff, 1962; Kiriya *et al.*, 1969; Chen *et al.*, 1981; Vorster *et al.*, 1985; Klopfenstein & Hosney, 1987; Beyers, 1988; Shinnick *et al.*, 1988). When hamsters (Sablé-Amplis *et al.*, 1987) and rabbits (Wells & Ershoff, 1962) were fed cholesterol-containing diets, pectin lowered liver cholesterol 50% and 12% respectively, but did not change or raise serum cholesterol. The response in experimental animals to dietary fibres is usually dependent on the species examined as well as the hypercholesterolaemic nature of the diet. Because of these variables, results of animal experiments are difficult to compare.

The percentage change in plasma cholesterol due to fibre treatment observed in several human clinical studies has been summarised by Anderson & Tietzen-Clark (1986) and Schneeman & Lefevre (1986). Oat bran, several gums, legumes and pectin reduce plasma cholesterol by 6-19%. The cholesterol-lowering ability of DF appears to be the greatest in hyperlipidaemic patients (Schneeman & Lefevre, 1986). Locust bean gum reduce plasma cholesterol by 10-17% in patients with familial hypercholesterolaemia (FH), and by 6-10% in normocholesterolaemic subjects (Zavoral *et al.*, 1983). However, patients with FH do not always show a greater response to DF than subjects with diet-induced hypercholesterolaemia. Konjac-glucomannan, a gel-forming fibre concentrate, lowered TC by 5.9% in patients with FH, but by 13.3% in subjects with diet-induced hypercholesterolaemia (Venter *et al.*, 1987). The decrease in serum cholesterol concentration observed with these water-soluble fibres usually occurs in the low-density lipoprotein (LDL) fraction, with no change or an increase in high-density lipoprotein cholesterol (HDL), resulting in a higher HDL/LDL cholesterol ratio (Chen & Anderson, 1986). According to Behall *et al.* (1984), the addition of 0.75 g fibre (gum)/100 Cal to a basal diet lowers TC in healthy subjects without decreasing HDL. The effect of DF on the protein and lipid composition of lipoprotein particles is not well documented (Schneeman & Lefevre, 1986).

Several mechanisms are probably involved in the hypocholesterolaemic effects of DF (Eastwood, 1987; Katan, 1987). Anderson & Chen (1979) reviewed and classified the proposed mechanisms into the following three major categories:

- altered intestinal absorption, metabolism and release of cholesterol;
- altered hepatic metabolism and release of cholesterol, and
- altered peripheral metabolism of lipoproteins.

The absorption of cholesterol is reduced by certain fibres (Vahouny *et al.*, 1980; Vahouny *et al.*, 1988). In addition, some soluble fibres, such as guar, pectin, and oat bran, increase faecal bile acid excretion, which may cause an increase in the conversion of cholesterol to bile acids and enhance the loss of cholesterol (Story, 1986). It has also been suggested that the ingestion of DF, especially guar gum, may retard the lymphatic transport of dietary triglycerides as large chylomicrons, thereby altering subsequent chylomicron metabolism (Imaizumi & Sugano, 1986). Schwartz *et al.* (1983) also reported alterations in cellular synthesis of cholesterol and phospholipid in the rat jejunum in response to diets containing pectin.

There are indications that not all soluble fibres increase faecal bile acid excretion (Chen & Anderson, 1986; Shuttler *et al.*, 1987), although results from studies with legumes are conflicting (Shuttler *et al.*, 1987). Furthermore, several studies indicate that the binding capacity of a fibre fraction is not necessarily related to its serum cholesterol-lowering ability (McLean Ross *et al.*, 1983; Anderson *et al.*, 1984, Lund, 1984). The magnitude of faecal loss of bile acids resulting from soluble fibre intake, is relatively small (Chen & Anderson, 1986). Thus, factors other than alterations in bile acid excretion and metabolism, contribute to the hypocholesterolaemic effects of soluble fibres. These fibres are readily fermented in the colon (see section 2.2). Anderson & Chen (1979) suggested that the "short chain fatty acid metabolites of certain fibres may alter (liver) cholesterol synthesis".

Effects of SCFAs on cholesterol metabolism

The effects of SCFAs on cholesterol metabolism in hindgut fermenting species have been investigated mainly in rats and pigs. The results of studies concerning the hypocholesterolaemic effects of propionate are summarised in table 2.3. Propionate, the SCFA which is cleared by the liver after absorption from the gut, is hypocholesterolaemic in rats (Chen *et al.*, 1984; Illman *et al.*, 1988) and in pigs (Boila *et al.*, 1981; Thacker & Bowland, 1981; Thacker *et al.*, 1981), either by inhibiting hepatic cholesterol synthesis (Boila *et al.*, 1981; Chen *et al.*, 1984) or redistribution of cholesterol from plasma to liver (Illman *et al.*, 1988). Inhibition of cholesterolgenesis by SCFAs in rat liver microsomes has been attributed by Ide *et al.* (1978) to decreased activity of the rate limiting enzyme of cholesterol synthesis, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, whilst Bush & Milligan (1971) reported inhibition of HMG-CoA synthase activity by propionic acid in bovine liver homogenates. From the secondary metabolites in the urine of patients with propionic acidemia it can be inferred that the enzyme inhibited by propionic acid metabolites is HMG-CoA reductase (Mienie *et al.*, 1988). In these patients propionyl-CoA condenses with acetyl-CoA to give 3-oxovaleryl-CoA (Kuhara *et al.*, 1983). Condensation with another propionyl-CoA gives 3-hydroxy-3-ethylglutaryl-CoA, as indicated in figure 2.3. Mienie *et al.* (1988) suggests that 3-OH- 3-ethylglutaryl-CoA acts as an inhibitor of HMG-CoA reductase, thereby decreasing the amount of cholesterol synthesised in the liver. Furthermore,

methylmevalonate, derived from 3-OH-3-ethylglutaryl-CoA, cannot be converted to cholesterol (Mienie *et al.*, 1988).

TABLE 2.3 EFFECT OF DIETARY PROPIONATE ON PLASMA CHOLESTEROL IN ANIMAL MODELS

Reference	Animal model	Study design	Results
Bush & Milligan, 1971	Bovine liver	Addition of 15 and 30 mM propionic acid.	Inhibition of HMG-CoA synthase activity (30 and 58% respectively).
Ide <i>et al.</i> , 1978	Rat liver microsomes	Rats fed various dietary fats at 10% level for 19 days. Activity of hepatic HMG-CoA reductase determined.	Fats with shorter chain fatty acids depressed HMG-CoA reductase activity.
Boila <i>et al.</i> , 1981	Pigs	Addition of 5% propionic acid to diets of pigs given a cholesterol synthesis inhibitor.	Lower plasma concentrations of 7-dehydrocholesterol (an intermediate in cholesterol synthesis) in pigs fed tallow & propionate than those fed tallow alone.
Thacker & Bowland, 1981	Pigs	Addition of 0, 3, 6 or 9% propionic acid (PA) to basic diets.	Dietary propionate (6 and 9%) results in reduction in serum TC, associated with reduction in HDLC. LDLC not affected. Liver cholesterol tends to be higher with inclusion of PA in the diet.
Thacker <i>et al.</i> , 1981	Pigs	Addition of 5% PA to a control diet and a diet containing 10% tallow.	Addition of PA to the control diet decrease plasma TC by 18.3% after 10 weeks. The tallow diet increases TC by 16.5%. PA prevents this increase.
Chen <i>et al.</i> , 1984	Rats	Addition of 0.3% cholesterol, with or without 0.5% sodium propionate, to control diet.	Serum and liver cholesterol concentrations lower in rats fed propionate. HDLC and TG levels not affected by propionate.
Illman <i>et al.</i> , 1988	Rats	Addition of 5% sodium propionate to basic diet for 10 days.	Propionate lowers plasma TC 15-19%, raises liver cholesterol 12%, and does not change hepatic cholestero-genesis and fatty acid synthesis rates.

It must be noted, however, that with respect to cholesterol metabolism, propionate may have a dual role. In addition to decreasing cholesterol concentration in serum, propionate also seems to be a substrate for cholesterolgenesis, as has been demonstrated in the rat, mouse,

chicken, cow and pig (Emmanuel & Robblee, 1984). All tissues tested used both acetate and propionate as cholesterol precursors, but the rate of propionate conversion was about 20% of the rate of acetate conversion to cholesterol. The net effect of propionate on cholesterol homeostasis will depend on the balance between its conversion to cholesterol and its regulatory effects on cholesterol synthesis, probably through inhibition of HMG-CoA synthase and/or reductase.

The possibility that propionate may inhibit hepatic cholesterolgenesis is not supported by *in vivo* results in rats fed oat bran (Illman & Topping, 1985) or propionate supplemented diets (Illman *et al.*, 1988). Concentrations of propionate in the hepatic portal vein, although raised by dietary oat bran, were less than 2% of those at which inhibition of hepatic cholesterolgenesis has been observed *in vitro* (Illman & Topping, 1985). In rats, as well as pigs, the absorption and metabolism of dietary propionate appear to occur in the upper gastro-intestinal tract (Illman *et al.*, 1988).

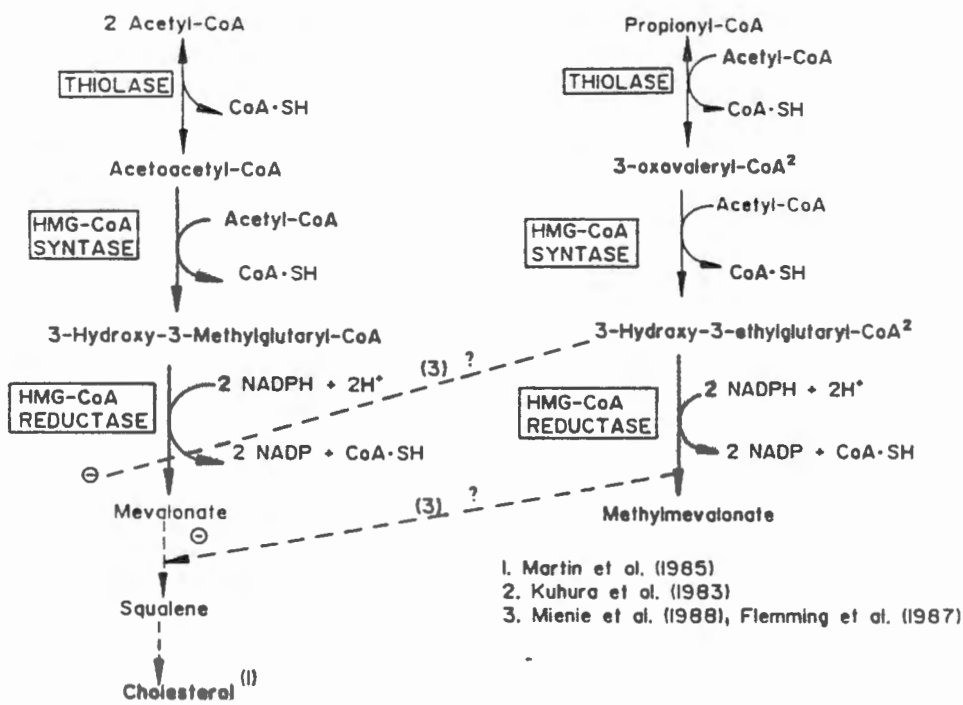


FIGURE 2.3 BIOSYNTHESIS OF CHOLESTEROL IN THE LIVER (left) AND PROPOSED INHIBITION OF HMG-CoA REDUCTASE BY 3-OH-3-ETHYLGLUTARYL-CoA (right)

As mentioned earlier (see section 2.3), propionate may influence a number of important enzymes involved in cellular metabolism, resulting *inter alia* in increased glycolysis. Similar effects have been reported for guar gum (Stanley & Newsholme, 1985). The increase in activities of some key enzymes of carbohydrate and lipid metabolism in liver of mice adapted to guar-supplemented diets might indicate increased glycolysis, and provision of NADPH and carbon sources for fatty acid and steroid synthesis. According to the authors their findings might explain the ability of guar gum to increase the rate of bile acid synthesis and eventually lower serum cholesterol concentration.

Total serum cholesterol concentration correlates positively with the concentration and/or coagulability of several blood clotting factors (Wilhelmsen *et al.*, 1984). The possibility that dietary factors which influence plasma lipids may have similar effects on plasma coagulation factors has been mentioned by Vorster (1987), and will be explored in the next section.

2.5 EFFECT ON COAGULATION FACTORS

There are epidemiological (Wilhelmsen *et al.*, 1984; Stone & Thorp, 1985; Meade *et al.*, 1986; Kannel *et al.*, 1987), clinical (Lowe *et al.*, 1980; Sugrue *et al.*, 1985) and experimental (Smith, 1984) evidence that raised plasma fibrinogen levels and factor VII coagulant activity are important in the pathogenesis of atherosclerosis and its clinical sequelae. Several mechanisms have been proposed to explain the atherogenic effect of hyperfibrinogenaemia, including contribution to the development of the atheromatous lesion itself (Kadish, 1979; Niewiarowsky & Rao, 1983; Smith, 1986), and alterations of platelet function (Marguerie *et al.*, 1986), blood viscosity (Turitto, 1982) and blood pressure (Letcher *et al.*, 1981). The mechanism by which raised factor VII activity may contribute to atherosclerosis is less clear. Meade *et al.* (1986) have suggested that levels of factor VII coagulant activity in plasma may be an index of the rate of activation of those components of the coagulation system with which it interacts. Activated factor VII (factor VII_a) involves both the intrinsic and extrinsic pathways of coagulation (see figure 2.4) (Jackson & Nemerson, 1980), which may possibly result in hypercoagulability and increased fibrin formation. Furthermore, evidence has been presented that activated factor VII is related to elevated plasma lipids, a known risk factor for CHD (Wilhelmsen *et al.*, 1984; Scarabin *et al.*, 1986). Hypercoagulability seems to be closely related to lipid abnormalities, as shown in several studies, the results of which are summarised by Smith (1986).

The mechanism of blood coagulation, and the properties of the coagulation factors, are fairly well delineated, and described in detail elsewhere (Hirsh & Brain, 1979; Jackson & Nemerson, 1980; Russel *et al.*, 1982; West, 1985; Vorster, 1987). The coagulation factors are listed in table 2.4, and their functions briefly summarised.

FIGURE 2.4 SCHEMATIC REPRESENTATION OF THE REACTIONS IN THE COAGULATION CASCADE*

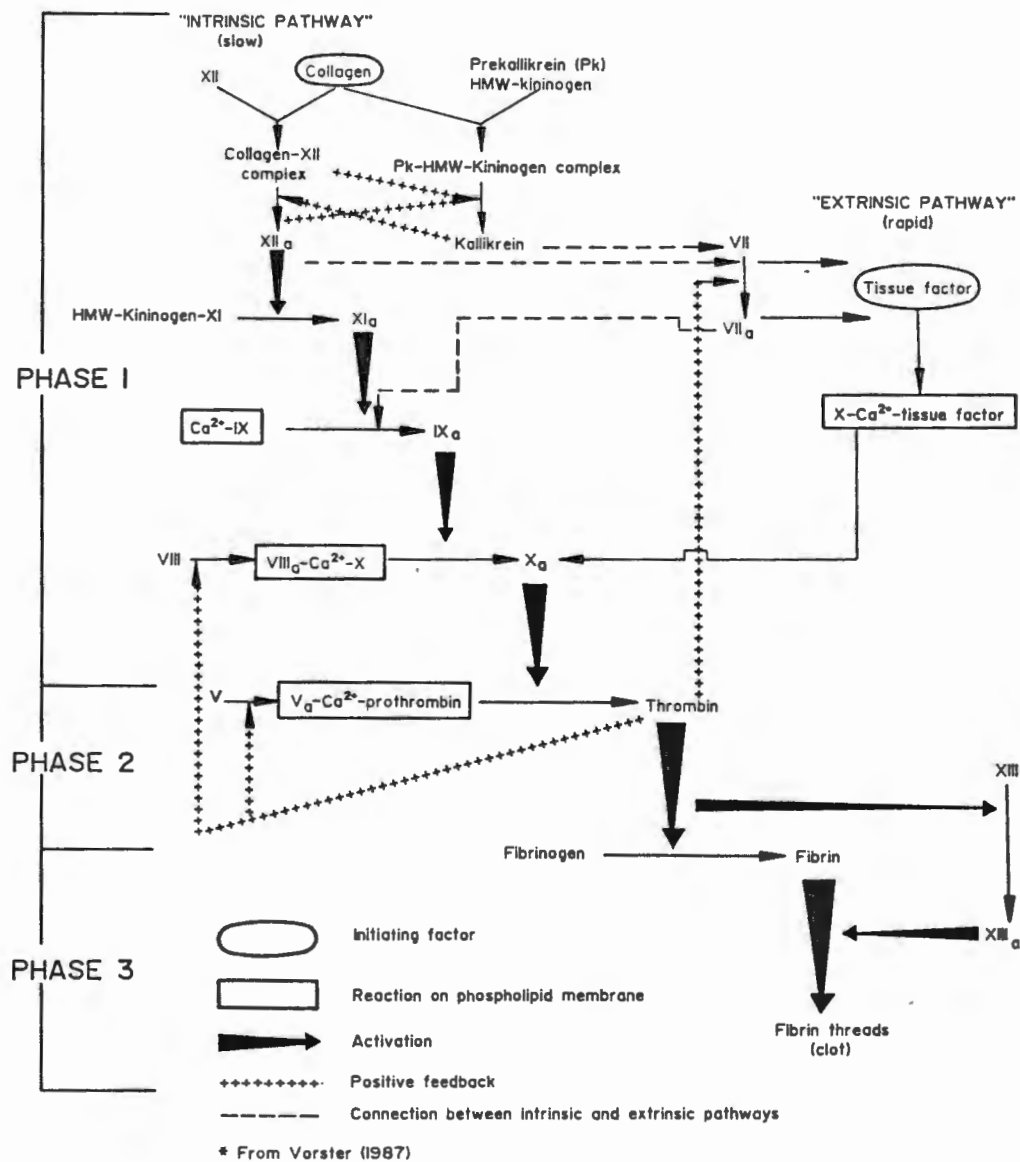


TABLE 2.4 GLOSSARY OF BLOOD COAGULATION FACTORS*

Common name and synonyms	Function
<u>Fibrinogen</u> Factor I	1. Soluble protein which after proteolysis by thrombin polymerises to form the fibrin clot. 2. Essential for normal platelet function.
<u>Prothrombin</u> Factor II	Circulating precursor of thrombin, converted to thrombin by activated factor X.
<u>Thromboplastin</u> (Sometimes tissue thromboplastin, or "tissue factor") Factor III	Forms a complex with factor VII, which acts enzymatically on factor X to form activated factor X in the presence of tissue phospholipids.
<u>Calcium ions</u> Factor IV	1. Forms "bridges" between vitamin K-dependent coagulation factors and acidic phospholipids of cell membranes. 2. Stabilises and activates several other coagulation factors.
<u>Factor V</u> Labile factor Proaccelerin	Activation of prothrombin
<u>Factor VII</u> Proconvertin	Activation of factor X
<u>Factor VIII</u> Antihaemophilic factor A Platelet cofactor 1 Antihaemophilic globulin	Controversial; participates in the activation of factor X.
<u>Factor IX</u> Christmas factor Antihaemophilic factor B Plasma thromboplastin component	Activation of factor X.
<u>Factor X</u> Stuart-Prower factor Prothrombokinase Autoprothrombin III	Conversion of prothrombin to thrombin
<u>Factor XI</u> Plasma thromboplastin antecedent	Activation of factor IX
<u>Factor XII</u> Hageman factor	Activation of factor XI
<u>Factor XIII</u> Fibrin-stabilising factor	Circulating precursor of transglutaminase which covalently cross-links fibrin monomers in the fibrin polymer.

*After Jackson & Nemerson, 1980 and Meyer (1983)

The clotting process, which is illustrated in figure 2.4, can be divided into three steps: an initial step in which a sequence of reactions results in the generation of an activator of prothrombin (factor X_a); a second step in which the prothrombin activator catalyses prothrombin in the presence of calcium ions and a cofactor (factor V_a) to thrombin; and a third step, in which thrombin, in the presence of calcium ions and factor XIII, acts as an enzyme to convert fibrinogen into fibrin molecules which polymerise to form fibrin threads (West, 1985).

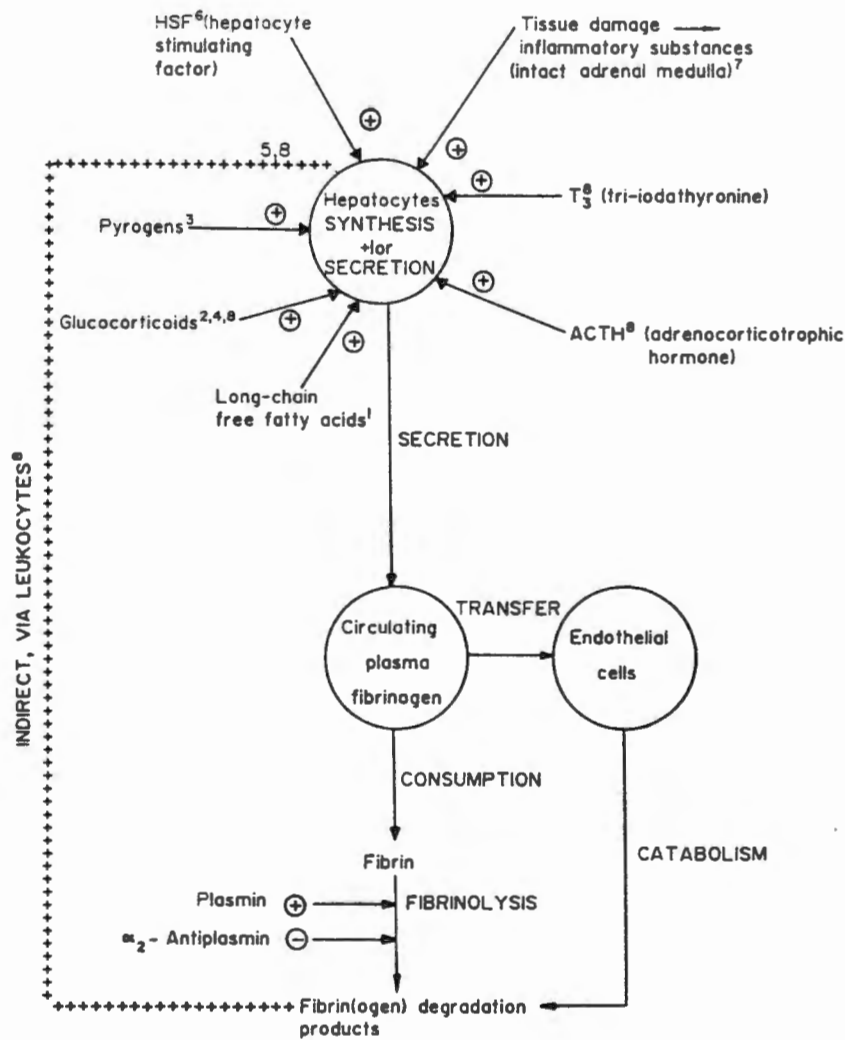
As indicated in figure 2.4, factor X may be activated by the intrinsic or extrinsic pathways. The first involves the factor XII (Hageman factor) - dependent reactions set off when blood is exposed to a negatively charged surface *in vitro*. This results in the generation of factor XI_a. The second pathway, the extrinsic system, involves formation of a complex between factor VII and a lipoprotein activity called tissue factor, 'extrinsic' to the circulation itself, present on the surface of altered or damaged cells. Activation of the extrinsic pathway results in much more rapid coagulation than activation of the intrinsic system (Jesty & Nemerson, 1977). Jackson & Nemerson (1980) presented evidence which questions some aspects of the traditional separation of the coagulation system into intrinsic and extrinsic pathways. The suggested crossover reactions which appear to couple the two pathways are indicated with dashed lines in figure 2.4.

The mechanisms controlling plasma fibrinogen levels are largely unknown and undoubtedly very complex. In an extensive review on fibrinogen synthesis, distribution and degradation the authors conclude that feedback between parameters related to plasma fibrinogen and rate of liver synthesis of fibrinogen seems to be managed by "hormonal information systems" (Reeve & Franks, 1974). The level of plasma fibrinogen itself does not exert any negative feedback (Regoeczi, 1974). There is indirect evidence that plasma levels of fibrinogen degradation products may stimulate fibrinogen secretion (Grieninger, 1983; Princen *et al.*, 1985), as indicated in figure 2.5. Other factors which have been found to influence the synthesis and/or secretion of fibrinogen are also shown in figure 2.5. The effect of pyrogens and other bacterial extracts (Limaos *et al.*, 1985) may be mediated through an effect on leucocytes (Grieninger *et al.*, 1983). In tissue culture, long chain saturated free fatty acids increase fibrinogen synthesis in human liver slices (Pilgeram & Pickart, 1968). Both plasma fibrinogen and free fatty acid (FFA) levels are increased after acute injury or stress, and in a number of chronic disorders, summarised by Pickart & Thaler (1980). On account of these observations, Pickart & Thaler (1980) proposed that hepatic fibrinogen production is stimulated by increased intrahepatic uptake of FFA from plasma. Whether any of these phenomena reflect truly specific regulation of fibrinogen synthesis remains uncertain. The concentration of plasma fibrinogen has been found to increase with age (Aillaud *et al.*, 1986), obesity (Balleisen *et al.*, 1985), smoking (Meade *et al.*, 1979), the use of oral contraceptives (Meade *et al.*, 1979), during pregnancy (Stirling *et al.*, 1984) and certain disease states such as coronary artery disease (O'Connor *et al.*, 1984), diabetes mellitus (Fuller *et al.*, 1979), and hypertriglyceridaemia (Simpson *et al.*, 1983).

Very little is known regarding the effect of dietary components and clotting factor levels. A possible association between DF intake and blood coagulation is indicated by the limited literature on this subject. The incidence of post-operative venous thrombosis in hospital patients was shown to be reduced by increasing the DF intake (Frohn, 1976; Latto, 1976). It is well known that vegetarians consume more dietary fibre than non- vegetarians (Hardinge *et al.*, 1958), and Haines *et al.* (1980) reported substantially lower factor VII and II- activity in

vegetarians than in non-vegetarians. Differences were not due to differences in age, smoking or obesity. Factor XIII and fibrinogen levels were very similar. The diets of vegetarians and non-vegetarians differ in respects other than dietary fibre content, e.g. amount of fat consumed. Consequently, conclusions concerning the effect of a specific nutrient can hardly be drawn from studies with vegetarians.

FIGURE 2.5 SCHEMATIC REPRESENTATION OF FIBRINOGEN TURNOVER*



+++++ Positive feedback
 * After Vorster, 1987

1. Pilgeram & Pickart (1968)	5. Princen et al. (1985)
2. Princen et al. (1984)	6. Fuller et al. (1985)
3. Limões et al. (1985)	7. Gravotto et al. (1985)
4. Kalvaria et al. (1986)	8. Grieninger et al. (1983)

Epidemiological studies in Wales demonstrated a significant negative correlation between plasma fibrinogen and the intake of cereal fibre (Fehily *et al.*, 1982; Yarnell *et al.*, 1983). However, in a randomised controlled trial, designed to investigate the effect of a high fibre diet on plasma fibrinogen, Fehily *et al.* (1986) reported no difference in fibrinogen levels of 201 healthy subjects while consuming high- versus low-fibre diets. Fibrinogen levels of these subjects were within normal range, which are probably difficult to improve.

It is known that the concentration and/or activity of several coagulation factors is raised in patients with diabetes mellitus (Jones & Peterson, 1981). Koepp & Hegewish (1981) reported decreased plasma viscosity and fibrinogen levels in diabetic children after supplementing their diet with guar gum. In another experimental study (Simpson *et al.*, 1982) a high fibre diet with generous amounts of leguminous vegetables reduced the mean levels of factors VIII and X substantially in non-insulin dependent diabetics. There were no changes in the concentration of coagulation factors in insulin-dependent diabetics, nor did fibrinogen levels change in the non-insulin dependent diabetics.

Miller *et al.*, (1986) investigated the association between dietary components and a number of coagulation factors in healthy adults. Plasma factor VII coagulant activity related positively and independently with fat and protein intake, whereas factor VII concentration was associated only with protein consumption. The authors observed raised factor VII coagulant activity within 12 hours after an increase in fat intake, but not after an increase in carbohydrate consumption. With prolonged high fat consumption, factor VII activity increased almost two-fold compared to the low-fat regimen. The results show that factor VII activity in the fasting blood sample may have been influenced by the extent of the preceding postprandial chylomicronaemia.

Hypolipidaemic diets cause a marked fall in the mean levels of several clotting factors, including factor VII in hyperlipidaemic patients (Elkeles *et al.*, 1980; Simpson *et al.*, 1983). In animal models (rats and monkeys) the feeding of cholesterol-containing diets significantly raises the plasma levels of factors II, V, VII, IX and X (Kim *et al.*, 1976). Positive correlations are reported by the authors between the levels of many coagulation factors (not including fibrinogen) and serum cholesterol.

The nutrient composition of the diets used in the above-mentioned studies has not been analysed in detail, and as in the case of the studies with vegetarians mentioned previously, conclusions regarding the effect of specific nutrients on coagulation factors can hardly be drawn. One of the dietary modifications in the study reported by Simpson *et al.* (1983) is an increased intake of dietary fibre. The inclusion of 5% gel-fibre concentrate (konjac-glucomannan) *per se* lowers fibrinogen and factor X levels of baboons on a Western diet (Vorster *et al.*, 1985).

As already mentioned, increased intake of leguminous vegetables (Simpson *et al.*, 1982), and supplementation of the diet with guar gum (Koepp & Hegewish, 1981) improved some of the coagulation factors in diabetic patients. The water-soluble fibre component, beta-glucan, present in leguminous vegetables, as well as guar gum and konjac-glucomannan are all readily fermented to SCFAs, which are absorbed from the colon and transported to the liver (see section 2.2). A possible inhibitory effect of short-chain fatty acids on blood coagulation has been suggested by Malhorta (1968), and investigated in the present study in baboons (chapter 5), rats (chapter 6) and healthy volunteers (chapter 7). This, and other mechanisms by which DF may influence coagulation, will be discussed in the next chapter.

2.6 SUMMARY

Dietary fibre never leaves the gut but has the ability to increase insulin sensitivity, lowers plasma cholesterol and possibly also plasma fibrinogen. The mechanisms proposed for the action of DF are indicated in figure 2.6 and includes firstly, a change in the rate of nutrient

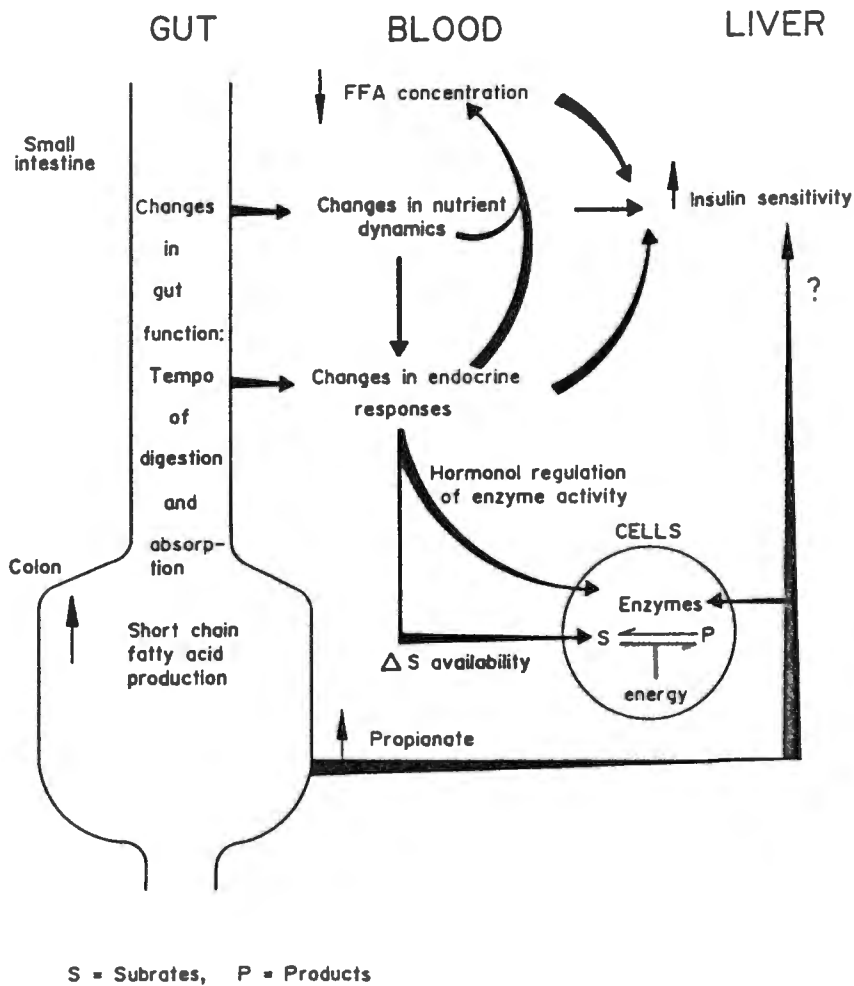


FIGURE 2.6 RELATION BETWEEN GUT AND METABOLIC EFFECTS OF DIETARY FIBRE

absorption, which results in altered endocrine response, cellular substrate availability and hormonal regulation of enzyme activities, and secondly a change in gut hormone or pancreatic glucagon secretion, which may enhance glucose metabolism in the liver and thereby decrease postprandial hyperglycaemia. However, this would not enhance glucose disposal. Therefore, other mechanisms must be activated to promote glucose utilisation and explain the long-term effects of DF on glucose metabolism. Furthermore, the cholesterol-lowering ability of DF cannot totally be ascribed to increased bile acid excretion. The digestion of DF and RS in the large intestine to SCFAs, including propionate, which are absorbed by man, seems to provide a very attractive alternative or additional mechanism.

CHAPTER 3

APPROACH TO THE INVESTIGATION

3.1 INTRODUCTION

The objective of this investigation was to determine the contribution of propionic acid to the physiological and metabolic effects of DF. If it could be demonstrated that propionate, derived in substantial amounts from other substrates, e.g. resistant or partially resistant starch, exerts similar effects, DF might partially be replaced by these substrates. Increasing the intake of DF is not always acceptable to everyone, in spite of the well-documented association between fibre-depleted diets and the development of Western diseases.

From the literature survey it is clear that changes in gut function resulting from increased DF intake, result in changes in blood nutrient dynamics, leading eventually to long-term modulations in metabolism. Increased intake of DF is associated with decreased serum and liver lipids; improved glucose tolerance accompanied by lower circulating insulin levels, indicating increased insulin sensitivity; and possibly with reduced plasma fibrinogen concentrations.

DF is fermented in the colon to SCFAs (see section 2.2), including propionate which is cleared by the liver. Propionate derived from gut bacterial metabolism in human beings has in the past been regarded as relatively unimportant quantitatively (Rosenberg, 1983). Nowadays, however, it seems as if propionate from gut bacterial metabolism contributes substantially ($\approx 60\%$) to total propionate turnover, the rest being contributed by other endogenous sources (Walter *et al.*, 1987; Baines *et al.*, 1988). The precursors and major catabolic pathways for propionate are shown in figure 3.1.

Propionyl-CoA is the catabolic intermediate of isoleucine, valine, methionine and treonine, as well as of phytanic acid, an odd-chain fatty acid, and the side chain of cholesterol (Kuhara *et al.*, 1983). Propionyl-CoA is normally degraded in the tricarboxylic acid cycle via methyl-malonyl-CoA and succinyl-CoA.

In recent years interest has grown in the physiological significance (Cummings, 1981b; 1984b) and metabolic consequences (Venter & Vorster, 1989) of SCFAs in man. Suggestions that propionate may mediate the hypocholesterolaemic effects of certain soluble plant fibres (Anderson & Chen, 1979, and many others) and may effect hepatic glucose metabolism as well (Anderson & Bridges, 1984) have led to many *in vitro* and *in vivo* experiments with propionate supplements in several animal species. The majority of studies on monogastric animals have been carried out on rats. In summary, it has been demonstrated that propionate increases hepatic glucose utilisation in rats (see section 2.3) and decreases plasma cholesterol concentration in rats and pigs (section 2.4). Rats adapted to a high fibre diet use mainly

propionate as a gluconeogenic substrate during periods of starvation, as opposed to rats on a fibre-free diet who predominantly utilise lactate (Demigné *et al.*, 1986). Additional evidence that propionate stimulates glycolysis in rats comes from the work of Veech *et al.* (1986) who measured enzyme activities in rat liver 30 min after intraperitoneal administration of SCFAs.

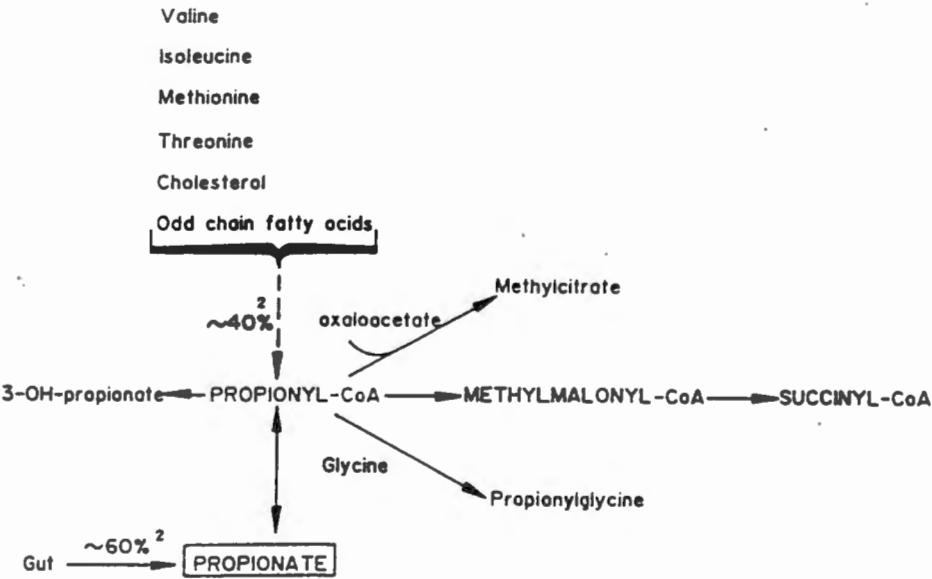


FIGURE 3.1 PRECURSORS AND MAJOR CATABOLIC PATHWAYS FOR PROPIONATE¹
 (THE FREE ACID IS DERIVED FROM ITS CoA ESTER BY HYDROLYSIS)
 Broken lines indicate the presence of several reactions.

¹Adapted from Rosenberg, 1983
²Walter *et al.*, 1987

3.2 HYPOTHETICAL MECHANISMS

It may be reasoned that if propionate facilitates glucose utilisation as energy substrate in fasting conditions to such an extent as to postpone a rise in circulating FFA, insulin sensitivity may improve. It is known that high serum concentrations of FFA are associated with hepatic (Strömblad & Björntrop, 1986) as well as peripheral (Randle *et al.*, 1963; Martin, 1985) insensitivity to insulin and inhibition of glucose uptake by cells. High serum FFA levels are also associated with increased fibrinogen synthesis (Pilgeram & Pickart, 1968). Furthermore, the association between raised serum concentrations of FFA and insulin sensitivity mentioned above, offers the possibility that fibrinogen synthesis may be influenced through a second mechanism. According to Regoeczi (1974) fibrinogen synthesis may be controlled on an inhibitory level. It has recently been suggested that a relative insulin deficiency (insulin resistance) with resultant increased circulating FFA may result in raised fibrinogen levels (Vorster *et al.*, 1988a). Circumstantial evidence that supports this supposition, comes from the work of Antoniadou & Westmoreland (1976) who demonstrated that *in vivo* intravascular coagulation and thrombosis in the rat were more pronounced in fasted, diabetic and obese animals, all characterised by a relative insulin deficiency and raised FFA circulating concentrations. The proposed relationships between insulin sensitivity, propionate and fibrinogen, are shown schematically in figure 3.2.

The effect of increased insulin sensitivity on lipid metabolism may be reflected by changes in serum HDLC levels. A close relationship between serum insulin and HDLC levels has been reported in the literature (Laasko *et al.*, 1987). The physiological regulation of HDLC metabolism by insulin as described by Fossati & Romon-Rousseaux (1987), is illustrated in figure 3.3.

Briefly, HDLC levels reflect two processes which are influenced by insulin: lipolysis of TG-rich lipoproteins and hepatic conversion of HDL_2 to HDL_3 , favoured by an excess of hepatic triglyceride-VLDL. Therefore, hyper- as well as hypo-insulinism may paradoxically lead to a decrease in HDLC levels: hypoinsulinism by decreasing lipoprotein lipase (LPL) activity, and hyperinsulinism associated with insulin resistance by increasing liver TG synthesis. Higher mean glucose stimulated plasma insulin levels are associated with lower HDLC levels and increased HDL-TG levels when compared to low post glucose plasma insulin (Stalder *et al.*, 1981). From this brief review it follows that if propionate or dietary fibre has the ability to improve insulin sensitivity, one could expect HDLC levels to be raised.

FIGURE 3.2 PROPOSED RELATIONSHIPS BETWEEN PROPIONATE, INSULIN SENSITIVITY AND FIBRINOGEN

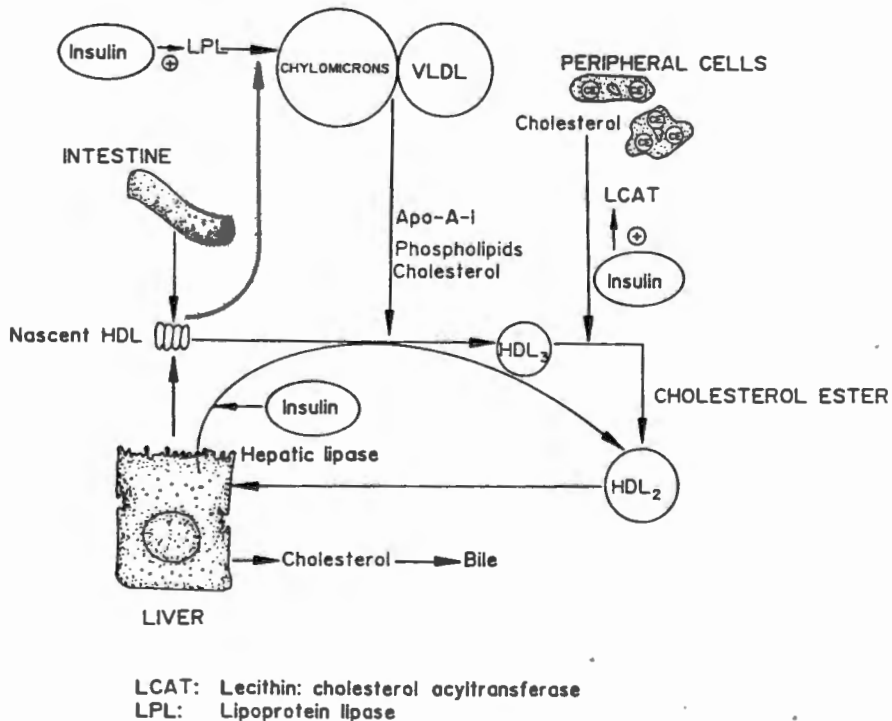
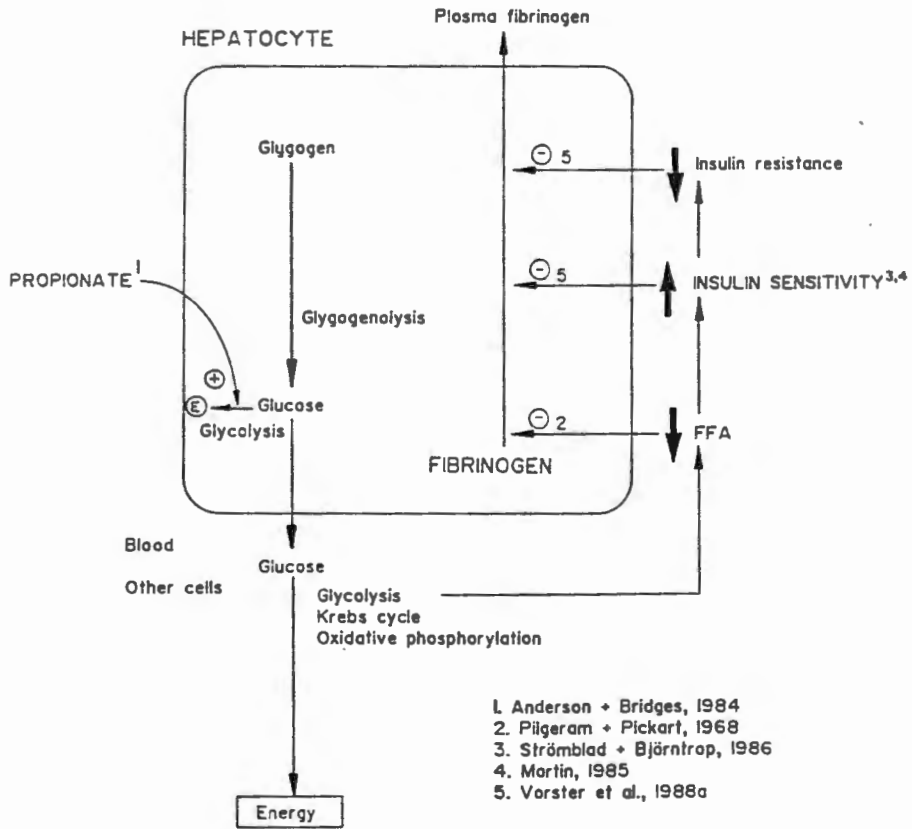


FIGURE 3.3 PHYSIOLOGIC ACTION OF INSULIN ON HDL METABOLISM*
 *Adapted from Fossati + Romon-Rousseaux, 1987

3.3 WAYS IN WHICH THE HYPOTHESIS WAS TESTED

To test the hypothesis that propionate, derived from gut bacterial metabolism, contributes to the well-known effects of fermentable DF on carbohydrate and lipid metabolism (increased insulin sensitivity and decreased serum cholesterol), the diet of healthy human volunteers was supplemented with sodium propionate (chapter 7). The effect of dietary propionate on carbohydrate and lipid metabolism, as observed from changes in glucose tolerance, insulin sensitivity, serum insulin and lipid levels, was compared with the known effects of DF on these variables. Plasma fibrinogen concentration and factor VII coagulation activity were also determined. As far as is known, this is the first study of propionate supplementation in man.

The study of the effects of propionate on metabolism in human subjects was, of necessity, confined to measurements of metabolites in the blood. From the literature survey it appears that the metabolism of tissues such as the liver may be modified by propionate. The next step in the investigation of the contribution of propionate to the effects of DF on metabolism thus appeared to be an *in vivo* study in experimental animals. Primates are regarded as the most appropriate animal model in dietary fibre digestion studies (Graham & Aman, 1987), as well as studies concerning the association of fibre-depleted diets and Western lifestyle diseases, including atherosclerosis (Kritchevsky, 1970). Practical difficulties of feeding a Western-style diet are easily overcome. The cost, which may be prohibitively high elsewhere, is well justified under local circumstances. It was therefore decided to compare in baboons (*Papio ursinus*), fed a semi-synthetic "Western" diet (SSW-diet) the long-term effects of propionate supplementation to that of konjac-glucomannan (K-GM), a soluble dietary fibre concentrate, on serum lipid profiles, liver lipid and glycogen content, glucose tolerance, immunoreactive insulin response, insulin sensitivity and plasma fibrinogen concentration (chapter 5).

Although the SSW-diet resulted in a marked, significant weight gain after 9 weeks in the baboons, compared to the background diet, evidence of an insulin resistant state was not found. The Zucker fatty rat, characterised by insulin resistance, served as a model in which therapeutic effects of DF and SCFAs, could be examined (chapter 6). The Zucker rat (Zucker & Zucker, 1961) displays insulin resistance in hepatic (Terrettaz & Jeanrenaud, 1983), muscle (Crettaz *et al.*, 1980), and adipose tissue (Cushman *et al.*, 1978). This rat is also known to be hyperfibrinogenaemic (Stolz *et al.*, 1981). The rat, unlike non-human primates, is not regarded as a suitable model for investigation of atherosclerosis. However, bacterial populations in the human and rat intestinal tract are very similar (Donaldson, 1968) and the rat is regarded as a useful experimental model in dietary fibre digestion studies (Nyman *et al.*, 1986). The effect of dietary propionate was compared with K-GM in Zucker rats, fed the same SSW-diet as the baboons (chapter 6).

Dietary fibre may quantitatively be less important than resistant starch (RS) and partially resistant starch (PRS) as substrates for fermentation and propionate production. The acute or short-term effects of maize porridge with varying amounts of digestible starch on blood

glucose and serum insulin response in volunteers on a Western diet were therefore investigated (chapter 8). This study was designed to give information on the physiological importance of PRS.

From the results of the abovementioned studies, the hypothesis regarding the physiological role of propionic acid could be evaluated, and the possible contribution of propionate to dietary fibre effects quantified.

CHAPTER 4

MATERIALS AND METHODS

4.1 INTRODUCTION

The protocol of the different studies as well as the motivation for the determination of specific parameters, is given in chapters 5 to 8. In this chapter the general care of the experimental animals, the dietary supplements which were given and the chemical methods are discussed.

In order to avoid contamination, especially during the determination of the coagulation factors, the test tubes used were either disposable or glass test tubes which were washed, rinsed several times in tap and distilled water, and finally in double distilled water. Double distilled water was also used for the preparation of reagent solutions.

The coefficient of variation (CV) of the method was determined for each set of measurements for control of accuracy and reproducibility of the method. Replicate determinations ($n = 5-10$) were done with a specific serum or plasma. The CV was calculated as:

$$[\text{Standard deviation} / \text{Mean}] \times 100.$$

4.2 CARE OF ANIMALS

4.2.1 BABOONS

Twelve male baboons (*Papio ursinus*) with body weights ranging from 14.5 kg to 27 kg (mean 18.96, SD 3.44), were obtained from three animal centres. Originally they were captured in different areas in the wild and then acclimatised and tested for tuberculosis at the respective animal centres. After receiving them, they were caged individually in the laboratory animal centre, allowed to stabilise, treated for internal parasites with mabendazole, and fed *ad libitum* dog cubes (Epol (Pty.) Ltd., Johannesburg, South Africa) and oranges. Epol® supplies 67% of the total energy as carbohydrate, 25% as protein and 8% as fat. It contains approximately 10% (w/w) dietary fibre. The baboons had free access to tap water.

After the stabilisation period of 5 weeks, they were randomised into 3 groups which were randomly allocated to one of three semi-synthetic "Western" diets (SSW-diet). The food was prepared up to 2 weeks beforehand in the form of balls, the necessary daily amount for each baboon weighed and kept at -20°C in plastic bags. The baboons were fed twice daily, after the food was warmed in a microwave oven to partly reverse retrogradation of starch (Schoch, 1965). The basic SSW-diet consisted of maize meal, beef dripping, sucrose and a commercially prepared protein-, vitamin- and mineral-containing dietary supplement, PVM®, formulated for primates by PVM Products (Pty.) Ltd. (Silverton, South Africa). PVM®, which is manufactured to specifications drawn up by the South African Bureau of Standards, results in

normal growth and excellent health when fed in combination with maize meal to young baboons (Sly *et al.*, 1980). Refined maize meal (Jabula Foods, Johannesburg), beef dripping (melted) and sucrose were mixed with the PVM ® powder. The composition of the SSW-diet is shown in table 4.1. Table 4.2 shows the nutrient analysis, which was done by computer using local food tables (Gouws & Langenhoven, 1986) except for dietary fibre which was analysed according to a modification of the procedure of the AOAC (1985). During the washout periods, the baboons were fed Epol dog cubes and oranges.

Final samples

One week after the final glucose tolerance tests were performed, the baboons were fasted for 4h {the time-course of the onset of large bowel fermentation (Pomare *et al.*, 1985; Illman *et al.*, 1988)} sedated with phencyclidine hydrochloride (1mg/kg body weight), weighed and anaesthetised with pentobarbitone sodium (25-30 mg/kg) given intravenously. Peripheral, portal and hepatic venous blood samples were taken. Livers were quickly excised, blotted dry, weighed and snap frozen. Samples were kept at -70°C .

TABLE 4.1 COMPOSITION OF THE SEMI-SYNTHETIC WESTERN DIETS

Ingredient	Quantity/100g
PVM ;	38.3g
Full-fat, heat-treated soybean meal	16.4g
Skimmed milk powder	7.3g
Deodorised whole fish flour	7.7g
Egg yolk powder (spray-dried)	4.2g
Table salt	0.7g
Potassium iodate	0.2g
Calcium carbonate	To yield 3 g Ca/100g PVM
Ferrous sulphate	To yield 36mg Fe/100g PVM
Vitamin A	4980 I.U.
Vitamin E	Equivalent to 38mg α -tocopherol acetate
Thiamin hydrochloride	1.6mg
Riboflavin	1.3mg
Nicotinic acid amide	8.8mg
Pyridoxine hydrochloride	1.6mg
Calcium pantothenate	6.4mg
Ascorbic acid	107mg
Vitamin D ₃	536 I.U.
Refined maize meal	38.3g
Beef tallow	13.5g
Sucrose	10.0g

TABLE 4.2 NUTRIENT ANALYSIS OF THE SEMI-SYNTHETIC WESTERN DIETS

Nutrient	Amount/100g
Total energy (kJ)	1832
(kcal)	438
Total protein (g)	20.6
Plant protein (g)	9.5
Animal protein (g)	11.1
% Energy: total protein	18.8
Total fat (g)	20.1
Saturated fatty acids (g)	6.9
Polyunsaturated fatty acids (g)	1.0
P:S ratio	0.14
% Energy: total fat	41.4
Cholesterol (mg)	134.8
Total carbohydrate (g)	43.5
% Energy: carbohydrate	39.7
Sugar (g)	10.0
Fibre*: insoluble (g)	3.1
soluble (g)	0.4
Calcium (mg)	1083.3
Iron (mg)	18.0
Zinc (mg)	0.53
Vitamin A (I.U.)	3147.8
Thiamin (mg)	2.16
Riboflavin (mg)	1.18
Nicotinic acid (mg)	11.50
Vitamin B ₆ (mg)	1.49
Folate (µg)	76.50
Vitamin B ₁₂ (µg)	2.10
Ascorbic acid (mg)	76.50

*Analysed by a modification of the procedure of the AOAC (1985). The AOAC procedure was modified in order to determine soluble and insoluble fibre separately. This was done by filtration after incubation with amyloglucosidase. The residue was used to determine insoluble fibre. The filtrate was treated with 95% ethanol according to the procedure described in section 43.A19 of the method. The precipitate was collected by filtration and used to determine soluble fibre.

4.2.2 ZUCKER RATS

Zucker rats, in conventional health status, originally obtained from a colony maintained at the Research Institute for Nutritional Diseases, South African Medical Research Council, Tygerberg, were allowed to breed in the local experimental animal centre in a temperature-

controlled room ($21.2 \pm 0.1^{\circ}\text{C}$, relative humidity $50 \pm 3\%$) with a 12-hour light/12-hour dark cycle. The rats were fed dog cubes (Epol (Pty.) Ltd., Johannesburg) *ad libitum* and allowed free access to tap water.

At the age of approximately 18 to 20 weeks obese (fa/fa) and lean litter mates (Fa/fa or Fa/Fa) of the same sex were grouped (7-10 rats per group, depending on availability). The rats, paired according to fenotype, sex and age (weight) were randomly allocated to the different experimental groups (chapter 6). Males and females were housed separately in wire bottom cages. The same basic experimental SSW-diet that was provided for the baboons, as indicated in tables 4.1 and 4.2, was fed to the rats, but the food was shaped into much smaller pellets and allowed *ad libitum*. The experimental diets were given for the same period as for the baboons, eg. 9 weeks.

4.3 DIETARY SUPPLEMENTS

Two different supplements were added to the SSW-diet which were fed to the experimental animals, namely 5% (w/w) of a gel fibre concentrate konjac-glucomannan (K-GM) and 2% (w/w) sodium propionate. Sodium propionate was also used in capsule form to supplement the diet of healthy female volunteers (chapter 7).

4.3.1 KONJAC-GLUCOMANNAN (K-GM)

K-GM is a hemicellulose which consists of a linear polymer of mannose and glucose in a proportion of 1:1 to 2.4:1 in the chain (Southgate, 1976). It is obtained from the tubers of *Amorphophallus konjac* (K. Koch) and has been used as a food in Japan for centuries in the form of *Konnyako* (Ebihara *et al.*, 1981). The K-GM granules used in these experiments contain 60 per cent pure glucomannan. It is produced by the Tsuruta Shokuhin Company of Japan and distributed by the Mannan Life Company. It was obtained from Birchacres Pharmacy, Kempton Park, South Africa.

The addition of K-GM made no difference as regards acceptability of the diets in both the rats and baboons. The soluble fibre content of the K-GM supplemented diet was 5.4g/100g.

4.3.2 SODIUM PROPIONATE ($\text{C}_3\text{H}_5\text{Na}_2\text{O}_2$)

The sodium salt of propionic acid was used to study some of the physiological and biochemical effects of the SCFA. Sodium propionate, which has antifungal properties, is used as a mould inhibitor in the food industry. According to the Merck Index (Stecher, 1968), the LD 50 for propionic acid, taken orally by rats, is 4.29g/kg. Sodium propionate in the form of a white odourless granular powder was purchased from SAARCHEM (Pty.) Ltd., Muldersdrift, South Africa. It is hygroscopic at relative humidities above 60% and sachets with silica gel crystals were used to keep dry the capsules, filled with sodium propionate for the human study (chapter 7).

4.4 GLUCOSE TOLERANCE TESTS (GTTs)

4.4.1 PROTOCOL

Baboon GTTs

The baboons were fasted for 18 hours before GTTs were done. All tests were performed between 08h00 and 11h00 in the theatre of the experimental animal centre (26°C, relative humidity $50 \pm 3\%$). Because of their vicious nature, GTTs were performed during anaesthesia. The baboons were immobilised with phencyclidine hydrochloride (1mg/kg body weight), weighed and anaesthetised with pentobarbitone sodium (25- 30mg/kg) given intravenously. A solution of 1g glucose in 5mL water per kg body weight was administered via a stomach tube. Venous blood samples were taken at the start of the GTTs and 15, 30, 45, 60, 90 and 120 min thereafter with an intravenous catheter (Jelco™ 20 Gauge, Johnson & Johnson). The catheter was placed in either the *V. cephalica* or *Vv. comitantes a saphena* of the baboon. A balanced solution of electrolytes (Ringer's lactate equivalent, Plasmalyte B® from Normosol R, Abbott Laboratories SA (Pty.) Ltd., Johannesburg) was administered intravenously in order to perform a 120min GTT.

Human GTTs

Glucose tolerance tests on humans were performed in the same standardised way usually followed in our laboratory. The subjects had consumed at least 150g carbohydrate per day for a minimum of 3 days beforehand, and fasted for 12h before they reported at 07h00 for the GTTs. They were not allowed to smoke and move around unnecessary during the tests. For the propionate supplemented study (chapter 7) a glucose solution of 1g in 5mL water per kg body weight was taken orally. The glucose load for control GTTs in the resistant starch study (chapter 8) was 50g in order to calculate the glycaemic index of maize porridge according to published standardised methods (Jenkins *et al.*, 1981). Venous blood samples were taken before glucose administration and every 30 min thereafter. Capillary blood samples were taken every 15 min.

The variability of a subject's response to replicate glucose tolerance tests was determined previously in our laboratory (Van Tonder, 1986). The mean intra-individual variation in the incremental area of 4 GTTs in 11 healthy female volunteers, aged 21.0 ± 1.8 yr (mean $\pm$ SD) and with body mass index (BMI) 20.0 ± 1.8 , is $10.68 \pm 4.87\%$.

4.5 CHEMICAL METHODS

4.5.1 BLOOD GLUCOSE

Capillary glucose concentrations were measured with the Glucometer II® reflectance photometer Model 5529 (Ames Division, Miles Laboratories, Elkhart, Indiana, USA) with Glucostix® reagent strips. The meter calibrates automatically for different batches of reagent strips. Accuracy was tested with "Dextro Check Calibrator" solutions of 2.78 and 16.67 mmol/L. The "Glucometer II" measures colour changes on the "Glucostix" reagent strip pad and a digital read-out displays the blood glucose level. Jarret *et al.* (1970) and Germer & Campbell (1982) reported excellent correlations between this method and other methods for the determination of glucose. For the determination of glucose in serum, the enzymatic method of Boehringer Mannheim (Boehringer Mannheim, West Germany) (Cat. no. 676543) was used. The CV of replicate measurements varied from 0.9 to 1.1%.

4.5.2 IMMUNOREACTIVE INSULIN (IRI)

IRI was determined in duplicate on non-haemolytic serum with a double-antibody enzyme-immunoassay kit (Enzymun-Test® Insulin, Cat. no. 199648, Boehringer Mannheim, West Germany). In the first incubation step, the insulin from the sample reacts both with the monoclonal antibody bound to the solid phase of the coated plastic tubes and also with the enzyme peroxidase(POD)-labelled monoclonal antibody to form a sandwich complex. The quantity of antibody-insulin-POD complex so formed is a measure of the insulin content of the sample. The unbound POD-conjugate is removed in a separation step. After the addition of hydrogen peroxide (H_2O_2) and a chromogen, a second incubation is carried out and the tube-bound POD activity then determined photometrically. The intensity of the colour reaction is directly proportional to the insulin concentration in the sample. A calibration curve is constructed with the aid of the standards contained in the kit. Extrapolation of the standard curve was necessary whenever the IRI values of the baboons were higher than 250 μ U/mL, the range of the kit being 0 – 250 μ U/mL. Dilution of the baboon serum with saline before starting the procedure gave unreliable results. The CV of repeated measurements of control sera varied between 6.7 and 11.0%.

4.5.3 SERUM LIPIDS

GENERAL

Fatty acids, lipids and lipoproteins were determined on serum of fasting experimental subjects or animals, which had been kept at -70°C . Except for short-chain and free fatty acids, commercial kits, based on enzymatic-colorimetric methods from Boehringer Mannheim (BM, Mannheim, West-Germany) were used for lipids and lipoproteins. Appropriate standards and control sera were used.

FREE FATTY ACIDS (FFA)

For the determination of FFA the colorimetric method of Duncombe (1963, 1964) was used. This method, as applied to 14 baboon serum samples, was compared to results obtained with an enzymatic kit (NEFA QUICK "BMY" ®, Boehringer Mannheim Yamanouchi, Minato-Ku, Tokyo. Cat. no. 450459 E). Oleic acid ($1000\mu\text{Eq/L}$) was used as the standard solution.

Duncombe's colorimetric method for long-chain fatty acids measures the extinction of a chloroform solution of the free fatty acids shaken with a copper nitrate-triethanolamine reagent. Sodium diethyldithiocarbamate is used as a colour developer.

Reagents

All reagents used were of analytical grade purity. The copper reagent consisted of 9 vol. of aqueous 1M triethanolamine, 1 vol. 1N acetic acid and 10 vol. of 6.45% (w/v) $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$. The diethyldithiocarbamate reagent was a 0.1% (w/v) solution of sodium diethyldithiocarbamate in butan- 2-ol. Both the reagents were stored in the refrigerator and used within a week.

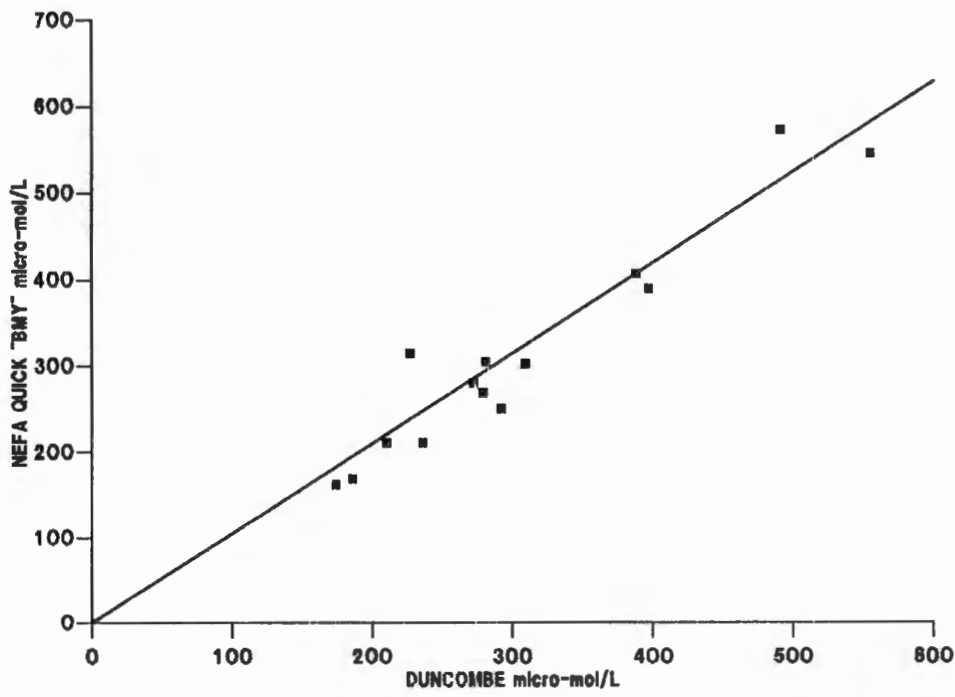
Calibration curve

This was prepared from palmitic standards made up in chloroform in the range of 10 – $100\mu\text{M}$. Sodium diethyldithiocarbamate was purchased from Aldrich Chemical Co., Milwaukee, Wisconsin, USA, palmitic acid from BDH Chemicals (Hickman & Kleber (Pty.) Ltd., Durban, RSA) and all the other reagents from E. Merck (Darmstadt, West Germany).

Procedure

Five mL chloroform was pipetted into a 10-15mL stoppered centrifuge tube, together with 0.5mL of the serum and 2.5mL of the copper reagent. After stoppering, the tube was shaken vigorously for at least 2 min and then centrifuged for about 40 min. The aqueous phase, which is separated from the chloroform phase by a disc of precipitated protein, was then aspirated and the disc of protein made to adhere to the wall of the tube without breaking up. Three mL of chloroform solution was then pipetted into a clean dry tube without touching the inner wall of either tube to avoid contamination by transfer of traces of copper solution. To this was added 0.5 mL of the diethyldithiocarbamate reagent and, after mixing, the extinction was read at $440m\mu$ against a reagent blank carried through the whole procedure. The extinction was referred to the calibration curve, the concentration of FFA in the chloroform read off and multiplied by 10 to get the concentration in the serum. The CV of replicate determinations varied from 1.1 to 5.4%. The correlation coefficient of the Duncombe and NEFA Quick "BMY" methods was $r = 0.9582$ (figure 4.1).

FIGURE 4.1 COMPARISON OF DUNCOMBE AND NEFA QUICK "BMY" METHODS



SHORT-CHAIN FATTY ACIDS

Peripheral venous serum acetate was determined in the resistant starch study (chapter 8) as described by Pomare *et al.*(1985) by vacuum transfer, using propionic acid as internal standard and acetate standards for preparing a standard curve. All chemicals used were either Aristar (BDH Chemicals Ltd., Poole, England) or the highest available purity. The CV for acetate measurements in 10 samples from the same fasting serum was 4.5%.

TOTAL CHOLESTEROL (TC)

TC was determined with the CHOD-PAP method (BM: Cat. no. 237 574). Cholesterol esterase hydrolyses cholesterol esters in the first step of the reaction to free cholesterol and fatty acids. The free cholesterol is then oxidised by cholesterol oxidase to Δ^4 -cholestenone and H_2O_2 . H_2O_2 reacts in the presence of peroxidase with 4-aminophenazone and phenol to give a

coloured complex, 4-(p-benzoquinone-mono-imino)-phenazone and water. The intensity of the colour developed is proportional to the amount of cholesterol (and cholesterol esters) in the serum sample, and is measured at 500 nm in a spectrophotometer. A standard curve was drawn for each set of determinations by using Preciset® cholesterol standards (BM: Cat. no. 125 512) with concentrations of 1.29 to 10.36 mmol/L. Precinorm® L (BM: Cat. no. 154 747), Precilip® (BM: Cat. no. 125 059), Precinorm® U (BM: Cat. no. 171 735) and Precipath® U (BM: Cat. no. 171 760) were used as control sera. The CV of the method was between 0.9 and 4.4%.

Intra-individual variations in serum or plasma concentration of TC occur in healthy populations. Kritchevsky (1985) summarised 12 studies conducted between 1924 and 1982, which clearly indicate a seasonal variation in cholesterol levels. In seven other studies where intra-individual variations were found, diurnal or seasonal variations did not occur (Kritchevsky, 1985). The magnitude of these variations may be 30% or more, although usual swings are closer to 15%. These variations are reflected in high-density lipoprotein cholesterol (HDLC) as well.

The reason for the intra-individual variation is not clear. It seems that diet does not play a significant role (Kritchevsky, 1985). Stress, variations in several hormones, lipoprotein lipase activity or other enzymes may be responsible (Kritchevsky, 1985; Gordon *et al.*, 1987).

We therefore decided to determine the intra-individual variation in TC and HDLC in 13 healthy young (21.0 ± 1.0 yr) volunteers of normal weight ($BMI\ 22.2 \pm 2.4$). The participants in the studies, which were done on humans, and reported in chapters 7 and 8, were from this age category and followed the same background diet. Fasting blood samples, taken four times over a period of 10 weeks, were kept at $-70^{\circ}C$ until all the samples were collected. TC and HDLC concentrations in all the samples were determined on the same day. The mean intra-individual variation for TC was found to be $4.99 \pm 1.40\%$ and for HDLC $5.38 \pm 1.49\%$.

HIGH-DENSITY LIPOPROTEIN CHOLESTEROL (HDLC)

Chylomicrons, very-low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) were precipitated by adding phosphotungstic acid and magnesium ions to the sample (HDL Cholesterol Precipitant, BM: Cat. no. 543 004). The cholesterol content of the supernatant was determined enzymatically with the method described for TC. For control of accuracy Special Control Serum for HDL Cholesterol (BM: Cat. no. 651 281) and Precinorm® L (BM: Cat. no. 154 747) were used. The CV of the method was 0.8 to 4.0%.

TRIGLYCERIDES (TG)

Triglyceride concentration was also determined with kits from BM (Cat. no. 701 882). In the first step of this enzymatic colorimetric test TG are hydrolysed by lipase to glycerol and fatty acids. Glycerol is then converted to glycerol-3- phosphate in the presence of ATP and then to dihydroxyacetone phosphate and hydrogen peroxide in the presence of oxygen by glycerokinase and L-glycerol-3-phosphate-oxidase respectively. The hydrogen peroxide reacts with 4-aminophenazone and 4-chlorophenol in the presence of peroxidase to form 4-(p-benzoquinone-mono-imino)-phenazone, water and hydrochloric acid. The intensity of the colour developed is proportional to the amount of glycerol (and thus TG) in the sample, and is measured at 500 nm in a spectrophotometer. Standard curves were drawn with Precimat ® Glycerol (BM: Cat. no. 166 588). The same control sera as for TC were used. The CV of the method varied between 1.4 and 2%.

LOW-DENSITY LIPOPROTEIN CHOLESTEROL (LDLC)

LDLC was calculated according to the formula of Friedewald *et al.*(1972).

4.5.4 LIVER LIPIDS

EXTRACTION

Liver lipids were determined on samples of fasting rats and 4 h-postprandial baboons, which had been kept at -70°C . Extraction of lipids from liver tissue was done in duplicate as described by Sodhi *et al.* (1979). Reagents and solvents used were of analytical grade purity and purchased from either E. Merck or Hickman & Kleber (BDH Chemicals). One gram of liver tissue was homogenised in 25mL of a chloroform:methanol (1:1 v/v) mixture and then filtered through Whatman no. 1 filter paper. The filtrate was collected in a calibrated measuring cylinder. The homogeniser and filter paper were washed twice with 5mL of the chloroform:methanol mixture. Chloroform:methanol (1:1 v/v) was added to the filtrate until the final volume reached 30mL. Of this lipid extract, 10mL was allowed to evaporate to dryness in nitrogen and dissolved in 2mL chloroform:methanol.

TOTAL LIPIDS

Total lipids were determined colorimetrically on the lipid extract after reaction with sulphuric and phosphoric acids and vanillin which gave a pink coloured complex. Kits from Boehringer Mannheim (Cat. no. 124 303), which include standards for total lipids were used. The CV of this method varied between 0.54 and 1.66%.

CHOLESTEROL

After extraction from liver tissue, cholesterol was determined colorimetrically on the principle that cholesterol forms a coloured complex with acetic anhydride and concentrated sulphuric acid (Liebermann-Burchard reaction, Tietz, 1976). The CV of the method varied between 0.76 and 2.69%.

4.5.5 LIVER GLYCOGEN

The glycogen content of the livers of fasting rats and 4 h-postprandial baboons, and which had been kept at -70°C , was determined by the principle that glycogen is released from the tissue by heating with strong alkali and precipitated on the addition of ethanol (Plummer, 1978). Sodium sulphate is added as a coprecipitant to give a quantitative yield of glycogen. The polysaccharide is then hydrolysed in acid and the glucose released is estimated.

For the isolation of glycogen, samples of 1g of liver tissue were placed in calibrated centrifuge tubes containing 2mL of KOH (300g/L) and heated in a boiling water bath for 20 min. with occasional shaking. The tubes were then cooled in ice, 0.2mL of saturated Na_2SO_4 added and mixed thoroughly. The glycogen was precipitated by adding 5mL of ethanol (95% v/v) and cooled on ice for 5 min. After centrifugation, the precipitated glycogen was dissolved in about 5mL of water with gentle warming, diluted with distilled water to the 10mL calibration mark and mixed thoroughly.

For the hydrolysis of glycogen, 1mL samples of the glycogen solutions were pipetted in calibrated test tubes, 1mL of HCl (1.2 mol/L) was added, marbles were placed on top of test tubes which were then heated in a boiling water bath for 2h. A drop (30 μL) of phenol red indicator was then added, and the solution neutralised carefully with NaOH (0.5mol/L) until the indicator changed from pink to a yellow colour. Distilled water was used to dilute the mixture to 10 mL. The glucose content was then determined by the glucose oxidase method of Folin & Wu (1920).

4.5.6 PLASMA COAGULATION FACTORS

PREPARATION OF PLASMA SAMPLES

Citrate plasma was prepared from peripheral venous blood (*V.cephalica*) from 12h-fasted humans, from *V.cephalica* or *Vv. comitantes a saphena* in 18h-fasted baboons and from blood drawn from the heart of 12h-fasted Zucker rats. Blood samples were drawn with disposable 5mL (rats) or 10mL (humans and baboons) syringes with 18-G needles. One part of citrate buffer solution (BM: Cat. no. 126 420, 0.11 mol/L) was drawn into sterile, disposable syringes before 9 parts of blood were drawn. The content was mixed carefully, transferred to a disposable plastic centrifuge tube, centrifuged and the supernatant divided into 4 portions

which were kept at -20°C until coagulation factor measurements. Samples were thawed within 2 min at 37°C in the heating block of the Fibrintimer ®.

APPARATUS

The Behring Fibrintimer ® (Behring Institute, Marburg, West Germany), which is a dual channel coagulometer with a light source and photo-detector, was used to determine fibrinogen concentration and factor VII coagulant activity in citrated plasma. The two measuring channels work independently of each other and tests can be done in duplicate. The coagulometer has a temperature controlled heating block for the incubation of reagents and samples at $37 \pm 0.2^{\circ}\text{C}$. Prepared samples are pipetted into disposable plastic cuvettes to which small disposable nickel coated mixers are added, incubated for 60s, and placed into one of the measuring channels. As soon as the starting reagent is added, the optical density of the sample changes, which triggers the timer and the stirring motor under the measuring channel. The sample is stirred during the entire clot formation procedure to prevent sedimentation of activating particles. This stirring action, combined with an optical density measurement, constitute the basic feature of the so called "turbo-densitometric" clot sensing method upon which the instrument operates. When a clot forms, the optical density of the sample increases. The timer and stirring motor stop when this change in optical density reaches a given value. The time (in seconds) that is required for a clot to form after the addition of the activating reagent, is indicated on the digital display. Even the most labile clot is concentrated by the small mixer rotating at the bottom of the cuvette and spindled on the long axis of the cuvette.

REAGENTS

All starting reagents, dilution buffers, factor-deficient plasmas, standard human and patho-plasmas were purchased from Behring Institute (BI).

Multifibren ® kit (BI: Cat. no. OTXG 21). The kit contains thrombin, which is prepared with a kaolin suspension (5g/L in a 5g NaCl solution), as well as barbital buffer solution and control plasma.

Thromborel ® S (BI: Cat. no. OUHP 35/47) was used as starting reagent for the determination of factor VII. This is a lyophilised thromboplastic extract from human placenta, containing calcium ions adapted to the test purpose.

Buffer solutions: Barbital buffer solution (BI: Cat. no. DIXM 57) and diethylbarbiturate acetate buffer solution (BI: Cat. no. ORHW 41) pH = 7.6 were used for the dilution of plasma for the determination of fibrinogen and factor VII respectively.

Clotting factor VII-deficient plasma (BI: Cat. no. OTXV 13). By using deficient plasmas in which a single factor is missing, the content of the other clotting factors is constant. The clotting time is then dependent only on the activity of the clotting factor which has to be determined. Evaluation is performed by reading the result from a reference curve directly in percentage of normal.

Standard and control plasmas

Control Plasma N (BI: Cat. no. ORKE 35) is obtained from a plasma pool from selected healthy male donors aged 20 to 40 years. It is stabilised with citrate buffer solution and is lyophilised. Control Plasma N was used for carrying out precision and accuracy control in the determination of fibrinogen.

Standard Human Plasma (BI: Cat. no. ORKL 07) is obtained from the pooled plasma of at least 100 selected healthy male donors aged between 20 and 40 years. It is also stabilised with citrate buffer solution and lyophilised. Standard Human Plasma was used as a reference for normal values for fibrinogen concentration and factor VII coagulant activity.

PROCEDURES

Fibrinogen (factor 1)

The concentration of fibrinogen in plasma samples was determined by a modified method of Clauss (Clauss, 1957) with the Fibrintimer®. Results obtained with this method were compared with a kinetic method (Becker *et al.*, 1984). The mean concentration of 50 samples as determined with the Fibrintimer® was 293.2 ± 68.7 mg/dL and with the kinetic method 283.8 ± 56.9 mg/dL. The coefficient of correlation of the two methods was $r = 0.87$ ($p = 0.0001$).

The principle of the Clauss method is that citrated plasma is clotted by addition of a relatively large amount of thrombin. The clotting time depends mainly on the fibrinogen content of the blood sample. The plasma was diluted 1:10 with barbital buffer solution. To 0.2mL diluted plasma was added 0.2mL fibrinogen reagent (Multifibren® test kit, BI: Cat. no. OTXG 21) and the clotting time determined with the Fibrintimer®. The fibrinogen concentration is reported in mg/dL. Conversion to the latter was done with the tabulation of values from the reference curve enclosed with the reagent. The values given only apply to reagents with the same lot number. The reference curves were checked with the aid of Control Plasma N (BI: Cat. no. ORKE 35) and Standard Human Plasma (BI: Cat. no. ORKL 07). When the coagulation time of duplicate samples differed more than 3%, the determination was repeated in duplicate. The CV of the method was between 1.1 and 3.0%.

For the kinetic method a test kit of Boehringer Mannheim (Cat. no. 524 484) with fibrinogen standard (BM: Precimat® Fibrinogen, Cat. no. 828 068) was used. The method is based on

the principle that batroxobin, an enzyme of the venom of the snake *Bathrops atrox*, breaks fibrinogen down to fibrin monomers. The turbidity that develops when the fibrin monomers polymerise to form fibrin, changes the absorbance of the sample, which can be read from a spectrophotometer at 340nm. The change in absorbance per unit of time is directly stoichiometric related to the fibrinogen concentration in the sample. The absorbance of a fibrinogen standard with a known concentration was used to calculate the concentration of the samples.

Factor VII coagulant activity

For the determination of factor VII (proconvertin) activity the samples were diluted with diethylbarbiturate acetate buffer solution (1:19 v/v). Equal volumes of diluted plasma and factor VII-deficient plasma were then mixed and incubated at 37°C. The coagulation reaction was triggered by Thromborel® S. Coagulation time of Standard Human Plasma was used to determine the ratio of factor VII in the samples. A ratio smaller than one indicates increased coagulant activity. Measurements on all the samples were done on the same day. The CV of eight measurements of control serum was $1.96 \pm 0.50\%$.

CHAPTER 5

COMPARISON BETWEEN PHYSIOLOGICAL EFFECTS OF KONJAC-GLUCOMANNAN AND PROPIONATE IN BABOONS

SUMMARY

The effects of the soluble dietary fibre concentrate konjac- glucomannan (K-GM) and of propionate on plasma fibrinogen, serum and liver lipids, glucose tolerance, insulin response, insulin sensitivity and liver glycogen were compared in baboons. Twelve male baboons were fed a semi-synthetic "Western" diet (SSW-diet) with and without K-GM (5%) or sodium propionate (2%) supplementation for periods of 9 weeks in a crossover, randomised order, with washout periods in between. Measurements were made at baseline and after 4 and 9 weeks of each study period. Total serum cholesterol (TC) increased by 25% ($p < 0.01$) and plasma fibrinogen by 18% ($p < 0.05$) on the unsupplemented SSW-diet. The propionate supplemented diet increased TC by 17% ($p < 0.05$), lowered serum free fatty acid (FFA) levels and prevented an increase in fibrinogen concentration. Konjac-glucomannan lowered TC, triglycerides and circulating FFA and also prevented an increase in fibrinogen concentration ($p < 0.05$). Changes in plasma fibrinogen levels correlated positively with changes in serum FFA concentration. The prevention of an increase in fibrinogen levels by the supplements may thus partly be ascribed to lower circulating FFA levels. Liver cholesterol content was 31-34% lower in the baboons fed the supplemented diets. It is concluded that the hypocholesterolaemic effect of soluble DF may partly be mediated through the ability of propionate to inhibit hepatic cholesterol synthesis. However, the baboon does not seem to be an appropriate model to study insulin sensitivity.

INTRODUCTION

Hypercholesterolaemia and glucose intolerance are important in the pathogenesis of atherosclerosis. Increased levels of fibrinogen are also related to the development of CHD (Meade *et al.*, 1986). In man as well as in animal models dietary supplements of soluble fibre lower serum cholesterol (Chen & Anderson, 1986; Kritchevsky, 1987; Venter *et al.*, 1987; Vorster *et al.*, 1985), improve glucose tolerance (Jenkins *et al.*, 1980; Vorster & De Jager, 1984; Vorster *et al.*, 1988b) and lower plasma fibrinogen (Koepp & Hegewisch, 1981; Vorster *et al.*, 1985). A number of possible mechanisms have been proposed (Anderson & Chen, 1979; Vorster *et al.*, 1988a), one of which is through an effect of the SCFAs derived from bacterial fermentation of DF. The suggestion that the cholesterol-lowering ability of DF may in part be ascribed to its fermentation products, is supported by the observation that the feeding of either sodium acetate (Beynen & Lemmens, 1987) or sodium propionate (Chen *et al.*, 1984; Illman *et al.*, 1988) has cholesterol-lowering activity in rats. Similar results have been reported for pigs fed diets supplemented with propionic acid (Boila *et al.*, 1981; Thacker & Bowland, 1981;

Thacker *et al.*, 1981; Thacker *et al.*, 1982) or calcium propionate (Thacker *et al.*, 1982). Less experimental evidence is available to support the hypothesis that DF improves insulin sensitivity and lowers plasma fibrinogen levels through effects of the SCFAs. The study reported here was therefore designed to compare in baboons fed a semi-synthetic "Western" diet (SSW-diet), the long-term effects of dietary propionate with that of konjac-glucomannan (K-GM) on glucose tolerance, immunoreactive insulin response, insulin sensitivity, liver glycogen and lipid content, serum lipid profiles and plasma fibrinogen concentration. The baboon is regarded as a suitable model for studies concerning the association of fibre-depleted diets and Western lifestyle diseases, such as atherosclerosis (Kritchevsky, 1970), as well as an appropriate model for DF digestion studies (Graham & Aman, 1987).

MATERIALS AND METHODS

The experimental diets, animals, protocol and chemical methods are described in chapter 4. Briefly, 12 male baboons (*Papio ursinus*) with average starting weight 18.96 kg were maintained in individual cages. Food intake was restricted to 400 g dry weight/d and water was provided *ad libitum*. The basic SSW-diet is described in table 4.1 and the daily nutrient intake given in table 5.1. Carbohydrate supplied 39.7% of the energy of the diet, protein 18.8% and fat 41.4%. To the basic diet was added 2% (w/w) sodium propionate or 5% K-GM. This amount of sodium propionate, when dissociated would form 6.2 g propionic acid, which is about double the maximum amount that could be derived from colonic fermentation of 5% (20 g) K-GM (Cummings, 1981b). Each group of baboons ($n = 4$) received each dietary treatment in a Latin square design for a period of 9 weeks with washout periods of 5 weeks in between. During the washout periods the baboons were fed *ad libitum* Epol® dog cubes and oranges. Two-hour GTTs were performed before a specific dietary treatment was given and again after 4 and 9 weeks. Venous blood samples were collected at the same time for the determination of immunoreactive insulin (IRI) response, serum lipids and plasma fibrinogen concentrations. All the animals remained in excellent health till the end of the experiment which lasted 43 weeks. One week after the final GTTs were performed, the baboons were fasted for 4 h, sedated and anaesthetised. Livers were excised quickly, blotted dry, weighed and snap frozen. Insulin sensitivity was calculated according to the method of Donahue *et al.* (1988) as the reciprocal of the fasting insulin and glucose product multiplied by 10 000. The study was approved by the Ethics Committee of the Potchefstroom University.

STATISTICS

In the statistical analysis a variety of variance/ covariance models were fitted to the data of individual parameters. It was found that the major factors influencing the responses were phases, diets and individual variation between baboons. A possible major contributing factor to the effect of the different phases seemed to be weight gain. However, models which included weight gain as a factor, along with phases, did not show a significant effect for weight gain. If weight gain was included as a factor and phases were excluded, then weight gain

turned out to be a very significant factor. It is clear that the effects of weight gain and phases are confounded and cannot be distinguished separately. Since phase showed a more significant effect, phase rather than weight gain was included as a factor in the analysis of variance.

TABLE 5.1 DAILY NUTRIENT INTAKE OF BABOONS

Nutrient	Amount
Total energy (kcal)	1750
(kJ)	7326
Total protein (g)	82.3
Plant protein (g)	37.9
Animal protein (g)	44.4
% Energy : total protein	18.8
Total fat (g)	80.5
Saturated fatty acids (g)	32.30
Mono-unsaturated fatty acids (g)	30.95
Polyunsaturated fatty acids (g)	13.61
P : S ratio	0.42
% Energy : total fat	41.4
Cholesterol (mg)	539
Total carbohydrate (g)	173.8
% Energy : total carbohydrate	39.7
Sugar (g)	40.0
Total dietary fibre	14.0
Fibre : insoluble (g)	12.4
soluble* (g)	1.6
Calcium (mg)	4333
Iron (mg)	71.8
Magnesium (mg)	328
Zinc (mg)	2.1
Vitamin A (IU)	12590
Vitamin D (μ g)	53.9
Vitamin E (mg)	104.5
Ascorbic acid (mg)	306.0
Thiamin (mg)	8.6
Riboflavin (mg)	4.7
Nicotinic acid (mg)	46.2
Vitamin B ₆ (mg)	6.0
Folate (μ g)	306.0
Vitamin B ₁₂ (μ g)	8.4
Pantothenic acid (mg)	46.0

* Soluble fibre content of the K-GM supplemented diet was 21.6 g.

In the case of the serum lipid parameters it was found that the baseline values differed significantly between the phases. Therefore the differences between 9 weeks and baseline for each phase was used as response variable. However, in the cases of mean fasting blood glucose and serum insulin concentrations, maximum glucose and insulin increments and areas under the glucose tolerance and IRI response curves, no significant differences between baseline values were found and in such cases the difference between the 9 week results and the mean of the three baseline values was used as response variable to reduce variation. In all cases residuals obtained from the fitted model were used to identify outliers among the data and the stability of conclusions with regard to elimination of such outliers was examined.

To determine the relationship between plasma fibrinogen and serum FFA levels, an analysis of covariance was done with fibrinogen as response variable and FFA as covariate. The liver lipid and glycogen content of baboons fed the different dietary treatments was compared with one-way analyses of variance. When significant differences were found, Tukey's Studentised range test was applied to determine which mean values were significantly different. All analysis were done with PROC.GLM of the SAS® system.

RESULTS

The addition of K-GM and propionate made no difference as regards acceptability of, and weight gain during the different experimental dietary treatments (table 5.2). Most of the baboons lost weight during the washout periods (mean weight change during the first and second washout periods was +0.16 and -0.88 kg respectively). When fed the SSW-diet, with or without supplements, the mean weight gain was the largest in phase 1 and the smallest in phase 3, with no difference between the dietary treatments.

TABLE 5.2 WEIGHT GAIN OF BABOONS ON THE SSW-DIET WITH OR WITHOUT K-GM AND PROPIONATE SUPPLEMENTS IN THE DIFFERENT PHASES OF THE EXPERIMENT (n = 4)*

Phase	Diet		
	SSW-diet	SSW-diet + K-GM	SSW-diet + propionate
Phase 1	4.60 ± 0.47	4.47 ± 0.54	4.80 ± 0.85
Phase 2	2.15 ± 0.53	2.70 ± 0.55	2.33 ± 0.29
Phase 3	1.65 ± 0.44	1.43 ± 0.26	1.38 ± 0.45
Mean	2.80 ± 0.48	2.87 ± 0.45	2.83 ± 0.53

* Values are expressed as mean ± SEM

Considerable variation in serum TC levels between baboons was observed, a phenomenon which has been reported previously (Strong *et al.*, 1964; Kritchevsky *et al.*, 1974). Mean serum TC values increased significantly when the baboons were fed the unsupplemented SSW-diet (25.4%) as well as the propionate supplemented diet (16.9%, table 5.3). Konjac-glucomannan prevented this increase. High-density lipoprotein cholesterol (HDLc) concentration increased during all the experimental dietary treatments (p < 0.05). Percentage HDLc of TC was consistently significantly higher during K-GM supplementation. Konjac-glucomannan also lowered triglyceride (TG) and FFA levels (p < 0.05). The decrease in FFA levels caused by propionate was statistically significant after 4, but not after 9 weeks. In contrast to the other diets propionate raised serum TG significantly by 5.6%. Liver cholesterol content was 31-34% lower in baboons fed the K-GM and propionate supplemented diets in the final phase of the experiment (p < 0.05). Liver glycogen content did not differ significantly.

TABLE 5.3 SERUM AND LIVER LIPIDS IN BABOONS FED THE SEMI-SYNTHETIC WESTERN DIET (SSW-DIET) WITHOUT OR WITH K-GM AND PROPIONATE SUPPLEMENTS*

Lipid	Phase	SSW-diet	SSW-diet + K-GM	SSW-diet + propionate
TC (mmol/L)	Baseline	2.36 ± 0.09	2.53 ± 0.15	2.42 ± 0.13
	Middle	2.99 ± 0.12 ¹	2.53 ± 0.08 ³	2.88 ± 0.16 ²
	End	2.96 ± 0.15 ¹	2.45 ± 0.11 ³	2.83 ± 0.12 ²
HDLC (mmol/L)	Baseline	1.24 ± 0.05	1.34 ± 0.08	1.39 ± 0.10
	Middle	1.71 ± 0.08 ¹	1.51 ± 0.06 ²	1.59 ± 0.08 ²
	End	1.69 ± 0.08 ¹	1.53 ± 0.06 ²	1.69 ± 0.07 ¹
% HDLC of TC	Baseline	52.9 ± 2.3	53.70 ± 2.8	56.9 ± 2.2
	Middle	57.6 ± 1.7	60.00 ± 1.8 ²	55.8 ± 2.2 ³
	End	57.5 ± 2.0	63.30 ± 1.9 ^{2 3}	59.9 ± 1.8
TG (mmol/L)	Baseline	0.42 ± 0.04	0.50 ± 0.05	0.36 ± 0.04
	Middle	0.33 ± 0.05	0.38 ± 0.06	0.31 ± 0.04
	End	0.36 ± 0.04	0.35 ± 0.04 ¹	0.38 ± 0.05 ³
FFA (μmol/L)	Baseline	212 ± 25.1	344 ± 58.0	210 ± 23.5
	Middle	243 ± 22.3	215 ± 13.2 ^{1 3}	197 ± 10.7 ³
	End	215 ± 13.4	195 ± 14.2 ^{1 3}	200 ± 15.7
Total liver lipids	End ⁴ (mg/g)	31.9 ± 2.5	31.5 ± 2.5	28.3 ± 2.7
Liver cholesterol	End ⁴ (mg/g)	3.2 ± 0.14	2.2 ± 0.20 ⁵	2.1 ± 0.06 ⁵

* Values are expressed as mean ± SEM (n = 12)

¹ Significantly different from baseline (p < 0.01, paired t-tests)

² Significantly different from baseline (p < 0.05, paired t-tests)

³ Change significantly different from change on other diets in the same line (p < 0.05)

⁴ n = 4 on each dietary treatment

⁵ Significantly different from SSW-diet (p < 0.05, one way ANOVA with Tukey's Studentised range test)

Because no significant differences were found between the means of the three baseline measurements for fasting glucose and insulin, maximum glucose and insulin increments and area under the glucose tolerance curve, the final values on the dietary treatments were compared with the mean of the three baseline values (table 5.4). The incremental area under the glucose tolerance curve was calculated with the fasting or lowest glucose concentration as baseline. The mean area under the glucose tolerance curve was smaller when the baboons were fed K-GM and propionate supplements (p < 0.05) and a decrease in maximum glucose increment with addition of K-GM (p = 0.06) and propionate (not significant) was observed.

The individual values for the calculated insulin sensitivity are represented in table 5.5. From the table it is clear that the variation in insulin sensitivity between baboons, as well as in the same baboon on different occasions, was too large to permit valid conclusions from the data.

TABLE 5.4 FASTING SERUM GLUCOSE AND INSULIN CONCENTRATIONS, MAXIMUM GLUCOSE AND INSULIN INCREMENTS AND AREA UNDER GTT-CURVE (MEAN $\pm$ SEM)

	Baseline	End of experimental period		
		SSW-diet	SSW-diet + K-GM	SSW-diet + propionate
Fasting glucose (mmol/L)	4.47 $\pm$ 0.14	4.27 $\pm$ 0.24	3.87 $\pm$ 0.24	4.40 $\pm$ 0.24
Fasting insulin (μ U/mL)	58.6 $\pm$ 5.90	89.8 $\pm$ 8.50	67.0 $\pm$ 7.90	87.2 $\pm$ 9.30
Maximum glucose increment (mmol/L)	2.28 $\pm$ 0.23	2.53 $\pm$ 0.36	1.73 $\pm$ 0.22	2.10 $\pm$ 0.28
Maximum insulin increment (μ U/mL)	144 $\pm$ 16	144 $\pm$ 11	140 $\pm$ 12	158 $\pm$ 13
Area under 90 min GTT-curve	118 $\pm$ 12	135 $\pm$ 10 ^a	103 $\pm$ 10 ^b	106 $\pm$ 10 ^b
Area under 120 min GTT-curve	145 $\pm$ 15	182 $\pm$ 12 ^a	133 $\pm$ 14 ^b	128 $\pm$ 12 ^b
Area under 120 min insulin-curve	8730 $\pm$ 2475	8080 $\pm$ 980	8380 $\pm$ 1171	10404 $\pm$ 1171
Liver glycogen (mg/g)*	-	291 $\pm$ 88	169 $\pm$ 16	154 $\pm$ 13

^{a,b} Means in a horizontal row with different superscript differ significantly (p < 0.05)

* n = 4

TABLE 5.5 INSULIN SENSITIVITY INDEX* OF INDIVIDUAL BABOONS BEFORE THE START AND AFTER 9 WEEKS OF EACH EXPERIMENTAL DIET

Baboon	Baseline			SSW-diet	SSW-diet + K-GM	SSW-diet + propionate
	Phase 1	Phase 2	Phase 3			
1	26.2	70.0	93.4	13.8	42.62	H
2	38.7	91.6	52.9	25.1	54.1	H
3	71.4	67.8	166.7	40.5	68.9	41.1
4	56.8	16.9	17.8	24.0	28.3	34.6
5	86.8	H	22.6	24.2	22.2	8.2
6	89.3	H	17.8	18.2	23.0	14.9
7	58.1	18.9	46.6	138.9	38.5	33.8
8	71.2	H	29.8	164.5	16.5	14.8
9	69.3	51.0	46.0	21.2	82.3	65.0
10	21.7	23.1	20.7	33.4	41.7	33.2
11	73.5	98.0	201.6	80.0	151.5	53.0
12	20.3	150.2	19.4	20.0	142.9	25.7
Mean	56.9	65.3	61.3	50.3	59.4	32.4
SD	23.5	41.5	59.2	48.6	43.4	16.8

*Donahue *et al.*, 1988

H = Haemolytic serum, not suited for determination of insulin concentration.

The mean plasma fibrinogen concentration before and after the respective dietary treatments is shown in table 5.6. The SSW-diet increased fibrinogen concentration by 17.8% whilst K-GM and propionate prevented an increase. The changes after 9 weeks were significant ($p < 0.10$). The results correspond with observations by Vorster *et al.* (1985) on baboons. The relationship between changes in fibrinogen and FFA concentrations, expressed as the coefficient of the regression function, i.e. the increase in fibrinogen per unit increase in FFA, was significant for the SSW-diet (0.51, $p = 0.0002$) and the propionate supplemented diet (0.34, $p = 0.001$) but not significant for the K-GM supplemented diet (0.04, $p = 0.29$).

TABLE 5.6 MEAN PLASMA FIBRINOGEN CONCENTRATIONS IN BABOONS ON THE SSW-DIET WITH OR WITHOUT K-GM AND PROPIONATE SUPPLEMENTS ($\pm$ SEM) (n = 12)

Diet	Baseline	4 weeks	9 weeks
		mg/dL	
SSW-diet	143.8 $\pm$ 13.5	146.6 $\pm$ 9.1	169.2 $\pm$ 8.5 ^a
SSW-diet + K-GM	143.7 $\pm$ 12.3	140.2 $\pm$ 7.4	142.4 $\pm$ 7.9 ^b
SSW-diet + propionate	153.0 $\pm$ 16.6	143.4 $\pm$ 9.4	148.3 $\pm$ 11.2 ^c

^a Significantly different from baseline value ($p < 0.05$)
^b Change significantly different from change on SSW-diet ($p < 0.05$)
^c Change significantly different from change on SSW-diet ($p < 0.10$)

DISCUSSION

Soluble DF lowers serum as well as liver cholesterol in several animal species (Kritchevsky, 1987) (see section 2.4). Dietary propionate lowers serum cholesterol in rats (Chen *et al.*, 1984; Illman *et al.*, 1988) and in pigs (Boila *et al.*, 1981; Thacker & Bowland, 1981), regardless of the cholesterol content of the diet. The mechanism of action of propionate remains uncertain but is believed to be mediated either by inhibition of hepatic cholesterol synthesis (Bush & Milligan, 1971; Boila *et al.*, 1981; Anderson & Bridges, 1984) or by redistribution of cholesterol from plasma to liver (Illman *et al.*, 1988). Results of the study reported here show that a SSW-diet increases serum TC concentration in baboons and that K-GM prevented this increase. Propionate supplementation resulted in a smaller increase in serum TC levels and lowered liver cholesterol content to the same extent as K-GM, which seems to indicate inhibition of cholesterol synthesis by propionate and not redistribution of cholesterol from plasma to liver.

The reason why the serum TC levels during propionate supplementation did not reflect the low liver cholesterol content is not clear. The dietary treatments lasted 9 weeks, which probably should have been long enough for cholesterol homeostasis to stabilise. Body cholesterol of baboons is metabolised according to a two-pool system, as in man. The half-life of the first pool, which normally comprises the free cholesterol and cholesterol esters of serum, red blood cell cholesterol and liver cholesterol is reported to be 8.5 days, and that of the second pool which comprises the remaining body cholesterol, 69.0 days in vitamin C-deficient baboons and 85.5 days in vitamin C-treated baboons (Kotzé *et al.*, 1974). These values are somewhat higher than half-life values reported by Kritchevsky (1970) and others for baboon cholesterol. The introduction of a Western diet may possibly require a longer period for stabilisation of cholesterol balance in baboons.

There are few reports of the effect of DF on lipid metabolism in non-human primates fed Western diets, and as far as is known, no reports of the effect of propionate. In Vervet monkeys fed Western diets supplemented with 10% cellulose or 10% pectin, serum cholesterol levels were elevated significantly after 34 weeks, compared to a control group which was fed a commercial ration with fruit and bread supplements (Kritchevsky *et al.*, 1988). Liver cholesterol content, which was the same in these three groups of Vervet monkeys, was slightly higher than in the baboons fed the SSW-diet in the study reported here. The mean starting serum TC concentration of the baboons, as well as concentrations when fed the SSW-diet corresponds with values reported in the literature for baboons before and after being fed an atherogenic diet (Kotzé & Kempff, 1973).

Konjac-glucomannan decreased serum TG and FFA levels significantly. Although not to the same extent, and statistically significant only after 4 weeks, propionate also lowered circulating FFA concentration. High serum concentrations of FFA are associated with hepatic (Strömblad & Björntrop, 1986) and peripheral (Martin, 1985) insensitivity to insulin. The mean area under the glucose tolerance curve decreased significantly during K-GM and propionate feeding which could have been a result of an increased insulin sensitivity associated with lower circulating FFA. In addition, propionate stimulates rates of glycolysis in isolated rat hepatocytes (Anderson & Bridges, 1984), facilitating glucose use, which may also contribute to dietary fibre's effect on the observed improved carbohydrate metabolism.

Little is known about the influence of diet on coagulation factors (see section 2.5). A possible inhibitory effect of dietary short-chain fatty acids on blood coagulation was first mentioned by Malhotra (1968). If fibrinogen synthesis in the liver is stimulated by raised circulating FFA levels as suggested by Pilgeram & Pickarl (1968), then it seems reasonable to conclude that the prevention of an increase in fibrinogen concentration by K-GM can in part be ascribed to lower circulating FFA levels during K-GM feeding. The lack of a significant relationship between fibrinogen and FFA levels during K-GM supplementation against the significant increase of 0.51 units fibrinogen per unit increase in FFA concentration when fed the

unsupplemented diet, supports this assumption. Propionate tended to lower FFA levels, prevented an increase in fibrinogen and resulted in a regression function of 0.35 for these two variables. The reason why increased circulating FFA increase plasma fibrinogen is not clear. It has been suggested (Vorster *et al.*, 1988a; Venter & Vorster, 1989, see chapter 3) that insulin may regulate aspects of fibrinogen synthesis and/or secretion on an inhibitory level, and that a hepatic insulin resistant state should be characterised by raised plasma fibrinogen. The influence of FFA on hepatic sensitivity to insulin, could be the mechanism through which FFA effects on plasma fibrinogen may be mediated. It is therefore conceivable that the effect of K-GM on circulating FFA levels and fibrinogen synthesis in the liver may in part be mediated through propionate. However, the baboon appeared to be a poor model for the investigation of insulin sensitivity in this study. It was therefore decided to test the hypothesis, stated in chapter 3, in an animal model known to be insulin resistant and hyperfibrinogenaemic, i.e. the Zucker fatty rat.

CHAPTER 6

COMPARISON OF THE EFFECTS OF KONJAC-GLUCOMANNAN WITH THE EFFECTS OF PROPIONATE SUPPLEMENTATION OF A WESTERN DIET IN ZUCKER RATS

SUMMARY

The effects of konjac-glucomannan (K-GM) (5%), and of propionate (2%) on plasma fibrinogen, serum and liver lipids, fasting serum insulin and glucose, as well as liver glycogen, were compared in 40 hyperfibrinogenaemic Zucker fatty rats and 40 lean litter mates, against a background semi-synthetic low-fibre, high-fat diet. After 9 weeks, significantly lower weight gain was observed in both fatty and lean rats fed K-GM. Mean plasma fibrinogen and fasting serum insulin levels of fatty and lean rats fed either the gel fibre or propionate diets, were significantly lower than those of animals fed the background diet alone. Both K-GM and propionate significantly increased insulin sensitivity, calculated as the reciprocal of the insulin and glucose product multiplied by 10 000. It seems as if the effect on fibrinogen and insulin levels was independent of effects on serum lipids since serum lipids were not influenced by the specific dietary treatments. The only exceptions were a decrease in % HDLC in both fatty and lean rats fed the SSW-diet without supplements, and an increase in TG levels in the same rats. Both K-GM and propionate significantly reduced liver glycogen stores. Total liver lipid content of rats fed the K-GM supplemented SSW-diet was more than 50% lower than in fatty rats fed the unsupplemented diet ($p < 0.05$). Propionate supplementation resulted in a decrease of 33.3% in total liver lipid content in fatty rats. Supplementation of the SSW-diet with K-GM or propionate tended to reduce liver cholesterol in fatty rats.

Significant correlations of plasma fibrinogen with weight gain, and effects on fibrinogen and insulin levels, suggest that dietary fibre's effect on fibrinogen levels may be mediated through effects on slower weight gain and an associated decrease in insulin resistance in this rat model. However, propionate did not influence weight gain and still exerted effects on fibrinogen levels and insulin sensitivity, similar to those of K-GM. This suggests that the reported ability of soluble DF to improve insulin sensitivity, and the proposed ability of DF to lower plasma fibrinogen, may in part be mediated through its fermentation product propionate.

INTRODUCTION

In the baboon model it was observed that a semi-synthetic low-fibre, high-fat (SSW-diet) increased plasma fibrinogen levels significantly and that the feeding of either K-GM or sodium propionate prevented this effect (see chapter 5). An increased intake of DF improves insulin function and lowers insulin resistance in diabetic patients (Anderson, 1986) as well as in

animal models (Zacharia, 1983; Ogunwole *et al.*, 1987). It has previously been hypothesised (Vorster *et al.*, 1988a, see chapter 3) that the hormone insulin may regulate aspects of fibrinogen synthesis and/or secretion on an inhibitory level, and that an insulin resistant state should be associated with raised plasma fibrinogen levels. The Zucker fatty rat (Zucker & Zucker, 1961) is characterised by hyperfibrinogenaemia (Stoltz *et al.*, 1981) and insulin resistance in hepatic (Terrettaz & Jeanrenaud, 1983), muscle (Crettaz, 1980), heart muscle (Eckel *et al.*, 1985), and adipose tissues (Cushman *et al.*, 1978). The rat might not be the most suitable model for studies regarding Western lifestyle diseases such as atherosclerosis, but it has been described that the extent of fibre degradation in man and rat is very similar (Nyman *et al.*, 1986) and that the SCFA profile in faecal material from the rat resembles that of man more closely than other animal models tested (Lupton & Villalba, 1988). The study reported here was designed to evaluate in Zucker rats the hypothesis that DF lowers plasma fibrinogen levels concomitantly with an improvement in insulin sensitivity, and that propionate contributes to this effect. To this extent, the effects of K-GM supplementation of the semi-synthetic Western diet (SSW-diet) fed to the baboons, were compared with the effects of propionate supplementation of the same diet. Results of Zucker rats fed commercially prepared laboratory food during the experimental period are also included.

MATERIALS AND METHODS

The experimental diets, animals and chemical methods are described in chapter 4. Briefly, locally bred Zucker fatty rats (fa/fa), phenotypically identified, and lean litter mates (Fa/?) were paired according to sex and age, and randomly allocated to one of 10 groups (7-9 rats/group; 5 groups of fatties; 5 groups of lean rats). Because the K-GM and propionate supplemented groups were studied at different times, an additional control group on the SSW-diet was included. The composition of the SSW-diet is described in tables 4.1 and 4.2. The dietary treatment of the different groups is indicated in table 6.1. The rats were fed *ad libitum* for 9 weeks. At the end of this period, 10h fasted rats were anaesthetised with 85-90 mg sodium pentobarbital/kg body weight. Blood samples were obtained by cardiac puncture for the preparation of citrated plasma (1:9 v/v) and serum, which were quickly frozen and kept at -70°C until analysis. Livers were excised, weighed, washed in ice-cold 0.9% NaCl and also kept at -70°C until analysis. The concentrations of plasma fibrinogen, serum glucose, -insulin and -lipids, total liver lipids, liver cholesterol and glycogen content were determined as described in chapter 4. Insulin sensitivity was calculated as the reciprocal of the insulin and glucose product multiplied by 10 000.

STATISTICS

Significant differences between groups (Student t-test) and Pearson correlation coefficients were calculated with the SAS® package. (SAS® Users' Guide, 1985. SAS Institute Inc. Cary, North Carolina). Fatty and lean rats were not compared. The study was approved by the Ethics Committee of the Potchefstroom University.

TABLE 6.1 DIETARY TREATMENT OF THE RESPECTIVE RAT GROUPS

Dietary treatment	Group number (n = 7-9)	
	fa/fa ¹	Fa/? ²
Epol ³	1	2
Semi-synthetic westernised diet (SSW-diet)	3	4
SSW-diet plus 5% K-GM	5	6
SSW-diet	7	8
SSW-diet plus 2% sodium propionate	9	10

¹ fa/fa designates the fatty rat which is homozygous with regard to the fat gene

² Fa/? designates the lean rat which may be homozygous with regard to the normal gene (Fa/Fa) or heterozygous with regard to the fat gene (Fa/fa)

³ Epol obtained from Epol (Pty.) Ltd., Johannesburg, South Africa, supplies 67% of the total energy as carbohydrate, 25% as protein and 8% as fat. It contains about 10% (w/w) dietary fiber. Rats were fed from one batch of Epol

RESULTS

Table 6.2 shows that in comparison with the commercial laboratory feed, the SSW-diet significantly increased weight gain in fatty and lean rats. No significant differences between the first and second control groups on the SSW-diet were observed. Konjac-glucomannan (K-GM) additions to the SSW-diet inhibited weight gain significantly in the fatty and lean rats, to such an extent that the lean rats gained significantly less weight during K-GM feeding than on the much higher fibre diet (commercial laboratory diet). This effect of K-GM on weight gain has been observed in Sprague-Dawley rats in spite of higher food intakes (Vorster *et al.*, 1985).

Total serum cholesterol was not affected by the dietary interventions (table 6.3). However, the SSW-diet significantly decreased the percentage of cholesterol carried by high-density lipoprotein (HDL) by 30.7% in the fatty rats. Mean percentage of HDLC was higher during K-GM feeding in lean rats (19,5%, $p < 0.05$) as well as fatty rats (12,8%, not statistically significant). Propionate supplementation of the SSW-diet tended to raise % HDLC (10.2% in both groups) but statistical significance was not reached. TG concentrations were higher in rats fed SSW-diet without supplements than in rats fed Epol ($p < 0.05$).

TABLE 6.2 MEAN $\pm$ SD OF BODY WEIGHT, WEIGHT GAIN AND RELATIVE LIVER WEIGHT OF ZUCKER RATS ON THE DIFFERENT DIETARY TREATMENTS

Dietary treatment	Body weight g	Fatty rats (fa/fa)		Body weight g	Lean Rats (Fa/?)	
		Weight gain g/rat/day	Relative liver weight g/100g		Weight gain g/rat/day	Relative liver weight g/100g
Epol	542.1 ¹ $\pm$ 61.7	2.1 ¹ $\pm$ 0.7	3.3 $\pm$ 0.4	303.7 ¹ $\pm$ 48.8	1.1 ¹ $\pm$ 0.6	2.8 $\pm$ 0.3
SSW-diet	619.3 ^{1,2} $\pm$ 43.4	3.9 ^{1,2} $\pm$ 0.3	3.6 ¹ $\pm$ 0.8	393.1 ^{1,2} $\pm$ 84.2	2.2 ^{1,2} $\pm$ 0.8	2.6 $\pm$ 0.3
SSW-diet + K-GM	553.5 ² $\pm$ 66.1	2.5 ² $\pm$ 0.9	2.7 ¹ $\pm$ 0.7	290.8 ² $\pm$ 19.6	0.4 ² $\pm$ 0.1	2.3 $\pm$ 0.2
SSW-diet	597.2 $\pm$ 81.2	3.5 $\pm$ 0.9	3.2 $\pm$ 0.7	334.7 $\pm$ 68.5	1.6 $\pm$ 0.5	2.6 $\pm$ 0.2
SSW-diet + propionate	540.1 $\pm$ 83.8	3.5 $\pm$ 0.8	2.9 $\pm$ 0.3	331.0 $\pm$ 88.6	1.8 $\pm$ 0.5	2.4 $\pm$ 0.3

^{1,2}Means in vertical columns with the same superscript differ significantly: Student-t-test, P < 0.05.

TABLE 6.3 MEAN $\pm$ SD SERUM LIPIDS OF ZUCKER RATS ON DIFFERENT DIETARY TREATMENTS

Dietary treatment	TC mmol/L	Fatty rats (fa/fa)		TC mmol/L	Lean Rats (Fa/?)	
		% HDLC TC	TG mmol/L		% HDLC of TC	TG mmol/L
Epol	4.8 $\pm$ 1.6	80.2 ¹ $\pm$ 12.1	6.7 ¹ $\pm$ 3.8	2.2 $\pm$ 0.4	72.9 $\pm$ 7.9	1.3 $\pm$ 0.7
SSW-diet	4.3 $\pm$ 0.7	55.6 ¹ $\pm$ 11.2	11.9 ¹ $\pm$ 3.7	1.9 $\pm$ 0.1	66.1 ¹ $\pm$ 9.0	1.3 $\pm$ 0.8
SSW-diet + K-GM	3.8 $\pm$ 1.7	62.7 $\pm$ 12.6	10.4 $\pm$ 8.4	2.0 $\pm$ 0.2	79.0 ¹ $\pm$ 2.5	0.7 $\pm$ 0.1
SSW-diet	6.1 $\pm$ 2.0	47.9 $\pm$ 6.4	12.4 $\pm$ 6.0	2.0 $\pm$ 0.2	74.9 $\pm$ 8.6	0.5 $\pm$ 0.1
SSW-diet + propionate	5.5 $\pm$ 1.6	52.8 $\pm$ 10.7	10.4 $\pm$ 4.2	1.8 $\pm$ 0.2	82.5 $\pm$ 5.7	0.4 $\pm$ 0.1

¹Means in vertical columns with the same superscript differ significantly: Student-t-test, p < 0.05

From table 6.4 it is clear that the SSW-diet increased total liver lipids, liver cholesterol and glycogen content of both fatty and lean rats. Total liver lipid concentration was 51.2% lower in fatty rats fed K-GM supplements than in rats fed the unsupplemented SSW-diet ($p < 0.05$). In lean rats K-GM lowered total liver lipids by 29.0%. K-GM tended to lower liver cholesterol in fatty as well as lean rats. The same trend was observed with propionate in fatty, but not in lean rats.

TABLE 6.4 MEAN $\pm$ SD LIVER TOTAL LIPIDS, CHOLESTEROL AND GLYCOGEN OF ZUCKER RATS ON DIFFERENT DIETARY TREATMENTS

Dietary treatment	Fatty rats (fa/fa)			Lean Rats (Fa/?)		
	Total lipids mg/g	Cholesterol mg/g	Glycogen mg/g	Total lipids mg/g	Cholesterol mg/g	Glycogen mg/g
Epil	28.8 ¹ ± 9.0	1.2 ¹ ± 0.2	36.8 ¹ ± 14.6	21.8 ¹ ± 3.9	1.3 ¹ ± 0.1	9.5 ¹ ± 7.5
SSW-diet	132.1 ^{1,2} ± 58.4	3.8 ¹ ± 2.3	289.6 ^{1,2} ± 119.7	58.2 ¹ ± 15.4	3.6 ¹ ± 2.4	59.5 ^{1,2} ± 46.4
SSW-diet + K-GM	64.4 ² ± 25.6	2.3 ± 0.5	136.3 ² ± 67.9	45.1 ± 21.2	2.6 ± 0.8	3.5 ² ± 1.9
SSW-diet	109.3 ± 67.4	3.9 ± 2.4	216.6 ³ ± 57.8	52.2 ± 7.6	2.9 ± 0.7	30.7 ± 31.1
SSW-diet + propionate	72.9 ± 25.4	2.4 ± 0.7	148.8 ³ ± 62.7	46.3 ± 8.2	3.1 ± 0.6	13.4 ± 18.3

^{1,2,3}Means in vertical columns with the same superscript differ significantly: Student-t-test, $P < 0.05$.

The SSW-diet resulted in significantly higher fasting circulating fibrinogen and insulin levels in the fatty rats, as indicated in table 6.5. Increases did not reach significance in the lean rats. K-GM additions lowered plasma fibrinogen concentration by 30.4% in fatty rats and 33.4% in lean rats ($p < 0.05$), compared to the unsupplemented SSW-diet. K-GM also lowered fasting serum insulin levels significantly (51.0% in fatty, and 67.2% in lean rats). The calculated insulin sensitivity index was nearly 200% higher in fatty rats fed K-GM and more than 200% higher in lean rats fed K-GM than in comparable control groups fed unsupplemented SSW-diets.

TABLE 6.5 MEAN $\pm$ SD PLASMA FIBRINOGEN, SERUM INSULIN AND -GLUCOSE OF ZUCKER RATS ON DIFFERENT DIETARY TREATMENTS

Dietary treatment	Fatty rats (fa/fa)					Lean Rats (Fa/?)				
	Fibrinogen mg/dL	Insulin μ U/mL	Glucose mmol/L	Insulin/ Glucose μ U/mmol	Insulin sensitivity index	Fibrinogen mg/dL	Insulin μ U/mL	Glucose mmol/L	Insulin/ Glucose μ U/mmol	Insulin sensitivity index
Epol	148.7 ¹	31.7 ¹	18.8	2.0	22.2	156.0	37.2	15.4	2.8	21.3
	± 31.3	± 4.4	± 10.6	± 0.7	± 12.3	± 33.6	± 5.5	± 7.1	± 0.9	± 10.3
SSW-diet	207.4 ^{1,2}	46.5 ^{1,2,3}	18.8	2.8	13.1 ³	162.8 ¹	41.1 ^{1,2}	11.8	3.81 ²	23.1 ³
	± 11.6	± 9.1	± 4.8	± 0.7	± 4.8	± 14.3	± 7.7	± 3.0	± 0.3	± 8.5
SSW-diet + K-GM	144.3 ²	22.3 ²	14.9	1.8	38.9 ³	108.4 ¹	13.5 ¹	10.7	1.3 ¹	72.3 ³
	± 17.8	± 11.1	± 4.0	± 1.7	± 14.4	± 9.7	± 1.4	± 2.2	± 0.4	± 11.1
SSW-diet	199.0 ⁴	30.3 ^{5,3}	14.5	2.4	25.7	157.0	21.1 ²	10.7	2.2 ²	50.9
	± 16.4	± 6.4	± 4.7	± 1.1	± 9.5	± 15.4	± 3.7	± 3.3	± 0.8	± 20.2
SSW-diet + propionate	178.7 ⁴	23.4 ⁵	14.8	1.8	± 33.7	147.3	18.5	11.4	1.6	58.2
	± 15.4	± 5.5	± 5.1	± 0.8	± 17.2	± 16.1	± 5.4	± 3.6	± 0.3	± 34.6

1,2,3,4,5 Means in vertical columns with the same superscript differ significantly: Student-t-test, $P < 0.05$

The effects of propionate supplementation on plasma fibrinogen and fasting serum insulin concentration, as well as calculated insulin sensitivity index were quite similar to the effects of K-GM addition, but less marked and did not always reach statistical significance. In the group of rats which were fed the unsupplemented SSW-diet at the same time when propionate- supplemented diets were fed, serum insulin values were significantly lower and insulin sensitivity much higher than in the first group of rats fed the SSW-diet. In the fatty rats fed propionate, serum insulin was 29.5% ($p < 0.05$) lower, and insulin sensitivity 31.1% higher than in rats fed SSW-diet simultaneously. In lean rats fed propionate, serum insulin concentration was 14.1% lower and calculated insulin sensitivity 14.9% higher, compared to rats fed SSW-diet without propionate addition. Propionate reduced plasma fibrinogen levels by 10.8% in fatty, and 6.2% in lean rats. Table 6.6 indicates that in the fatty rats, plasma fibrinogen correlated positively with weight gain, liver weight (absolute and relative) as well as the lipid, cholesterol and glycogen content of the liver. A negative correlation between fibrinogen and insulin was observed in the lean rats.

DISCUSSION

Differences in means of the lean rats reflected those in the fatty rats, although the same level of significance was not always reached. The groups of lean rats included rats which were either homozygous or heterozygous. When serum triglycerides were used as an indicator of the presence of the fat gene, it was clear that differences in the heterozygous lean rats were mainly responsible for observed differences in means.

TABLE 6.6 SIGNIFICANT CORRELATIONS BETWEEN SOME VARIABLES OF THE ZUCKER RATS

Variables		Fatty rats		Lean rats	
		r	p	r	p
Serum cholesterol with:	weight	+0.4	0.004	NS ¹	
	triglycerides	+0.5	0.001	+0.4	0.01
Fibrinogen with:	weight	NS		+0.6	0.0001
	weight gain	+0.6	0.001	+0.7	0.0001
	liver weight	+0.5	0.01	+0.7	0.0001
	relative liver weight	+0.5	0.01	+0.4	0.03
	HDLC	NS		-0.4	0.02
	% HDLC	-0.4	0.02	-.06	0.003
	insulin	+0.3	0.09	+0.5	0.005
	liver lipids	+0.7	0.0001	NS	
	liver cholesterol	+0.6	0.0001	NS	
	liver glycogen	+0.7	0.0001	+0.4	0.4
	weight	NS		+0.4	0.04
Insulin with:	liver weight	+0.4	0.03	+0.5	0.007
	% HDLC	NS		-0.6	0.001
	triglycerides	NS		+0.5	0.002

¹NS Not significant

The absence of an effect of the dietary interventions on total serum cholesterol, and especially in the case of K-GM, a known hypocholesterolaemic gel fibre (Vorster *et al.*, 1985), is not surprising. The rat is known to be resistant to serum cholesterol changes if a bile acid is not included in the diet. The low-fibre, high-fat diet (SSW-diet) had a detrimental effect on percentage of HDLC and liver lipids, which was counteracted by the K-GM and propionate additions to some extent.

The increased plasma fibrinogen levels of these rats when a low-fibre, high-fat diet was fed, confirms the observations in baboons (chapter 5) and humans (Vorster *et al.*, 1988b). The significant lower values observed during K-GM feeding, suggests that gel fibres may prevent this effect. The fact that propionate feeding also resulted in lower plasma fibrinogen levels, further suggests that the fermentation of gel fibre may contribute to this effect.

The insulin resistant Zucker fatty rat is characterized by raised circulating insulin levels (Terrettaz & Jeanrenaud, 1983). The lower fasting insulin levels observed during K-GM and propionate feeding may indicate that these dietary treatments improved insulin sensitivity. This is supported by the lower mean insulin/glucose ratios (table 6.5) as well as the higher insulin sensitivity index observed in these rats. Although statistical significance was not

reached, propionate tended to increase the insulin sensitivity index. Fasting serum glucose concentrations did not differ in rats on different dietary treatments. However, marked differences were observed in liver glycogen content. In spite of large individual variations, reflected in the large standard deviations of the mean, liver glycogen was statistically significantly and markedly reduced in all rat-groups fed K-GM or propionate supplements, except in lean rats fed propionate. This fits in with the hypothesis stated in chapter 3, that propionate, derived from fermentation of DF, stimulates glycogenolysis and glucose utilisation, thereby postponing the mobilisation of FFA in the post-absorptive phase, without increasing serum insulin levels. Unfortunately, circulating FFA levels could not be determined in this study, because of the relatively large volume of serum needed for the method. The fact that significant lowering of plasma fibrinogen levels was accompanied in all instances by significant lower insulin levels, suggests that insulin may influence fibrinogen secretion on an inhibitory level, and supports the hypothesis that dietary factors which lower insulin resistance will also lower raised plasma fibrinogen levels.

The possibility that the K-GM effect was secondary to effects on weight gain should be considered. Plasma fibrinogen correlates with body weight index (Balleisen *et al.*, 1985) and obesity is associated with an insulin resistant state (Flier, 1983). It is therefore conceivable that weight loss or slower weight gain should lower both insulin resistance and plasma fibrinogen levels. The fact that the propionate supplemented rats also showed significant reductions in circulating insulin and fibrinogen levels, shows that the slower weight gain of the K-GM fed rats was not the only mechanism through which this gel fibre influenced plasma fibrinogen.

In conclusion, the soluble DF component, K-GM, improves the insulin resistant state and hyperfibrinogenaemia of the Zucker fatty rat, possibly partly through effects of propionate on metabolism. It remains to be established whether propionate improves insulin sensitivity and lower plasma fibrinogen levels in man. This possibility was investigated in the next study (chapter 7).

CHAPTER 7

COMPARISON OF THE EFFECTS OF PROPIONATE SUPPLEMENTATION AND KNOWN EFFECTS OF DIETARY FIBRE IN HEALTHY VOLUNTEERS

SUMMARY

Propionate produced in the colon from the fermentation of α -amylase resistant starch and non-starch polysaccharides, is cholesterol lowering and glucogenic in animal models. In man little is known about the effect of propionate on metabolism. In a double blind, placebo controlled study the diet of 10 healthy female volunteers, aged 20-22 yr was supplemented for a period of seven weeks with 7.5g sodium propionate daily in capsule form. The control group, paired according to percentage of high-density lipoprotein cholesterol (HDL), body mass index and area under baseline glucose tolerance curve, received dibasic calcium phosphate in identical capsules as placebo. Propionate supplementation increased HDL (9.5%) ($p < 0.05$) and decreased fasting serum glucose and maximum insulin increments during glucose tolerance tests ($p < 0.05$). The results suggest that the improvement in glucose tolerance and insulin sensitivity and the known beneficial effect of dietary fibre on HDL metabolism may in part be mediated through effects of propionate on hepatic carbohydrate metabolism.

INTRODUCTION

Dietary supplements of soluble fiber have been shown to improve glucose tolerance (Jenkins *et al.*, 1980; Vorster *et al.*, 1988b), lower serum cholesterol (Chen & Anderson, 1986; Venter *et al.*, 1987) and lower plasma fibrinogen (Koepp & Hegewisch, 1981) in man. A number of possible mechanisms have been proposed (Anderson & Chen, 1979; Vorster *et al.*, 1988a), one of which is through an effect of the short chain fatty acids (SCFA), acetic, propionic and butyric acid, which are derived from colonic fermentation of DF and RS (see section 2.2). Studies in man indicate that acetate is metabolised by muscle (Lindeneg *et al.*, 1964; Lindquist *et al.*, 1973; Skutches *et al.*, 1979) whilst butyrate is metabolised by colonic epithelium (Roediger, 1980). Propionate seems to be cleared by the liver (Cummings *et al.*, 1987) but very little is known about propionate metabolism in healthy man.

Propionate metabolism has been extensively studied in rats (Chen *et al.*, 1984; Illman *et al.*, 1988) and in pigs (Boila *et al.*, 1981; Thacker & Bowland, 1981). The results from the studies in baboons and Zucker rats, described in the previous two chapters, seem to indicate that dietary propionate may have an inhibitory effect on hepatic cholesterol synthesis and a regulatory effect on hepatic carbohydrate metabolism, resulting in improved insulin sensitivity and lower circulating FFA levels. The objective of the study described here was to determine the effect of dietary propionate on blood glucose and serum insulin response after glucose challenge, serum lipid profile, and plasma fibrinogen concentration and factor VII coagulant

activity in healthy volunteers on a Western diet. Circulating FFA levels were determined because of the reported association of FFA concentrations with insulin sensitivity (Martin, 1985) and plasma fibrinogen levels (Pilgeram & Pickart, 1968).

METHODS

Twenty healthy non-smoking female volunteers aged 20-22yrs, body mass index (BMI) 18.0 - 25.5 kg/m² participated in this double blind, placebo controlled study. Before commencement of the study the subjects were stabilised on a diet which supplied at least 150 g carbohydrate daily, to minimise variation in glucose tolerance tests. They were then paired according to BMI, area under baseline glucose tolerance test (GTT) and percentage high density lipoprotein cholesterol (HDLC) of total serum cholesterol (TC) ratio (see **Statistics**).

The study lasted 7 weeks during which the subjects consumed their usual diet. At the start of the experimental period a validated food frequency questionnaire was administered to determine the habitual background diet of the subjects. Information was coded and analysed by computer using local food tables (Gouws & Langenhoven, 1986). One subject of each pair was then randomly allocated to the experimental and the other to the control group. The experimental group took 7.5 g sodium propionate daily in 15 capsules with meals and the placebo group took dibasic calcium phosphate in identical capsules. Sodium propionate and dibasic calcium phosphate were purchased from SAARCHM (Pty) Ltd, Muldersdrift, RSA. At the start and end of the study period 2h glucose tolerance tests were performed under standardised conditions at 07h00 on subjects who had fasted for 12h. A solution of 1 g glucose in 5 mL water per kg body weight was taken orally. Venous blood was obtained at the start of the glucose tolerance tests and 30, 60 and 120 minutes thereafter for measurement of glucose, insulin, lipids, free fatty acids (FFA) and coagulation factors. The chemical methods are described in chapter 4. Insulin sensitivity was calculated according to the method described by Donahue *et al.* (1988). The incremental glucose area above the fasting or other lowest value observed during the GTT was calculated.

At the end of the study period subjects completed a questionnaire on the side effects of, and compliance with the treatments, as well as lifestyle changes. The study was approved by the Ethics Committee of the Potchefstroom University.

STATISTICS

The pairing of the subjects was done as follows: BMI, percentage HDLC and area under glucose tolerance curve were obtained for each subject. Each of these three sets of measurements were standardised by subtraction of their means and division by their standard deviations. For any proposed pairing an index of closeness can be obtained by summing over the three variables the sum over the pairs of squares of differences between the standardised measurements of individual pairs. The pairing which minimises this index was calculated

numerically. This pairing represents the best possible compromise between pairings that could be based on the individual variables. Baseline and end values of each group were compared with paired Student-t-tests. It was also tested whether the changes between baseline and end values in the experimental group differed from the changes between baseline and end in the control group. All analysis were done with PROC.GLM and PROC.TTEST of the SAS-system.

RESULTS

Diets

The mean habitual intake of macronutrients did not differ significantly ($p > 0.05$) between the two groups at the start of the study (table 7.1). However, some of the subjects reported increased food consumption during the Easter holidays, a week before the study ended. A significant increase ($p < 0.005$) in BMI was observed in both groups. The mean BMI changed from 21.9 to 22.6 in the control group and from 22.1 to 22.9 in the experimental group. An analysis of covariance with BMI as covariate was thus performed to compensate for increased BMI.

TABLE 7.1 MEAN HABITUAL INTAKE OF MACRONUTRIENTS

NUTRIENT	Experimental group		Control group	
	n = 9		n = 9	
	$\bar{x}$	SD	$\bar{x}$	SD
Total energy (kJ)	8083	1035	8325	1285
(kcal)	1929	246	1987	306
Total protein (g)	78.1	11.8	83.6	19.4
plant protein (g)	23.3	6.3	20.1	6.2
animal protein (g)	54.7	12.2	63.4	22.1
Total fat (g)	84.0	13.3	82.7	22.7
saturated fatty acids (g)	31.9	6.6	29.6	8.4
mono-unsaturated fatty acids (g)	28.2	5.7	29.1	8.5
polyunsaturated fatty acids (g)	17.4	3.9	18.5	6.4
Cholesterol (mg)	291.2	73.6	281.7	65.4
Total carbohydrate (g)	201.7	41.7	217.2	40.9
Dietary fibre (g)	21.6	6.5	19.6	4.8
Sucrose (g)	56.0	28.4	64.7	26.8

Compliance, side effects and lifestyle changes

A score for compliance was calculated (amount of capsules taken daily x frequency). The mean score for the control group was 69.7 and that of the experimental group 53.1 (table 7.2). Subjects from both groups reported nausea (experimental: 6; control: 3), constipation (2 in each group), flatulence (1 in each group), vomiting (4 in the experimental group) and increased appetite (2 in the control group). Nausea was eliminated by taking the capsules in the middle of meals, and was not of such an extent that it could prevent increase in BMI. One of the subjects in the propionate supplemented group did not complete the study because of nausea. The results of the corresponding subject in the control group could thus not be used. No change in alcohol intake nor in activity was reported, but 5 subjects in the control group and 2 subjects in the experimental group reported increased food consumption during the Easter holidays.

TABLE 7.2 SCORE* FOR COMPLIANCE TO TREATMENT IN EXPERIMENTAL AND CONTROL GROUPS

Experimental group		Control group	
n = 9		n = 9	
Mean	SD	Mean	SD
53.1	19.3	69.7	9.2

*Calculated as follows:

Number of capsules taken per day x 5 (every day)
(maximum 15) x 3 (every other day)
 x 1 (less than half the time)

Carbohydrate metabolism

Statistically significant decreases in the mean area under the glucose tolerance curve as well as the maximum glucose increment was observed in both groups (table 7.3, figures 7.1 and 7.2). Although not statistically significant the mean area under the insulin response curve decreased by 17% in the experimental group without changing in the control group (figures 7.3 and 7.4). The decrease in maximum insulin increment in the experimental group was significantly different from the change in the control group (as can be seen from table 7.3). Fasting serum glucose decreased by 9.5% in the experimental group (p < 0.02).

TABLE 7.3 MEAN ($\pm$ SEM) AREA UNDER GTT- AND IRI-CURVES, MAXIMUM GLUCOSE AND INSULIN INCREMENTS AND FASTING SERUM GLUCOSE AND INSULIN LEVELS

	Experimental group			Control group			Difference [*] in changes
	Baseline	End	Change	Baseline	End	Change	
Area under GTT-curve	162 ± 19	107 ± 15	$p < 0.02$	172 ± 24	116 ± 72	$p < 0.05$	NS
Area under IRI-curve	4938 ± 922	4092 ± 638	NS	4049 ± 563	4073 ± 685	NS	NS
Maximum glucose increment	2.5 ± 0.5	1.3 ± 0.3	$p < 0.02$	2.6 ± 0.3	1.5 ± 0.2	$p < 0.01$	NS
Maximum insulin increment	71.3 ± 11.8	59.6 ± 8.8	NS	60.9 ± 6.7	70.4 ± 8.8	NS	$p < 0.05$
Fasting glucose (mmol/L)	4.2 ± 0.1	3.8 ± 0.1	$p < 0.02$	4.0 ± 0.2	3.9 ± 0.1	NS	NS
Fasting insulin (μ U/mL)	22.1 ± 5.5	24.9 ± 7.9	NS	32.7 ± 10.6	37.2 ± 10.7	NS	NS

* ANOVA and paired t-tests

Lipid metabolism (table 7.4).

High-density lipoprotein cholesterol rose in the propionate supplemented group and this was significantly different from the change in the control group. Serum triglycerides also rose in the experimental group ($p < 0.02$) and free fatty acid levels fell, although not statistically significant. Serum obtained before and after the experiment from two of the subjects who received propionate supplements, was analysed for FFA concentration by gas-liquid chromatography at the Department of Biochemistry. The mean fasting concentration of circulating long-chain free fatty acids ($C_{16:0}$ and $C_{18:0-2}$) was $169.4 (\pm 12.7) \mu\text{mol/L}$ before propionate supplementation and $61.8 (\pm 14.4) \mu\text{mol/L}$ seven weeks later. Changes in FFA and TG levels were not observed in the control group. Analysis of covariance with BMI as covariate shows that if BMI remains constant, then LDLC will be significantly ($p < 0.01$) lower in the propionate supplemented group and percentage HDLC higher ($p = 0.002$) compared to baseline values.

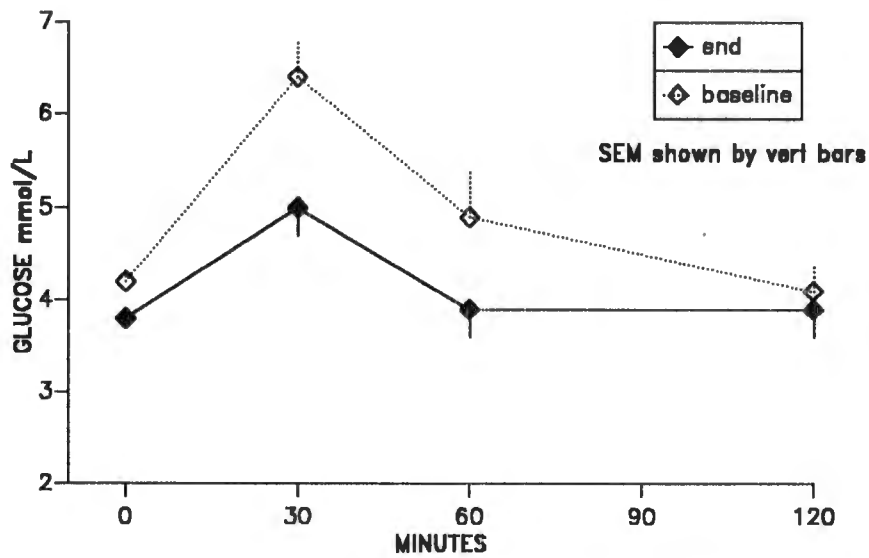


FIG 7.1 MEAN GTT-CURVE OF EXPERIMENTAL GROUP
Baseline differs significantly from end ($p < 0.02$)

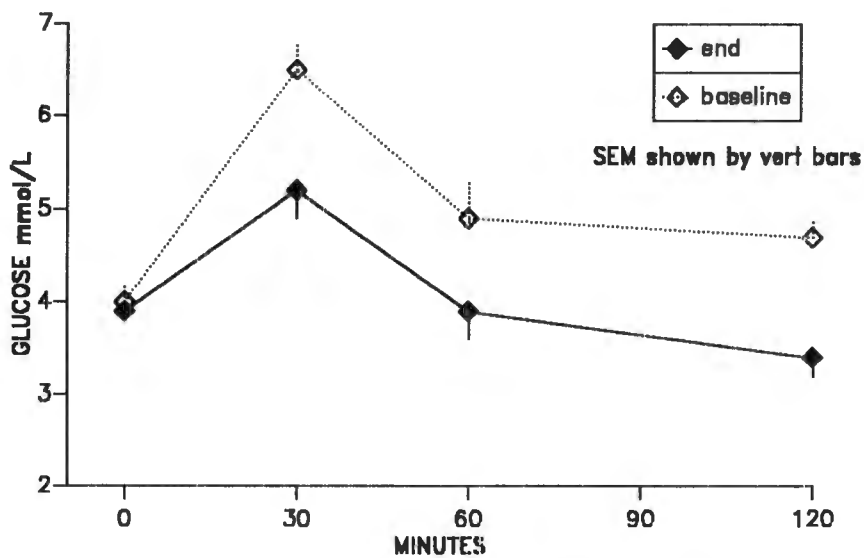


FIG 7.2 MEAN GTT-CURVE OF CONTROL GROUP
Baseline differs significantly from end ($p < 0.05$)

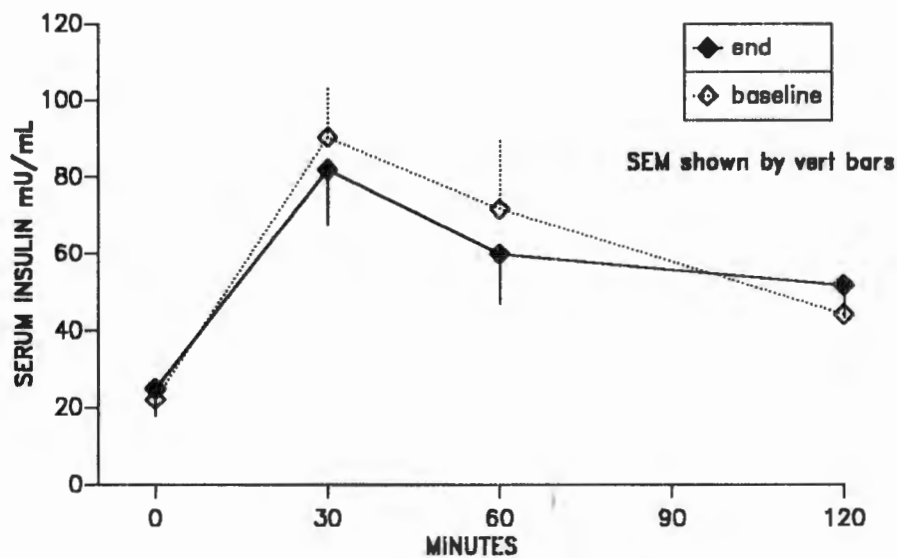


FIG 7.3 MEAN IRI-CURVE OF EXPERIMENTAL GROUP

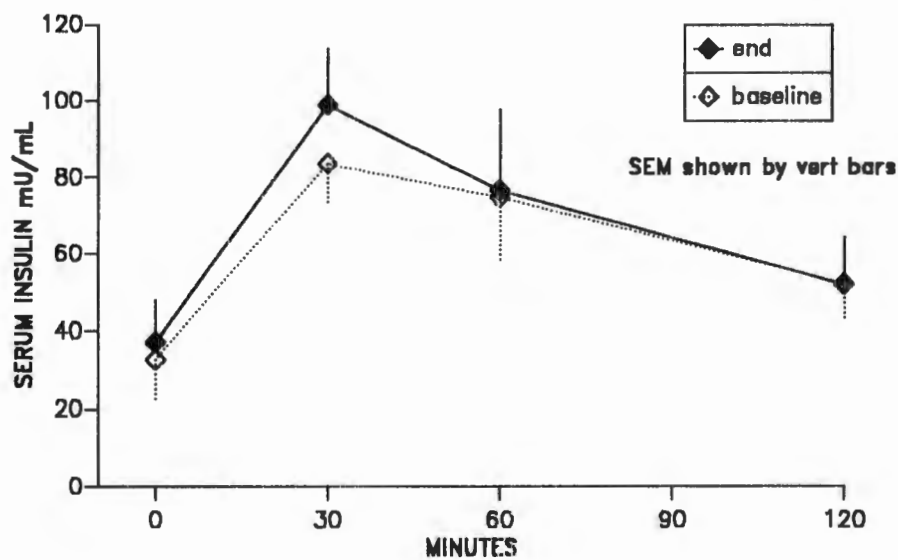


FIG 7.4 MEAN IRI-CURVE OF CONTROL GROUP

TABLE 7.4 MEAN (±SEM) SERUM LIPID AND LIPOPROTEIN LEVELS (mmol/L)

	Experimental group			Control group			Difference* in changes
	Baseline	End	Change	Baseline	End	Change	
Total cholesterol (TC)	4.55	4.81	NS	4.33	4.37	NS	NS
	±0.17	±0.21		±0.19	±0.18		
HDLC	1.36	1.51	NS	1.40	1.39	NS	p<0.05
	±0.06	±0.05		±0.06	±0.06		
% HDLC of TC	30.2	31.8	NS	3.29	32.5	NS	NS
	±1.33	±1.40		±2.21	±2.20		
LDLC	3.01	3.12	NS	2.78	2.87	NS	NS
	±0.16	±0.19		±0.20	±0.19		
Triglycerides	0.77	0.90	p<0.02	0.74	0.75	NS	NS
	±0.12	±0.13		±0.08	±0.07		
FFA (μmol/L)	561	471	NS	561	559	NS	NS
	±75.3	±39.8		±55.6	±40.5		

* ANOVA and paired t-tests

Haemostatic variables

Fibrinogen concentrations in both groups were in the normal range (table 7.5). Although not significant, the mean concentration in the control group increased during the study period. Factor V11 coagulant activity fell significantly in both groups.

TABLE 7.5 MEAN (±SEM) PLASMA FIBRINOGEN CONCENTRATION AND FACTOR VII ACTIVITY

	Experimental group			Control group			Difference* in changes
	Baseline	End	Change	Baseline	End	Change	
Fibrinogen (mg/dL)	238	240	NS	196	232	NS	NS
	±23	±8		±20	±12		
Factor VII (ratio)**	0.89	1.08	p<0.05	0.91	1.04	p<0.05	NS
	±0.07	±0.02		±0.07	±0.01		

* ANOVA and paired t-tests

** A ratio smaller than 1.0 indicates increased coagulant activity

DISCUSSION

Both the control and experimental group showed a fall in fasting blood glucose at the end of the experimental period. In the propionate supplemented group the fasting glucose levels were significantly lower at the end of the experiment and the mean increment in serum insulin during GTT significantly smaller compared to the control group, which may indicate improved insulin sensitivity. Lower FFA levels, which were observed in the experimental group, might have been responsible for the apparent increase in insulin sensitivity with propionate feeding. High circulating levels of FFA are known to be associated with insensitivity to insulin and inhibition of glucose uptake by muscle cells (Martin, 1985). However, the results of this study do not provide conclusive evidence that propionate improves insulin sensitivity in man. The improvement of glucose tolerance in the control group cannot be explained. The subjects were under free living conditions and although they were asked to keep their diet constant, changes could have occurred. Intra-individual variation in the oral glucose tolerance test, even carried out under controlled conditions, must also be considered. It has been shown in a group of males twenty to sixty years of age who were not known to have diabetes (McDonald *et al.*, 1965) that some individuals exhibited highly stable values for the same glucose tolerance test reading in six tests administered over a one-year period, whilst other individuals varied greatly on one or more of the readings. The mean coefficient of variation in the incremental area of four glucose tolerance tests in healthy female students aged 21.0 ± 1.8 yr (mean $\pm$ SD), with body mass index 20.0 ± 1.8 and who followed the same background diet as the subjects who participated in this study, is reported to be $10.7 \pm 4.9\%$ (Van Tonder, 1986). One of the subjects in the control group showed a reduction of 60% in area under the glucose tolerance test, which influenced the results to a great extent.

Dietary propionate is hypocholesterolaemic in animal models (Chen *et al.*, 1984; Illman *et al.*, 1988). As far as is known, propionate supplements have not been given to human volunteers before. In interpreting the results of this study, it should be kept in mind that the values of variables measured were in normal ranges and that it would probably be difficult to improve a normal parameter. Furthermore, the results were confounded by the significant increase in body weight in both groups. Although serum TC values did not decrease, analysis of covariance with BMI as covariate predicted a significant decrease in LDLC. The observed increase in HDLC after propionate supplementation in this study has not been reported for animals (Thacker & Bowland, 1981; Chen *et al.*, 1984). The close relationship between insulin and HDLC levels which has been reported (Stalder *et al.*, 1981; Laasko *et al.*, 1987) may explain the results. It has been suggested (Fossati & Romon-Rousseaux, 1987) that insulin acts directly at the hepatic level by favouring conversion of HDL_2 to HDL_3 , by its action on hepatic lipase. In the peripheral circulation, it induces HDL_2 formation by allowing TG-rich lipoprotein catabolism by lipoprotein lipase and free cholesterol esterification by lecithin : cholesterol acyltransferase (see chapter 3).

The increase in TG in the experimental group may be due to the increase in BMI. However, the control group also gained weight during the study without significant change in TG levels. Compared to the control group, the change in TG in the experimental group was not statistically significant. In pigs, dietary inclusion of propionic acid resulted in lower serum triglycerides ($p < 0.10$) (Thacker & Bowland, 1981). Propionate supplementation did not influence serum TG concentrations in rats and lowered liver TG content significantly (Chen *et al.*, 1984). Some of our subjects reported increased food but not alcohol consumption during the Easter holidays. The increased TG levels of the propionate supplemented group could not be explained by improper fasting, because their fasting blood glucose levels were significantly lower.

Little is known about the influence of diet on coagulation factors. A possible inhibitory effect of the short-chain fatty acids on blood coagulation was first mentioned by Malhotra (1968). In recent review articles possible mechanisms whereby dietary fibre, the main substrate for SCFA, may influence plasma coagulation factors have been proposed (Vorster *et al.*, 1988a; Venter & Vorster, 1989). The normal coagulation profiles of the subjects in the study reported here may explain the absence of an effect of propionate, or dietary fibre's effect on coagulation may be mediated through other mechanisms.

In summary, dietary propionate supplementation decreased the fasting blood glucose levels and maximum insulin increments, and increased HDLC in these subjects. The putative ability of propionate to improve insulin sensitivity remains to be firmly established in man. It should further be kept in mind that the metabolic effects of rapid intestinal absorption of dietary propionate and gradual colonic absorption of propionate derived from microbial fermentation of dietary fibre and alpha-amylase resistant starch, may not be the same (Illman *et al.*, 1988). Another approach may be to study the effects of starch known to be resistant to digestion in the small intestine and fermented in the colon on glucose tolerance. This approach was followed in the next study (chapter 8).

CHAPTER 8

COMPARISON OF THE EFFECTS OF MAIZE PORRIDGE WITH SUSPECTED VARYING AMOUNTS OF DIGESTIBLE STARCH ON BLOOD GLUCOSE, INSULIN AND ACETATE LEVELS IN HEALTHY VOLUNTEERS

SUMMARY

In the first part of the study, blood glucose and serum insulin responses after ingestion of 50 g carbohydrate as glucose, oats, sorghum, or maize porridge, were compared in 11 healthy volunteers on a Western diet. The glycaemic indices (GIs) of the porridges were comparable to published values. The lowest blood glucose value observed during glucose tolerance tests (GTTs) was used as baseline in calculating the incremental glucose area. This method incorporates hypoglycaemic incidents during GTTs and gives a better reflection of GTT curves and glucose increments than existing methods. Large individual variations were observed.

A homogenous group of 20 healthy volunteers participated in the second part of the study, in which blood glucose, serum insulin and acetate levels were compared after ingestion of hot, cooled and reheated maize porridge. Results showed that the GI of maize porridge could be lowered from 66.2 to 50.0 by eating it cold, probably because during cooling, resistant and partially resistant starch (PRS) are formed from the amylose and amylopectin molecules of maize starch respectively. Reheating probably improved the digestion of PRS and resulted in a GI of 55.7. This effect may have health benefits for the population at large and be of particular importance to patients with diabetes mellitus. It is further suggested that the method used for calculating the GI has advantages over existing methods.

INTRODUCTION

The incidence of degenerative Western type diseases appears to be exceptionally low in blacks in South Africa (Walker & Walker, 1985; Segal & Walker, 1986) compared to American blacks in whom the incidence of coronary heart disease (Curry *et al.*, 1984) and noninsulin-dependent diabetes mellitus (Harris *et al.*, 1987) is even higher than in Caucasians. Previously the low incidence in South African blacks was attributed to increased fibre in the diet (Trowell & Burkitt, 1981), but it was shown recently that the intake of DF of rural (Vorster *et al.*, 1987) as well urban (Segal & Walker, 1986) blacks in South Africa is surprisingly low (≈ 11 g/day). The consumption of starch, however, is reported to be very high, with carbohydrate supplying $\approx 70\%$ of the energy in the diet (Vorster *et al.*, 1987).

It is known that maize porridge is often eaten cooled by blacks in South Africa. Recent research has shown that moist-heated starchy foods are incompletely digested when cooled because of retrogradation of the starch dispersed during cooking (Englyst & Cummings, 1987a,

b) (see section 2.2). Maldigested starch, fermented in the large intestine to SCFAs, is expected to share certain properties of DF which is similarly fermented. Therefore, it can be reasoned that the consumption of large quantities of resistant and partially resistant starch in the form of cooled maize porridge may protect South African blacks against the development of Western diseases. It would then follow that the postprandial blood glucose and serum insulin responses after the consumption of cooled maize porridge will be smaller, compared to the response after hot porridge. This was tested experimentally by determining the blood glucose and serum insulin responses and acetate levels in healthy young Caucasians after the consumption of hot, reheated and cooled maize porridge, and calculating the glycaemic indices of the porridge. Glucose was used as carbohydrate reference because of the reported presence of RS in white bread (Englyst & Cummings, 1985).

METHODS

Study design

In a preliminary study the effect of a carbohydrate load of 50 g in the form of maize porridge on the glycaemic and insulinogaenic response of 11 healthy volunteers was compared with the effect of 50 g carbohydrate portions of glucose, sorghum, and oats porridge during 3 h glucose tolerance tests (GTTs).

In the main study a comparison was made of the effects of 50 g carbohydrate in the form of glucose, hot, reheated, and cooled maize porridge on blood glucose levels and insulin response of 20 healthy young volunteers during 4 h GTTs. The subjects were divided into 4 groups. Each group ($n = 5$) received each treatment in a Latin square design, at least one week apart.

Subjects and background diet

Eleven volunteers (4 men and 7 women), aged 20 to 46 years (mean 27.3, SD 8.9), with body mass indices (kg/m^2) ranging from 19.4 to 26.8 (mean 23.0; SD 2.5) participated in the preliminary study. The subjects in the main study (3 men, 17 women) were more homogenous with regard to age (mean 21.0 ± 1.0) and BMI (mean 22.2 ± 2.4). Two of the men were smokers (10 to 30 cigarettes per day). All the participants were in everyday good health, and gave informed consent. The studies were approved by the Ethics Committee of the Potchefstroom University.

Habitual food intake was determined before the inception of the main study with a validated frequency questionnaire and analysed with a computer program based on local food tables (Gouws & Langenhoven, 1986). Mean habitual nutrient intake (table 8.1) shows that the subjects consumed at least 150 g carbohydrate per day, which allowed optimal substrate-induced enzyme synthesis and thus minimum variation in glucose tolerance.

TABLE 8.1 MEAN HABITUAL NUTRIENT INTAKE OF SUBJECTS (n = 20) IN MAIN STUDY

Nutrient	Mean	SD
Total energy (MJ)	10.5	4.2
Total protein (g)	94.2	47.8
Plant protein (g)	26.7	10.8
Animal protein (g)	67.4	41.3
% Energy: protein	15.2	
Total fat (g)	108.8	49.6
Saturated fat (g)	34.6	18.5
Mono-unsaturated fat (g)	35.3	18.2
Poly-unsaturated fat (g)	30.2	11.4
P/S ratio	0.87	
% Energy: fat	39.3	
Cholesterol (mg)	401.1	318.6
Total carbohydrate (g)	274.7	107.4
% Energy: carbohydrate	44.3	
% Energy: sucrose	13.6	
Dietary fibre (g)	27.6	12.9

Carbohydrate loads

In the preliminary study a solution of 50 g glucose in 400 mL water was administered for the control (reference) GTT. The porridge was prepared from 79.0 g unsifted maize meal, 75.4 g unsifted sorghum meal (Mabella) or 68.7 g oat meal, cooked in individual portions in the conventional way in 400 mL water with 2 mL salt added. Each portion supplied 50 g carbohydrate, according to local food tables (Gouws & Langenhoven, 1986). The porridge was cooked 2 h before the GTT and consumed without milk or sugar.

In the main study the control GTT was performed with 50 g glucose dissolved in 250 mL H_2O . Individual portions of refined maize meal (62.7 g) was soaked in 150 mL lukewarm water with 2 mL table salt added. The porridge was then cooked in a microwave oven at maximum power for three periods of 2 min each, and stirred for 2 min in between. Hot porridge was consumed immediately afterwards. Porridge intended to be consumed cold or reheated was prepared 36 h beforehand, kept at 4 degree C and served as such or heated for 2 min in the microwave oven. The porridge was consumed with 150 mL skimmed milk at the same temperature. The carbohydrate load was 50 g in all instances.

Glucose tolerance tests (GTTs)

GTTs were performed under standardised conditions, as described in chapter 4. Venous as well as capillary blood samples were obtained at the start of the GTTs. Capillary blood samples were obtained after 15, 30, 45, 60, 90, 120, 180 and 240 min for the determination of glucose, and venous blood samples after 30, 60 and 240 min for the determination of serum immunoreactive insulin and acetate. In the preliminary study final samples were obtained after 360 min.

Glycaemic index (GI)

The GI of the different porridges was calculated according to the method of Jenkins *et al.* (1981a) by dividing the incremental area under GTT curve with 50 g carbohydrate as porridge by the incremental area under GTT curve with 50 g glucose and multiplying the ratio by 100.

The incremental glucose area above the fasting or other lowest value observed during the GTT was calculated, thus avoiding negative areas. The inclusion of negative areas results in smaller total incremental areas. Because it disguises hypoglycaemic incidents it does not reflect the GTT-curve accurately.

Chemical methods

Blood glucose and serum insulin concentrations were determined as described in chapter 4. The serum concentration of acetate, the only SCFA that reaches the peripheral circulation in measurable quantities after absorption from the large intestine (Pomare *et al.*, 1985), is expected to rise during fermentation. Venous blood samples, obtained at the start of the GTT (t_0) and 4 h later (t_{240}) were used to determine acetate concentrations in duplicate with the method described by Pomare *et al.* (1985), as mentioned in chapter 4.

Statistics

Significant differences were calculated with paired t-tests, using the SAS package (SAS Users' Guide, 1985. SAS Institute Inc. Cary, North Carolina).

RESULTS

Preliminary study

The mean glucose tolerance curves after the ingestion of 50 g glucose and the same amount of carbohydrate as oats, sorghum and maize porridge are illustrated in figure 8.1. Glucose resulted in the highest curve, as expected, and oats porridge in the flattest curve, probably due to the soluble fibre content of oats. A smaller curve was observed with maize than with sorghum porridge. The same pattern emerges in the comparison of the maximum glucose and

insulin increments (table 8.2), except for a lower mean insulin response with glucose compared to sorghum and maize. It is known that some amino acids stimulate insulin secretion (Martin, 1985). The protein content of these two types of porridge might have contributed to this effect. Insulin secretion was possibly inhibited by the gelforming fibre in oats (Vorster *et al.* 1987b).

TABLE 8.2 MEAN GLUCOSE AND INSULIN INCREMENTS AFTER INGESTION OF GLUCOSE, AND PORRIDGES MADE OF OATS, SORGHUM AND MAIZE MEAL (n = 11) (±SD)

	Control	Oats	Sorghum	Maize
Glucose increment (mmol/L)	5.5±1.8	1.9±0.8	3.7±0.9	3.1±1.3
Insulin increment (μU/mL)	52.2±22.9	19.6±18.8	60.9±26.7	52.0±20.07

The mean GI of the three porridges, calculated with the incremental areas of $t^0 - t^{90}$, $t^0 - t^{120}$ and $t^0 - t^{180}$, is given in table 8.3. The GI for 90 and 120 min corresponds well with the data in table 8.2, with oats having the lowest and sorghum the highest GI. Little difference was observed between oats and maize after 180 min.

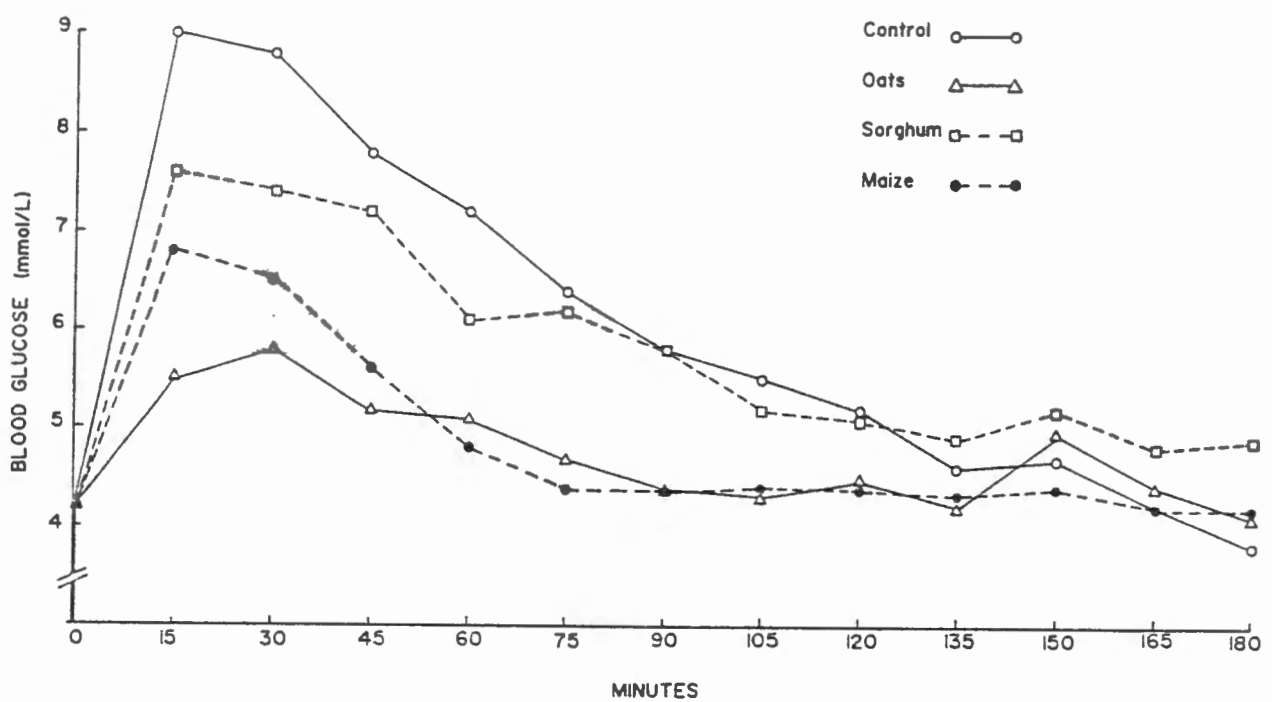


FIGURE 8.1 Mean glucose tolerance curves after ingestion of 50g carbohydrate in the form of glucose (○—○), oats porridge (Δ—Δ), sorghum porridge (□—□), and maize porridge (●—●), preliminary study (n=11)

TABLE 8.3 GLYCAEMIC INDEX OF OATS, SORGHUM AND MAIZE PORRIDGE

Porridge		$t_0 - t_{90}$	$t_0 - t_{120}$	$t_0 - t_{180}$
Oats	$\bar{x}$	50.8	49.5	54.3
	SD	22.5	20.1	23.5
Sorghum	$\bar{x}$	62.1	60.7	61.5
	SD	16.3	14.0	19.0
Maize	$\bar{x}$	57.4	56.3	55.8
	SD	14.5	15.1	15.9

Main study

As shown in table 8.1, the energy distribution of the background diet of the subjects was typical Western, with fat supplying nearly 40% of the total fat intake. Cooled maize porridge clearly resulted in significantly ($p < 0.05$) lower mean blood glucose response than hot or reheated porridge (figures 8.2 and 8.3), and lower mean GI values (table 8.4). The insulin incremental areas after the different carbohydrate loads are shown in figure 8.4.

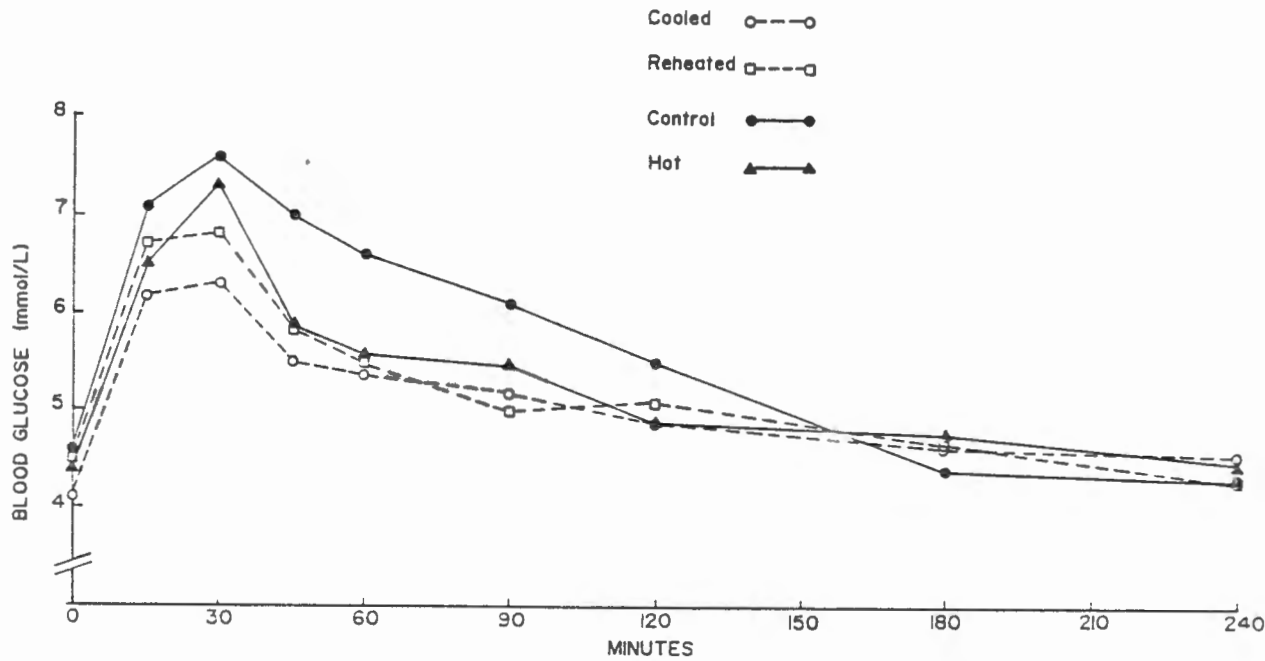
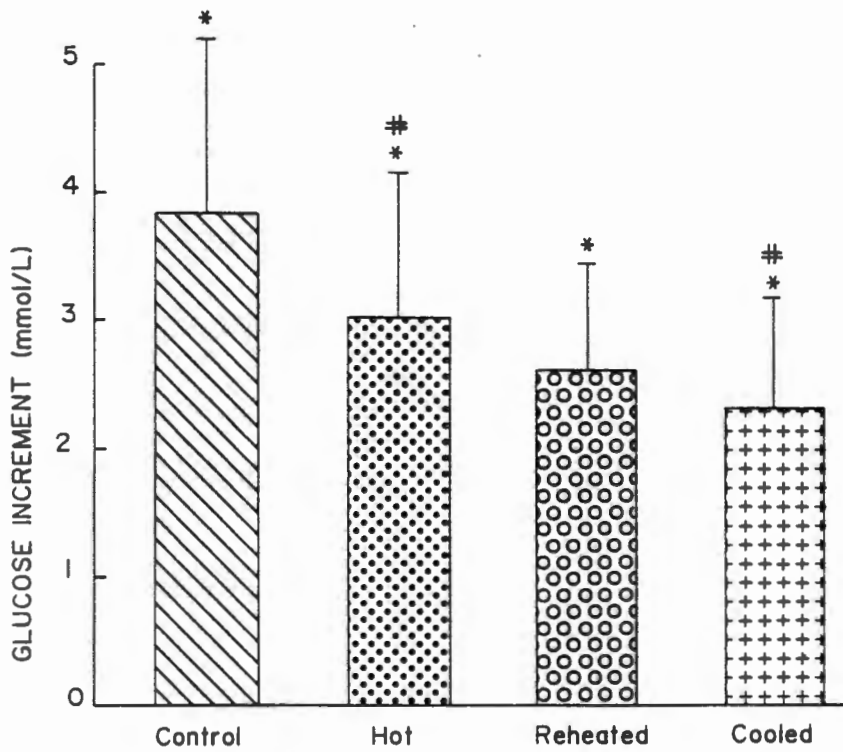


FIGURE 8.2 Mean glucose tolerance curves after ingestion of 50g carbohydrate in the form of glucose (○---○) and maize porridge: hot (▲---▲), reheated (□---□) and cooled (●---●) (n=20)



* and #: Significantly different ($P < 0.05$)
 Figure 8.3 Mean ($\pm$ SD) glucose increment after ingestion of 50g carbohydrate in the form of glucose (control), hot, reheated and cooled maize porridge (n=20).

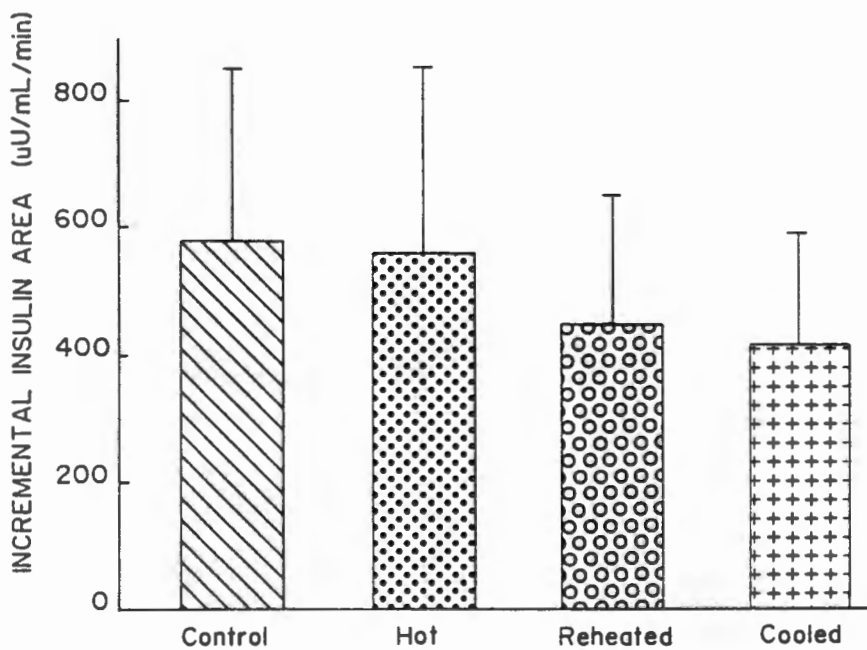


Figure 8.4 Area under IRI-response curve after ingestion of glucose (control), hot, reheated and cooled maize porridge (n=20)

TABLE 8.4 MEAN GLYCAEMIC INDEX (0 - 120 min) OF HOT, REHEATED AND COOLED MAIZE PORRIDGE (n = 11)

Food	Glycaemic index	
	Mean	SD
Hot maize porridge	66.2	26.1
Reheated maize porridge	55.7	20.5
Cooled maize porridge	50.0	14.5

Table 8.5 shows the mean change in serum acetate levels 4 h after glucose or maize porridge with different amounts of digestible starch was administered. Acetate levels decreased the least after cooled porridge was consumed, but statistical significance was not reached, possibly as a result of the large individual variation.

TABLE 8.5 MEAN SERUM ACETATE CONCENTRATION BEFORE AND 240 MIN AFTER INGESTION OF GLUCOSE, HOT, REHEATED AND COOLED MAIZE PORRIDGE (n = 10)

Carbohydrate		Acetate ($\mu\text{mol/L}$)		
		t_0	t_{240}	Change
Glucose	$\bar{x}$	43.6	32.6	-11.0
	SD	16.8	4.7	
Hot porridge	$\bar{x}$	43.7	31.9	-11.8
	SD	8.8	6.9	
Reheated porridge	$\bar{x}$	38.3	31.0	-7.3
	SD	7.1	5.3	
Cooled porridge	$\bar{x}$	38.3	31.6	-6.7
	SD	13.3	4.9	

DISCUSSION

The most important observation in the preliminary study was the low GI of the three South African types of porridge. The results indicate clearly that the consumption of complex carbohydrate as porridge results in a blunted blood glucose response compared to a glucose solution containing the same amount of carbohydrate. The calculation of incremental glucose area with the lowest blood glucose value, used as baseline, resulted in GI values which reflected the pattern of the curves (figure 8.1) as well as the maximum glucose increments (table 8.2). Walker & Walker (1984) determined the GI of maize porridge in black schoolchildren and reported mean values and standard deviations of 74 (± 7) for refined maize porridge and 71 (± 6) for porridge made with unrefined maize meal. The GI of oats

porridge in patients with diabetes mellitus is reported to be $98 (\pm 9)$ (Jenkins *et al.*, 1983). These authors calculated the incremental glucose area above the fasting concentration, as most investigators do. Using the same method, the mean GI of the oats, sorghum and maize porridges in this study were $75 (\pm 86)$, $109 (\pm 63)$ and $73 (\pm 86)$ respectively. The mean values in this study were thus very similar to those reported in the literature. However, the standard deviation was much larger than reported values. This could be the result of large inherent individual variation in glycaemic response or due to the heterogeneity of the experimental group. The first explanation is possibly more correct, since the same individual variation with a very homogenous group in the main study was observed.

The results of the main study clearly indicated that the consumption of cold maize porridge results in smaller blood glucose and insulin responses than hot porridge. This corresponds with the observations of Englyst & Cummings (1987a) with cooled potato in ileostomy patients. A considerable amount (12%) of starch was recovered in the effluent after consumption of cooled potato, whilst only 3% of starch from freshly cooked potatoes was recovered, reflecting the extent to which starch resisted hydrolysis after cooling. When cooled potato was reheated and consumed, the starch components recovered in the effluent were reduced to 7% of the starch fed. The resistance of 12% of cooled potato starch to amylase was ascribed to the presence of retrograded amylopectin (i.e. PRS 3, see table 2.1) as well as retrograded amylose (RS) (Englyst & Cummings, 1987a). Reheating of the amylopectin fraction partly reverses retrogradation (Schoch, 1965) and increases digestibility (Englyst & Cummings, 1987a, b). The proportion of amylopectin and amylose varies in different starches. Potato starch contains 20-23% amylose (Wurzburg & Szymanski, 1970), whereas the amylose content of maize starch varies from 25% in common varieties to 70% in varieties genetically manipulated to increase the amylose content, so-called "high-amylose corn" (Whistler, 1984). The maize starch used in the preparation of the porridge in this study is reported to contain 26% amylose and 74% amylopectin (Cronje, 1985). It seems reasonable to conclude that the differences in blood glucose and insulin responses observed with cooled and reheated maize porridge may be ascribed to the presence of PRS 3 and RS. It is reported that the amylose fraction of maize starch has particular gel-forming ability with cooling. These gels cannot be reliquified with reheating (Sweetman & MacKellar, 1959).

Starch escaping digestion in the small intestine is readily fermented by bacteria in the colon to SCFAs, which are rapidly absorbed (McNeil *et al.*, 1978) (see section 2.2). Butyrate is used by colonic epithelial cells, propionate is largely cleared by the liver, and acetate passes to the peripheral circulation (Cummings, 1983). Instead of an increase in peripheral acetate concentration, which was expected after consumption of cooled porridge, a decrease was observed from fasting value to concentration 4 h after the carbohydrate loads were administered. The decrease with cooled maize porridge was smaller. Pomare *et al.* (1985) reported peak blood acetate levels 2-4 h after the administration of lactulose. With pectin administration, however, acetate levels started to rise only after 6 h. An increase in acetate

levels as result of fermentation of maldigested maize starch may therefore possibly occur later than 4 h after consumption.

In conclusion, the results show that the GI of maize porridge is low, and can further be lowered by cooling the porridge. This observation may be ascribed to the composition of the starch of maize and may be of importance in the diets of patients with diabetes mellitus and/or obese subjects. The method for the calculation of incremental glucose areas used incorporates the functional hypoglycaemia during GTTs in healthy subjects and may give a better indication of mean glucose tolerance curves and blood glucose increments. This method is recommended for calculation of incremental glucose areas in the future. It is further recommended that the GI of cooled and reheated porridge made with high-amylose maize meal should be investigated. Reheating of starch does not improve the digestibility of the retrograded amylose fraction in the small intestine, and was reported to be more acceptable than cold porridge starch by the subjects who participated in this study.

CHAPTER 9

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

9.1 DISCUSSION

Introduction

In this chapter, the results of the reported studies (chapters 5-7) will be used to compare the biochemical effects of konjac-glucomannan (K-GM) and propionate in an attempt to quantitate the contribution of propionic acid to the biochemical effects of K-GM and to delineate the mechanisms through which these effects are mediated.

The effects of the long-term feeding of K-GM and propionate against the background of a Western diet are summarised in table 9.1. The calculated changes in the total amount of liver and serum cholesterol of the two animal models are shown in tables 9.2 and 9.3.

In interpreting the results, the following should be kept in mind:

- The baboons and rats were fed the same diets for the same experimental period. Therefore, it seems reasonable to compare the results obtained with these two animal models qualitatively. Although such comparisons may not be valid quantitatively because of interspecies differences, the percentage changes in parameters after dietary propionate may provide useful information in an attempt to quantify the contribution of propionate to the effects of DF.
- Further, the metabolic effects of rapid intestinal absorption of dietary propionate and gradual colonic absorption of propionate derived from fermentation of K-GM may not be the same. As far as is known, the time-course of gut fermentation of K-GM has not been determined. On account of the similarity in physical and physiological properties of K-GM and pectin (another soluble DF component), fermentation of K-GM may be as slow as that of pectin. According to Pomare *et al.* (1985), blood acetate levels start to rise 6 h after 20 g of pectin has been administered, and remain elevated at about twice the fasting levels for the subsequent 18 h. The baboons were fed the same amount of K-GM daily for 9 weeks, resulting in a slow, continuous formation and absorption of propionate, extending well into the fasting state.
- Metabolic events, especially in the liver, differ markedly during the fed and fasting state, and are controlled by two different sets of hormones. From the literature it is clear that the effect of propionate on hepatic metabolism differs widely in fed and fasted animals. Therefore, difference in fasting values between control and K-GM or

propionate treated animals should be compared separately from differences observed during glucose tolerance tests (fed state).

- It should be emphasised that the acute effect of the two supplements (fed with a meal) was not investigated in this study.
- Further, the supplements were added to a Westernised background diet with the objective to establish environmental conditions comparable to man. It may therefore not be reasonable to compare the results of the dietary propionate studies in animals with those of others such as Boila *et al.* (1981) and Thacker & Bowland (1981) with pigs, and Chen *et al.* (1984) and Illman *et al.* (1988) with rats, since the experimental diets differ markedly. The addition of 10% tallow to the diet fed to pigs (Thacker *et al.*, 1981), however, bears some resemblance to the SSW-diets fed to baboons and Zucker rats, and allows comparisons to some extent. No comparable data are available for effects of dietary propionate in man.
- Finally, the observation of Demigné *et al.* (1986), namely that rats adapted to a high fibre diet had a more efficient hepatic uptake of acetate than rats fed a basal fibre free diet, may be of importance in the comparison and interpretation of reported effects of SCFAs, especially those found in isolated hepatocytes. Thus, results obtained with acute SCFA administration may be influenced by the pre-experimental background diet of the experimental animals.

Effects in the fasting state

Carbohydrate metabolism

Carbohydrate metabolism during the fasting state is characterised by hepatic glucose production, a homeostatic mechanism to ensure adequate blood glucose levels for the function of the nervous system. During this state, glucagon, catecholamines, growth hormone and the corticosteroids stimulate glycogenolysis (breakdown of glycogen to glucose), as well as gluconeogenesis, the conversion of lactate, pyruvic acid, glucogenic amino acids, and glycerol to glucose (West, 1985).

From table 9.1 it can be seen that in comparison with control diets, both K-GM and propionate supplemented diets lowered liver glycogen stores. K-GM also lowered fasting blood glucose levels, but propionate tended to increase these levels, although not significantly. In the human study, propionate significantly decreased fasting blood glucose. An increase in blood glucose levels after administration of propionate to rats has also been reported by Williams *et al.* (1971). The effect of K-GM and propionate on fasting serum insulin in the animal models were in the same direction, although K-GM had a greater

lowering effect than propionate. In the human study, propionate tended to increase fasting insulin values, which may explain the lower glucose levels observed in these subjects.

From these results it seems reasonable to conclude that, at least in the animal models, both K-GM and propionate stimulated glycogenolysis (lower glycogen stores) and that propionate additionally stimulated gluconeogenesis (increased blood glucose levels), either by acting as a substrate or by enhancing enzyme activities in the various metabolic pathways.

TABLE 9.1 PERCENTAGE CHANGES IN VARIABLES WITH PROPIONATE AND K-GM DIETS

Variable	Baboons ²		Fatty rats ²		Lean rats ²		Humans ³
	K-GM	Propionate	K-GM	Propionate	K-GM	Propionate	Propionate
FFA(μ mol/L)	↓9.3 ¹	↓7.0	-	-	-	-	↓16.0
TC (mmol/L)	↓17.2 ¹	↓4.4	↓11.6	9.8	↑5.3	↓10.0	↓7.3 ⁴
% HDL of TC	↑10.1 ¹	↑4.2	↑12.8	↑10.2	↑19.5 ¹	↑10.1	↑9.0 ^{1,4}
TG (mmol/L)	↓2.8	↑5.6 ¹	↓12.6,	↓16.1	↓46.2	↓20.0	↑16.9 ¹
Total liver lipids (mg/g)	↓1.3	↓11.3	↓51.2 ¹	↓33.0	↓22.5	↓11.3	-
Liver cholesterol (mg/g)	↓31.3 ¹	↓34.4 ¹	↓39.5	↓38.5	↓27.8	↑6.9	-
Liver glycogen (mg/g)	↓41.9	↓47.1	↓52.9 ¹	↓31.3 ¹	↓94.1 ¹	↓56.4	-
Fasting glucose (mmol/L)	↓9.4	↑3.0	↓19.9	↑2.1	↓7.8	↑6.5	↓9.5 ¹
Fasting insulin (μ U/mL)	↓25.4	↓2.9	↓51.0 ¹	↓29.5 ¹	↓67.2 ¹	↓14.1	↑12.7
Maximum glucose increment (mmol/L)	↓31.6	↓17.0	-	-	-	-	↓48.0 ¹
Maximum insulin increment (μ U/mL)	↓2.8	↑9.7	-	-	-	-	↓16.4
Insulin sensitivity index	↑18.1	↓35.6	↑196.9 ¹	↑31.1	↑67.2 ¹	↑14.9	↑1.6
Insulin/glucose	↓7.9	↑8.9	↓30.8	↓25.0	↓63.9	↓27.3	↑19.4
Fibrinogen (mg/dL)	↓15.9 ¹	↓12.5	↓30.4 ¹	↓10.8 ¹	↓33.4 ¹	↓6.2	↑0.8

¹ Change statistically significant ($p < 0.05$)
² Compared to SSW-diet after 9 weeks.
³ Compared to baseline values
⁴ Corrected values of analysis of covariance with BMI as covariate
 ↑ increase , ↓ decrease

**TABLE 9.2 MEAN TOTAL LIVER AND SERUM CHOLESTEROL IN BABOONS FED
PROPIONATE AND K-GM SUPPLEMENTED WESTERN DIETS (n = 4)**

Variable	SSW-diet	SSW-diet + K-GM	SSW-diet + propionate
Mean serum TC (mg/dL)	114.6	94.8	109.5
Mean body weight (kg)	27.6	26.8	25.8
Calculated blood volume (dL) ¹	16.6	16.1	15.5
Calculated serum volume (dL) ²	9.9	9.7	9.3
Calculated total serum cholesterol (mg)	1134.5	919.6	1018.4
Calculated total serum cholesterol (mg/kg)	41.1	34.3	39.5
Liver cholesterol concentration (mg/g)	3.2	2.2	2.1
Mean liver weight (g)	427	418	442
Calculated total liver cholesterol (mg)	1362	914	923
Calculated liver cholesterol (mg/kg)	49.3	34.1	35.8
Serum + liver cholesterol (mg)	2496.5	1833.6	1941.4

¹ 50 – 70 mL/kg (Canadian Council on Animal Care, 1980)

² Packed cell volume: 30-43%

**TABLE 9.3 MEAN TOTAL LIVER AND SERUM CHOLESTEROL IN ZUCKER FATTY RATS
FED PROPIONATE AND K-GM SUPPLEMENTED WESTERN DIETS (n = 7-9)**

Variable	SSW-diet	SSW-diet + K-GM	SSW-diet	SSW-diet + propionate
Mean serum TC (mg/dL)	166.0	146.7	235.5	212.4
Mean body weight (g)	619.3	553.5	597.2	540.1
Calculated blood volume(dL) ¹	0.356	0.318	0.343	0.311
Calculated serum volume(dL) ²	0.214	0.191	0.206	0.187
Calculated total serum cholesterol (mg)	35.52	28.02	48.51	39.72
Calculated total serum cholesterol (mg/kg)	57.4	50.6	81.3	73.6
Liver cholesterol concentration (mg/g)	3.8	2.3	3.9	2.4
Mean liver weight (g)	22.5	15.2	19.3	16.1
Calculated total liver cholesterol (mg)	85.5	35.0	75.3	38.6
Calculated liver cholesterol (mg/kg)	138.1	63.2	126.1	71.5
Serum + liver cholesterol (mg)	121.0	63.0	123.8	78.3

¹ 50 – 65 mL/kg (Canadian Council on Animal Care, 1980)

² Packed cell volume: 40-50%

Evidence is presented in the literature that although propionate inhibits glucose production from lactate (Anderson & Bridges, 1984), in rats adapted to a high fibre diet, propionate is the main glucogenic substrate (Demigné *et al.*, 1986). Veech *et al.* (1986) also reported that the intra-peritoneal administration of either acetate or propionate to 48 hour starved rats, elevated the portion of the gluconeogenic pathway from fructose 1,6-diphosphate (FDP) to glucose 1.5 to 3.5 fold in livers freeze-clamped one half hour later. Veech *et al.* (1986) mention that ... "malate, citrate, and glucose are elevated only in propionate and acetate treated groups where presumably inorganic pyrophosphate (PPI) is being generated in both cytoplasm and the mitochondria". However, they demonstrated that in the initial portion of the gluconeogenesis pathway, the metabolites from phosphoenolpyruvate (PEP) to FDP were elevated by acetate, propionate and butyrate. They further mention that the pattern of elevation of this portion of the pathway is the classic pattern seen with increased cAMP formation as a result of hormonal (e.g. glucagon) stimulation. Butyrate therefore differs from acetate, propionate or glucagon by not elevating glucose production in the starved animal. It is possible that the different effects of propionate and butyrate on glucose production may explain the different effects of K-GM (which supply all SCFAs) and propionate on blood glucose levels observed in the present study.

A possible mechanism of the proposed effect of K-GM and propionate on glycogenolysis (which will explain the observed lower glycogen levels in the present study) is less clear. It could be a secondary effect, resulting from the lower circulating insulin levels with K-GM and propionate treatment and a resultant higher glucagon : insulin ratio. However, a direct or primary effect of propionate on liver metabolism in this regard cannot be excluded. The study of Veech *et al.*, (1986) demonstrated large increases in the hepatic content of PPI and calcium with SCFA administration to fed or fasted rats. Increases were much higher in the fasted rats (PPI 100 fold and calcium 2 to 3 fold, compared to PPI 10 fold and calcium 1.2 to 2 fold in fed rats). Their study indicates that PPI is linked to the reduction of the hepatic free cytoplasmic $NADP^+ : NADPH^+$ ratio after the administration of SCFAs. This effect is similar to that seen after the administration of glucagon, previously reported by these authors (Veech *et al.*, 1975), a treatment known to cause an increase in hepatic cAMP. Clearly, the effect of propionate on hepatic cAMP levels should be examined, since glucagon stimulation of glycogenolysis is mediated through the production of cAMP (West, 1985).

The ability of K-GM (and other dietary fibre components) to lower fasting (and non-fasting) serum insulin levels, is usually ascribed to the effect of fibre on glucose absorption, the resultant lower insulin secretion, up-regulation of insulin receptors and increase in insulin sensitivity (Vorster, 1987). The observation in the present study that propionate also lowered fasting serum insulin levels in the animal models studied, suggests an additional mechanism, independent of the physical effects of dietary fibre on glucose absorption in the small intestine (see section 2.2). The effect of the SCFAs on the pancreatic output of

insulin and glucagon, and thus on the insulin : glucagon ratio in both the fasted and fed state, should be examined in more detail in monogastric animals before possible mechanisms in this regard could be suggested.

Lipid metabolism

During the fasting state, the decrease in circulating insulin levels and the increase in the lipolytic hormones (glucagon, growth hormone, catecholamines and corticosteroids) result in an increased lipolysis, raised circulating FFA levels and FFA oxidation (West, 1985).

In addition to the beneficial effects on total serum cholesterol and percentage of HDL cholesterol of K-GM and propionate (table 9.1), the present study clearly demonstrates a lowering of circulating FFA levels and of liver total lipids and liver cholesterol in the animal models by both supplements. Interestingly, propionate and K-GM had opposite effects on serum triglyceride (TG) concentrations. K-GM tended to decrease fasting levels, while propionate significantly increased TG levels in the baboon model as well as in the humans.

The SSW-diet fed to the baboons and rats in the studies reported here contained 13.5% beef tallow. The effect of the diet on serum cholesterol may therefore be compared with the observations of Thacker *et al.* (1981) in pigs which were fed diets containing 10% tallow. Total serum cholesterol increased by 16.5% in pigs fed the tallow diet. Addition of 5% propionic acid to the diet prevented this increase. Addition of 2% sodium propionate (1.5% propionic acid) to the Western diet fed to baboons and Zucker rats in the present study resulted in smaller increments in total serum cholesterol levels than the control diet.

The calculated total serum and liver cholesterol (mg/kg body weight) of the baboons and fatty rats are shown in tables 9.2 and 9.3. Changes with K-GM and propionate treatment in the ratios of the serum : liver cholesterol pools are summarised in table 9.4. K-GM and propionate, while decreasing both the serum and liver pools in the baboon and rat, increased the ratio of the serum : liver pool. Therefore, the lowering of total serum cholesterol by K-GM and propionate cannot be ascribed to a shift from the serum to the liver pool. The data fit a model in which decreased hepatic synthesis and secretion of cholesterol may (or may not) be accompanied by an increased excretion of cholesterol. It is suggested here that the greater effect of K-GM on serum cholesterol levels (table 9.1) is a result of two mechanisms: firstly, an increased cholesterol excretion because of the gastro-intestinal binding of bile acids (salts) by the gel fibre, and the inhibition of the entero-hepatic circulation of bile acids, as suggested by Story & Kritchevsky (1976, see section 2.2.3). Secondly, by an additional inhibition of hepatic cholesterol synthesis by propionate, derived from the colonic fermentation of the gel fibre. There is substantial evidence in the literature that propionic acid inhibits liver cholesterol synthesis (see section 2.4). It seems as if this effect of propionate is mediated through inhibition of HMG-CoA reductase. The results of the animal studies clearly indicate that the

hypocholesterolaemic effect of K-GM can only partly be ascribed to its fermentation product, propionic acid.

TABLE 9.4 COMPARISON OF CHANGES IN THE RATIO OF SERUM : LIVER CHOLESTEROL POOLS WITH K-GM AND PROPIONATE TREATMENT

Baboons	SSW-diet	SSW-diet + K-GM	SSW-diet + propionate	
Serum TC (mg/kg)	41.1	34.4	39.5	
Liver (mg/kg)	49.3	34.1	35.8	
Ratio (serum/liver)	0.83	1.00	1.10	

Rats	SSW-diet	SSW-diet + K-GM	SSW-diet	SSW-diet + propionate
Serum TC (mg/kg)	57.4	50.6	81.3	73.6
Liver (mg/kg)	138.1	63.2	126.1	71.5
Ratio (serum/liver)	0.42	0.80	0.64	1.03

The lowering of the fasting circulating FFA levels by K-GM and propionate (table 9.1) is intriguing. The mechanism is not clear, but it could be associated with the glucogenic effect of propionate. In the fasting state, glucose is used as energy substrate mainly by insulin independent tissues, such as the nervous system, because of the lower insulin levels and the further inhibition of insulin function and glucose uptake by FFA in insulin sensitive tissues (Randle effect). It is conceivable that an increase in glucose production, as was indicated in the present study with propionate feeding, could increase the relative amount of glucose used by insulin sensitive tissues (muscle and adipocytes), decreasing the amount of FFA needed for energy production. The shift in this balance in favour of glucose oxidation will be associated with a relative reduction in lipolysis, and thus lower FFA levels.

The lower FFA levels, and the relative smaller inhibition of insulin function, are possibly responsible for the lowering of plasma fibrinogen levels by K-GM and propionate (table 9.1). Another advantage of lower plasma FFA was shown by Jenkins *et al.* (1980). Their study demonstrated an improved glucose tolerance of a fibre-free meal, 4 hours after a guar supplemented meal which resulted in lower plasma FFA levels.

A very important observation from the dietary propionate studies both in the animal models and humans is the consistently higher percentage of HDLC in serum. The design of

these studies does not allow valid conclusions regarding the mechanism whereby this effect is mediated. However, the association between high HDLC levels and low serum insulin concentrations (increased serum insulin sensitivity) is well documented, and illustrated in figure 3.3.

Effects in the fed state

The metabolic events during the fed state are characterised by raised blood glucose concentrations, resulting in an increase in the ratio of insulin to glucagon; oxidation of glucose to provide energy (glycolysis); synthesis of glycogen from glucose (glycogenesis); synthesis of fat (lipogenesis) and lowered circulating FFA levels.

From a survey of the literature (see section 2.3) it appears as if propionate stimulates hepatic glycolysis (Anderson & Bridges, 1984; Veech *et al.*, 1986) in the fed state. Glycolytic rates are influenced by the activity of phosphofructokinase. Citrate, which appears to be an inhibitor of this enzyme (Pilkes *et al.*, 1976), is reduced by propionate metabolites (Blair *et al.*, 1973). The result is a prevention of inhibition of phosphofructokinase and thus increased rate of glycolysis. Additional evidence that propionate stimulates the activity of phosphofructokinase is presented by Stanley & Newsholme (1985) and Stanley *et al.* (1986). These authors reported increased activity of phosphofructokinase in liver extracts prepared from mice adapted to a guar-supplemented diet (Stanley & Newsholme, 1985). The lack of such an effect of wheat bran, is ascribed to its poor fermenting ability (Stanley *et al.*, 1986). Other key enzymes in carbohydrate and lipid metabolism, of which the activities are increased by guar gum, is glucose-6-phosphate dehydrogenase, malate dehydrogenase (oxaloacetate-decarboxylating) ($NADP^+$), and ATP-citrate(*pro*-3S)-lyase. Enzymatic analysis of the hepatic metabolites after SCFA administration to fed rats by Veech *et al.* (1986), shows that SCFAs raises the portion of glycolysis from fructose 1,6-biphosphate to phosphoenolpyruvate 2 to 4 fold in liver from fed rats.

The present studies were not designed to investigate the effect of propionate on carbohydrate metabolism in the fed state. However, the response to a glucose challenge was studied in baboons and in man. Propionate supplementation, as well as propionate substrates (K-GM and cooled porridge) reduced the maximum glucose increment during GTTs (table 9.1). This effect may probably be ascribed to increased insulin sensitivity, an effect which is very well documented for gel-fibres in the literature. The mechanism is possibly an up regulation of the insulin receptor associated with chronic lowered circulating insulin levels (Anderson, 1986b). The mechanism through which propionate might improve insulin sensitivity is probably mediated through the observed lowered FFA levels.

Contribution of propionate to the mechanism of action of K-GM

From table 9.1 it is clear that variables influenced by K-GM feeding were, with certain exceptions, also influenced in the same direction by propionate feeding. Propionate effects were, however, not always of the same magnitude and therefore not always significant. From this it seems reasonable to conclude that propionic acid, derived from the colonic fermentation of K-GM, contributes to some extent to those effects where variables were changed in the same direction. In those instances where variables were changed by propionate in an opposite direction (e.g. fasting serum TG and glucose levels), it is conceivable that effects of K-GM resulting from changes in small intestine function, are of such a magnitude that they override the effects of propionic acid.

In table 9.5, an attempt is made to quantify the contribution of propionic acid to K-GM effects. The amount of propionate fed in these studies was 8 g/400 g diet (6.15 g propionic acid) and of K-GM 20 g/400 g diet. It is calculated that this amount of K-GM will provide 3.1 g propionic acid (from the equation proposed for man by Miller & Wolin, 1979). Therefore, the magnitude of the effect of propionate (% change : table 9.1) was divided by 1.98, and expressed as a percentage of the K-GM effect.

TABLE 9.5 CALCULATED CONTRIBUTION OF PROPIONATE TO KONJAC-GLUCOMANNAN EFFECTS

Effect	Baboons	Zucker rats		Humans ¹
		Fatties	Lean	
↓FFA	37.6%	ND ²	ND	ND
↓TC	12.9%	42.7%	NA ³	26.1%
↑ HDLC	21.0%	40.2%	26.2%	12.6%
↓TG	0%	64.5%	21.9%	0%
↓ Total liver lipids	100%	32.6%	25.4%	NA
↓Liver cholesterol	55.5%	49.2%	0%	NA
↓Fasting glucose	0%	0%	0%	ND
↓Fasting insulin	5.8%	29.2%	10.6%	ND
↓Glucose increment	27.2%	ND	ND	ND
↑Insulin sensitivity index	0%	8.0%	11.2%	ND
↓Insulin/glucose	0%	41.0%	21.6%	ND
↓Fibrinogen	38.8%	17.9%	9.4%	ND

¹ Compared to effects of K-GM in hypercholesterolaemic patients (Venter *et al.*, 1987)

² Not determined

³ Not applicable

Values in table 9.5 reflect a possible contribution of propionate to effects found in the present study. This contribution may be influenced by a wide variety of factors, and could conceivably differ in different situations. The contribution to the lowering of plasma fibrinogen is a good example. In the baboon model, a value of 38.8% was found. In the rat model propionate contributed 17.9% (fatty rats) and only 9.4% (lean rats) to the hypofibrinogenaemic effect of K-GM. This can be explained by an additional effect of K-GM on weight gain in the rat model, especially in the lean rats. Vorster *et al.* (1989) recently showed that obesity and weight reduction influence plasma fibrinogen levels. K-GM did not affect weight gain in the baboon, and thus a secondary effect on plasma fibrinogen was not present. Therefore, although the effect of K-GM on the plasma fibrinogen of the baboons was smaller than the effect in the fatty rats (table 9.1 : 15.9% and 30.4%), the contribution of propionic acid to the total effect in the baboon was greater than in the rats (table 9.5 : 38.8% and 17.9%).

In addition to possible secondary or "separate" effects of the dietary fibre, other factors such as species differences, pre-experimental diets, initial values of parameters influenced, etc., could conceivably influence the contribution of propionic acid to the effect of the dietary fibre in a particular model.

From table 9.5 it can be concluded that in this study, propionic acid contributed to the majority of the biochemical consequences of gel fibre intake.

9.2 CONCLUSIONS AND RECOMMENDATIONS

The first conclusion from the results of this investigation is that propionate seems to stimulate glycogenolysis, in addition to the reported effect on gluconeogenesis. This conclusion is substantiated by reduced liver glycogen stores observed in the animal models.

Secondly, the administration of propionate decreases fasting circulating FFA levels which were associated with increased insulin sensitivity and reduced plasma fibrinogen levels. The lower circulating FFA levels have various beneficial effects on haemostasis, carbohydrate and lipid metabolism.

Thirdly, the results of the investigation clearly support the hypothesis that propionate, derived from fermentation of K-GM, contributes to some of the known effects of this gel fibre. These include lowering of cholesterol distributed between liver and serum, increasing HDLC in serum, and resulting in smaller increments during GTTs. This contribution may be influenced by a wide variety of factors.

Fourthly, the results support the hypothesis that dietary factors which lower insulin resistance or improve insulin sensitivity will also lower raised plasma fibrinogen levels.

Recommendations for further work

Propionate metabolism has been studied extensively in ruminants, where it is a major glucose precursor. Recently, the fermentation of polysaccharides and absorption of SCFAs have also been studied in several hindgut fermenting species (Bugaut, 1987; Rechkemmer *et al.*, 1988), including the dog, pig, pony, rabbit, rat, and guinea-pig. *In vivo* fermentation studies should be done in non-human primates, which are regarded as the most suitable model for studies regarding Western lifestyle diseases. It has already been established *in vitro* that the SCFA profile of the baboon differs less from man than that of the pig (Lupton & Villalba, 1988). Besides the choice of an animal model for the investigation of polysaccharide fermentation and the physiological and metabolic consequences for man, it is regarded of importance to feed the animals a background diet similar to that associated with diseases in industrially developed countries. It has further been suggested by Storer *et al.* (1984) that sex may be an important variable in fermentation studies that should be taken into account.

As far as is known, the fermentability of the viscous fibre K-GM has never been studied. We have proved K-GM to be effective in lowering serum cholesterol concentrations (Venter *et al.*, 1987) and improving diabetic control (Vorster *et al.*, 1988b) in smaller doses than pectin, increasing the acceptability for long-term use by hypercholesterolaemic and diabetic patients. Long-term use of viscous fibre is only possible if palatable supplements are available. Incorporation of guar into crispbread (Jenkins & Wolever, 1981), and beta-glucan into white pan bread (Klopfenstein & Hosney, 1987) proved to be an acceptable and effective way to administer viscous fibre supplements over the long-term. The incorporation of K-GM into bread or other baked products should be investigated.

The effects of propionate on serum lipid profile in man may possibly emerge more clearly in subjects with raised TC concentrations in serum. In a pilot study (Venter, 1988, unpublished results) it has been observed that 7.5 g sodium propionate per day in capsule form tended to lower TC in 6 hypercholesterolaemic men after 4 weeks. Although the nauseating effect of sodium propionate was much less when the capsules were taken in mid meal, and better compliance was reported than by the normocholesterolaemic subjects (see chapter 7), the problem may be solved completely by incorporating the sodium propionate in capsules which are dissolved in the small intestine or in enteric-coated tablets.

The suggested inhibition of cholesterol synthesis in liver by 3-OH-3-ethylglutaryl-CoA, an intermediate metabolite of propionate (Mienie *et al.*, 1988), can be studied *in vitro* by measuring the effect thereof on the effect of HMG-CoA in hepatocytes. Another possible

approach may be the administration of ^{14}C -labelled propionic acid to non-human primates, e.g. baboons, and measuring the concentration of intermediate metabolites in plasma, urine, liver, and other tissues. To delineate the mechanism whereby propionate stimulates glycogenolysis, it seems necessary to determine the effect of propionate on cAMP in hepatocytes. The effect of the SCFAs on the pancreatic output of insulin and glucagon, and thus on the insulin : glucagon ratio in both the fasted and fed state, should be examined in more detail in monogastric animals before possible mechanisms whereby propionate lowers fasting serum insulin could be suggested.

Substances which influence fibrinogen synthesis may be identified by *in vitro* studies with hepatocytes, in an attempt to delineate the mechanism(s) whereby fibrinogen synthesis and secretion are regulated. Further, *in vitro* studies of the digestibility of cooled and reheated maize porridge seem necessary to confirm the conclusions drawn from the results of the study reported in chapter 8.

Dietary recommendations

- *Dietary measures to lower fasting FFA levels*

This study gave clear indications that lowered circulating FFA levels are beneficial. Although an examination of 440 middle-aged men in Prague (Fabry *et al.*, 1964) showed that those who ate one to three meals daily were heavier, and had higher plasma cholesterol and more impaired glucose tolerance compared to those who ate five or six meals daily, the importance of small, frequent meals seems to be underestimated. Recommendations to reduce significant overweight and to reduce mean serum cholesterol in adults to 5.17 mmol/L, are incorporated in the Dietary Guidelines for Americans and in the current health objectives for the American population (U.S. Department of Health and Human Services, 1980). However, no recommendation is made regarding meal frequency. From an analysis of 17 sets of dietary guidelines by Truswell (1987) it appears that small frequent meals are recommended by German authorities only. Five or six small meals a day, instead of one to three meals, may result in a beneficial reduction of circulating serum FFA levels between meals.

- *The use of dietary fibre and/or propionate supplements*

From the results of this investigation, supplementation of the diet with sodium propionate to improve glucose tolerance and lower serum cholesterol seems to be less effective than a concentrated gel fibre supplement. The use of purified fibre supplements is not recommended for the population at large. However, supplementation of a high-fibre, high-carbohydrate, low-fat diet with K-GM proved

to be meaningful in insulin-treated diabetic subjects (Vorster *et al.*, 1988b). Gel fibre supplements may well be justified for hypercholesterolaemic and/or diabetic patients for whom high-fibre diets are not acceptable or practicable.

Instead of propionate supplementation, an increased consumption of substrates for polysaccharide fermentation, i.e. DF, resistant and partially resistant starch is highly recommended. The food industry should contribute to the production of palatable starchy food products containing fractions of starch resistant to hydrolysis in the small intestine. It goes without saying that ordinary, wholesome foods will always be preferable to dietary supplements.

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