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MAN'S PHYSIOLOGICAL RESPONSES
TO FRUCTOSE.

by

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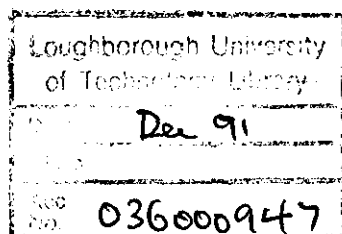
A Doctoral Thesis

Submitted in partial fulfilment of the
requirement for the award of

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~~Doctor~~ of Philosophy
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September 20th, 1987.

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MAN'S PHYSIOLOGICAL RESPONSES TO FRUCTOSE.

All aspects of the work undertaken to complete this thesis were conducted by the author. The main stages included literature searches, experimental design, organisation of studies, blood sampling, setting-up and use of techniques for blood and urine analysis, statistical analysis, and thesis presentation. The ideas and views expressed in this thesis, that contribute to the general areas of nutrition and physiology, are those of the author.

R.C. Parker (1987).

DEDICATION

To subjects and friends who were not subjects
who combined to make this thesis possible.

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ABSTRACT.

Dietary changes that have occurred during the last one hundred years have resulted in an increased contribution of fructose to the diet in the United Kingdom. Over this period the total carbohydrate intake has declined and in effect a partial dietary substitution of glucose sources by fructose has occurred. These dietary events have coincided with the emergence of certain affluent diseases, the most significant being obesity, gout, diabetes mellitus and in particular coronary heart disease (CHD). The extent of these diseases, their interrelationships and the possible involvement of increased dietary sugar in the manifestation of CHD is discussed.

Evidence is drawn from national statistics, retrospective and prospective studies, experts and expert committees, and from dietary studies examining the influence of high sugar diets on the CHD risk factor cholesterol and related metabolites. From most areas of study the evidence is often contradictory and reflects the complexity of the situation under examination. Expert committees have recommended a reduction of dietary sugar but this is intended to reduce the incidence of obesity and does not represent a belief that high dietary sugar contributes directly to other forms of affluent disease save in the provision of excess calories.

However, dietary studies have demonstrated that fructose is metabolised in a different manner to glucose and can result in elevated fasting blood levels of triglycerides and cholesterol. This finding indicates that fructose, by virtue of its influence on the internal environment, may contribute more directly to the development of degenerative disease. It clearly identifies the need to study further the physiological effects of fructose.

The experimental work covers blood absorption profiles and the examination of certain physiological effects of short-term low level additions of fructose and glucose to the free-choice diets of healthy young people. The effect of increased dietary sugar on the composition of the free-choice diet is also examined. The physiological effects selected for study provide an indication of the quality of the internal environment with respect to degenerative disease development.

The first study, examining blood absorption profiles of orally administered fructose and glucose, demonstrates the major initial differences in the physiological treatment of these sugars. Substitution of glucose sources by fructose initially results in a prolonged disturbance of blood sugar composition that is a consequence of the failure of physiological mechanisms geared to deal with dietary glucose to respond to the

introduction of elevated fructose levels. The importance of these events is discussed in light of dietary exchanges of fructose and glucose sources.

Studies, involving elevated sugar intake, revealed adverse physiological effects in male subjects receiving fructose supplements. The detrimental effects observed were an elevation of blood triglycerides, cholesterol esters, low density lipoproteins (LDL) and cortisol, a reduction of blood coagulation time and a rise in the urinary output of free cortisol. Essentially these effects represent, or give rise to, a shift in the internal environment in a direction which is believed to lead to degenerative disease. A "dual" action of fructose giving rise to these changes is proposed involving metabolic changes which favour hepatic triglyceride synthesis and endocrine changes leading to elevated cortisol levels and consequent antagonism of the actions of insulin.

Hepatic uptake of the majority of dietary fructose, elevated hepatic triglyceride and very low density lipoprotein (VLDL) synthesis, and the subsequent increased level of peripheral VLDL degradation and LDL formation is proposed to account for the metabolic action of dietary fructose. An adrenocortical stress-type response occurred as the result of either

persistently high levels or large fluctuations of blood fructose. The physiological mechanisms geared to deal with dietary glucose do not respond in a similar manner to the "replacement" sugar fructose and at a high dietary fructose level may necessitate a stress response to preserve overall physiological function. The insulin antagonistic actions of cortisol augment the metabolic action of fructose and in the short-term produce a shift in the internal environment in a direction characteristic of maturity onset diabetes that has, as a consequence of impaired carbohydrate metabolism, a metabolic lesion that is a risk factor for CHD. In the long-term insulin antagonism not only produces an environment favourable for CHD development but may also lead to pancreatic β -cell exhaustion.

Addition of fructose and glucose to the free-choice diet caused disruption to the composition of the pre-sugar increase typical diet. In comparison to free-choice levels fructose addition resulted in a reduction of fat and carbohydrate (other than sugar) intake and glucose addition a reduction of fat intake only. In comparison to the change in protein intake observed during fructose additions elevated dietary glucose levels also resulted in a reduction of protein intake. The differences in the metabolic handling of fructose

and glucose resulting in different primary tissue locations and endocrine responses and the effect this has on the physiological mechanisms controlling energy balance is put forward to account for the different dietary modifications.

Examination of the diet, for the purpose of recommending dietary changes to improve the quality of the internal environment, should include the assessment of fructose intake. In a comprehensive attempt to achieve an improvement in metabolic status a reduction in the contribution of fructose to the diet should be advised. Future dietary studies should aim to examine the physiological effects of a reduction of dietary fructose from free-choice levels that are currently considered normal but which would certainly be viewed as abnormal one hundred years ago.

GENERAL INTRODUCTION

The background, essential considerations and layout of this thesis are identified in this section. This has involved description of the broad and specific areas within which later study has taken place and highlighting the research approach adopted. In combination these aspects serve to "set the scene" for this thesis.

Work conducted for this thesis was funded by a three year research and training grant provided by Loughborough University of Technology. This grant was awarded to the author to examine man's physiological responses to fructose. The initial step in this research necessitated the identification of a precise area where a valuable practical contribution to knowledge could be achieved. After careful examination of the literature indications were clear that this contribution could be achieved within the sphere of dietary change and affluent disease.

An increasingly large section of modern nutrition is directed towards obtaining a better understanding of the impact of recent dietary changes on the altered disease pattern in affluent societies. The diseases that now cause most concern in this area are coronary heart disease (CHD), diabetes mellitus, gout and obesity. Many aspects of the affluent diet have been examined in relation to the increased prevalence of these diseases, but dietary fibre, fat, and sugar intake have received the most intense attention.

Sugar has received considerable attention in the branch of modern nutrition due to the dramatic increase in sugar consumption that has occurred this century. More accurately sucrose intakes have risen. This thesis deals specifically with a component sugar of sucrose - fructose, and its possible involvement with affluent disease. This possible involvement has been examined in four parts.

Part 1 covers the basic chemistry and occurrence of fructose in the diet. Part 2 begins by asking the question, why look at man's physiological responses to fructose? This question is comprehensively answered by thorough consideration of the change in fructose intake, the prominence of affluent disease, the CHD risk factor cholesterol, dietary surveys and studies, national statistics of diet and disease, retrospective studies and the opinion of experts and expert committees.

Part 3 covers all the experimental work completed. The first section of part 3 is devoted to experimental techniques set up by the author for use in later studies. The remaining four sections include the dietary studies that have been set up to investigate the possible role of fructose in degenerative disease development. The design of these studies is based on the information gained from part 2 and adds to it where knowledge is lacking. Emphasis has been placed on the influence of fructose on the internal environment with respect to

changes observed in degenerative disease. For this purpose the physiological parameters selected for study are those that provide information on the relative risk of disease development.

Part 4 consists of a comprehensive overview of the entire thesis.

PART 1
FRUCTOSE

1.1 STRUCTURE AND NOMENCLATURE.

Fructose (laevulose or fruit sugar) has the empirical formula $C_6 H_{12} O_6$ and is thus a hexose sugar and an isomer of glucose. Due to the positioning of the carbonyl group fructose is classed as a ketohexose and glucose an aldohexose.

Naturally occurring fructose is correctly denoted as D(-)("small" capital D) fructose. The small capital indicates that in nature fructose is based on D-glyceraldehyde, the negative sign showing the direction in which plane polarized light is rotated by the fructose molecule. The rotation of plane polarized light negatively (or to the left) occurs as a result of the presence of asymmetric carbon atoms. Unless otherwise stated the use of fructose in this thesis refers to that form found naturally, i.e. D(-) fructose.

The fructose molecule can be represented in two main forms, as a straight chain or as a 5 membered ring structure. In solution the vast majority of fructose occurs in the ring configuration. Two types of the ring form of fructose occur, termed α and β , which differ only in the configuration about the carbonyl,carbon atom. They are termed anomers.

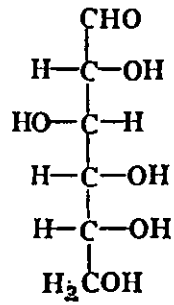
The 5 membered ring formed by fructose is a derivative of furan and is hence named a furanose ring. Glucose, unlike fructose, most commonly forms a ring structure

in solution based on pyran a 6 membered ring and is termed a pyranose sugar. The straight chain, α , and β forms of fructose and glucose are illustrated in figure 1.1.1. Glucose is considered since frequent comparisons are made in the literature and in this thesis between the physiological effects of this isomer of fructose. A very important dietary source of fructose is the disaccharide sucrose. Sucrose consists of one molecule of glucose and one molecule of fructose joined by a glycosidic linkage. The structure of sucrose is illustrated in figure 1.1.2.

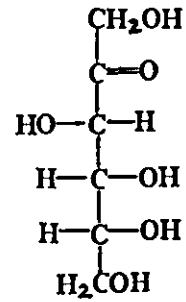
1.2 DIETARY SOURCES OF FRUCTOSE.

In developed countries sucrose is the most important dietary source of fructose and although intakes in the U.K. may have reached a plateau, approximately 60g - 75g of fructose/head/day is provided by sucrose (Davidson, Passmore, Brock and Truswell, 1975; Van Schaik, 1976). However, with advancing technologies and the resultant decrease in the cost of pure fructose it has been predicted that fructose will compete with sucrose in the sweetener market (Aminoff, 1974).

The properties of sucrose and fructose including sweetness, crystallinity, preservation, thickening, bodying, fermentation and humectancy make these sugars widely used in the food industry. As a result of this use fructose can be found in a wide variety of products including soft

Figure 1.1.1. Structure of fructose and glucose.

glucose



fructose

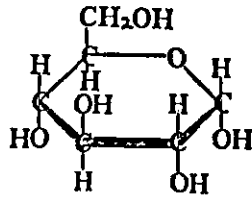
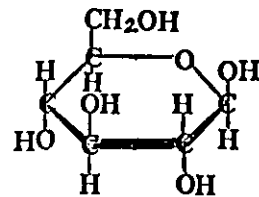
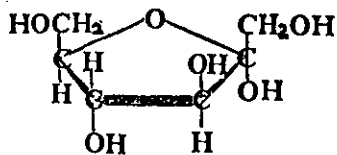
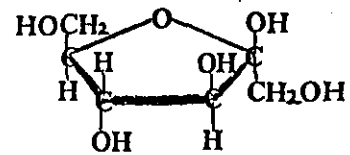
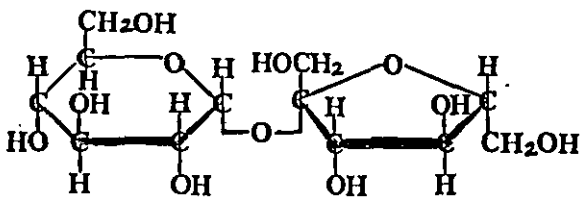
 α -D(+) glucose β -D(+) glucose α -D(-) fructose β -D(-) fructose

Figure 1.1.2. Structure of Sucrose.



drinks, breakfast cereals, confectionery, biscuits, cakes, jams, tinned foods and beer. Examples of the fructose and sucrose content of various foods and beverages are listed in table 1.2.1. (data extracted from Southgate, Paul, Dean, and Christie, 1978).

Fructose is found naturally in honey and in low concentrations in many fruits and vegetables usually in the presence of an equal combination of glucose or as part of sucrose. Table 1.2.2. lists the fructose and sucrose content of a variety of common fruits and vegetables (extracted from Hardinge, Swarner, and Crooks; 1965). For some individuals an important contribution to the intake of fructose comes from the addition of either fructose or sucrose to tea or coffee. An individual ingesting four cups of tea or coffee per day containing two rounded teaspoons of sugar would be increasing their daily intake of sugar by approximately 48g. In terms of a yearly period this would amount to 0.25kg sugar/kg BW for an individual at 70 kg from this source alone, probably half of this amount being fructose.

Fructose in pure form can be obtained from chemists and health food shops for human consumption and can be used for the same purposes as sucrose. Fructose is 1.7 times as sweet as sucrose (Dermer, 1946) a quality that is made use of by manufacturers to promote the sale of this sugar. However, fructose is sixteen times as expensive as sucrose and for the purpose of sweetening drinks,

alternatives are available, (Hough and Emsley, 1986). In addition fructose may only be detected as sweeter, in comparison to sucrose, in solution and not in prepared foods such as cakes or puddings (Hardy, Brennard, and Wyse; 1979).

TABLE 1.2.1. FRUCTOSE AND SUCROSE CONTENT OF PREPARED
FOODS.

	Fructose	Sucrose
	Range (g/100g)	Range (g/100g)
Alcoholic beverages*	0 - 16.1	0 - 27.4
Biscuits	0 - 0.9	0 - 42.9
Breakfast cereals	0 - 6.0	0 - 45.6
Cakes and pies	0.5 - 11.3	20.5 - 47.8
Cola	4.8	0.5
Confectionery	0	52.0 - 58.7
Fruit juices	2.8 - 5.3	0.3 - 44.2
Milk products	0 - 2.5	0 - 45.3

* (g/100 ml)

TABLE 1.2.2. FRUCTOSE AND SUCROSE CONTENT OF FRUITS
AND VEGETABLES.

Food	Sugar content	
	Fructose (g/100g)	Sucrose (g/100g)
<u>Fruits</u>		
Apple	5.0	3.1
Apricot	0.4	5.5
Banana	3.5	11.9
Blackberry	2.9	0.2
Cherry	7.2	0.1
Currants(Black)	3.7	0.6
Dates	23.9	0.3
Gooseberry	4.1	0.7
Grapes(Black)	7.3	-
Grapes(White)	8.0	-
Grapefruit	1.2	2.9
Lemon	1.4	0.4
Orange(Valencia)	2.3	4.2
Peaches	1.6	6.6
Pears(Bartlett)	5.0	1.5
Pineapple(ripened on plant)	1.4	7.9
Plums(Damson)	3.4	1.0
Prunes(uncooked)	15.0	2.0
Raspberry	2.4	1.0
Strawberry(ripe)	2.3	1.4
Tangerine	-	9.0
Tomatoes	1.2	-

TABLE 1.2.2. (continued)

Food	Fructose (g/100g)	Sucrose (g/100g)
<u>Vegetables</u>		
Cabbage(raw)	-	0.3
Carrots(raw)	-	1.7
Cauliflower	-	0.3
Cucumber	-	0.1
Lettuce	-	0.2
Onions	-	2.9
Parsnips fresh	-	3.5
Peas(green)	-	5.5
Potatoes	0.1	0.1
Radishes	-	0.3

PART TWO

WHY LOOK AT MAN'S PHYSIOLOGICAL RESPONSES TO FRUCTOSE?

PART 2. WHY LOOK AT MAN'S PHYSIOLOGICAL RESPONSES TO
FRUCTOSE?

- 2.1 INTRODUCTION.
- 2.2 CHANGING PATTERNS OF FRUCTOSE CONSUMPTION.
- 2.3 MAJOR AFFLUENT DISEASES.
 - 2.3.1 CORONARY HEART DISEASE
 - 2.3.2 OBESITY.
 - 2.3.3 DIABETES MELLITUS.
 - 2.3.4 GOUT.
- 2.4 SUGAR INTAKE AND AFFLUENT DISEASE.
 - 2.4.1 NATIONAL STATISTICS.
 - 2.4.2 SUGAR INTAKE OF INDIVIDUALS WITH AFFLUENT DISEASE.
 - 2.4.3 DIETARY STUDIES AND CHD RISK FACTOR-CHOLESTEROL.
 - 2.4.4 OPINIONS OF EXPERTS AND EXPERT COMMITTEES.
- 2.5 AN ANSWER - SUMMARY.

2.1 INTRODUCTION.

Part 2 provides a valid answer to the above question by consideration of several integral factors of the diet/disease debate. Additionally part 2 serves the important purpose of directing the reader to the precise problem that this thesis considers - Man's physiological responses to fructose with respect to diseases of affluence.

Initially the rise in fructose consumption to a position where it is recognised as a significant contributor to the UK diet is described. Other changes within the carbohydrate composition of the diet are also highlighted. Consideration switches to the altered disease pattern that has emerged during the period when dietary changes have taken place. CHD, obesity, diabetes mellitus, and gout are discussed. These affluent diseases are considered in isolation at this stage and emphasis is placed on their prominence in Western societies.

The discussion continues by the examination of possible associations between increases in sugar intake and affluent disease. Three main experimental approaches to the problem of investigating the possible associations are included: 1) examination of gross national statistics of food consumption and post mortem records, 2) analysis of the diet of individuals known to be afflicted with affluent disease, 3) precise dietary

studies examining the effects of individual components of the diet on recognised risk factors. Additional valuable information relating to the importance of sugar and affluent disease has been gained by the consideration of the views of experts and expert committees who have necessarily drawn their conclusions from examination of material arising from the above areas.

The final section provides a summarised answer to the title question of part 2. Evidence is extracted from the previous sections to contribute towards this answer.

In the following sections, where possible, discussions are focussed on fructose. However, in some cases sugar, sugars or sucrose are discussed since these carbohydrate-divisions have been used in the cited materials and their inclusion is either necessary for comparison with fructose or because of their fructose content.

2.2 CHANGING PATTERNS OF FRUCTOSE CONSUMPTION.

The change to an "affluent diet" that has occurred in the United Kingdom has involved a reduction in the energy derived from carbohydrates (Hollingsworth, 1974). In addition to this change the proportion of the various classes of carbohydrate consumed has altered, so that in affluent times refined sugars have increased their contribution to the total carbohydrate intake. Yudkin,

(1964) estimates that sucrose contributes 35% of the total carbohydrate intake (and 20% of all calories), in comparison with less than 2% 200 years ago.

Although a recent decline in the amount of sucrose purchased as packet sugar has been recorded, the true intake of refined sugar, which allows for "hidden sugar" consumption, in prepared foods, is approximately 125g/person/day (Cannon, 1983). This high level of refined sugar intake has been maintained during the last 10 years and it is possible that a plateau has been achieved. The steady increase in sucrose consumption to this figure that occurred during the years 1900 to 1970 (apart from the declines during both World Wars) is clearly illustrated in the Department of Health and Social Security (DHSS) report concerning diet and CHD (1974).

The result of the above changes is an increase in the intake of fructose and a decrease in the amount of glucose consumed. A rise in fructose consumption from as little as 1% to 10% of total calorie intake has occurred (based on Yudkin's data, 1964). Essentially a change of this magnitude represents the introduction of a new "energy carrier" to the body which must be dealt with by existing physiological mechanisms.

The use of fructose corn syrups in prepared products (Cantor, 1975; Kushnir, 1979) coupled with the results of advancing technology, which has reduced the production cost of fructose, will increase further the amount of

fructose consumed (Aminoff, 1974).

In view of the increasing contribution of fructose to the diet the physiological responses evoked by this sugar warrant investigation.

2.3 MAJOR AFFLUENT DISEASES.

The emergence of the diseases of affluence during the period of dietary change, indicates further the need to explore the physiological responses to fructose. The diseases of affluence that are of particular importance are CHD, obesity, diabetes mellitus, and gout. The extent of the problem that affluent disease poses is highlighted below.

2.3.1 CORONARY HEART DISEASE.

CHD was an uncommon disorder in the 1920's that usually only affected members of the professional and upper classes. Women were rarely affected. CHD is now the leading cause of death in Britain (Truswell, 1981). In the United Kingdom CHD accounts for forty per cent of all deaths in males aged from 45 to 54 years of age (DHSS, 1981).

The incidence of CHD in pre-menopausal women is low, in comparison to males of the same age range, but rises rapidly following the menopause. Figures from the DHSS

(1981) illustrate how the ratio of male to female deaths from CHD (per 100,000 population) decreases with increasing age. The greatest increase in female deaths due to CHD occurs when the proportion of post-menopausal women included in the age range would be expected to be very high (Beck, Moffat, and Lloyd; 1973). The fact that the ratio of male to female deaths from CHD in the 65-74 age group remains high (2:4:1) could be accounted for by the age difference between the sexes at which the disease started.

The prevalence of CHD, although high in all affluent societies demonstrates varying trends in different countries. For example, in some developed nations a decline in the incidence of CHD has been recorded in recent years, although this is not the case in Britain and most European countries (Dwyer and Hetzel, 1980; Editorial, 1980).

2.3.2 OBESITY.

With increasing affluence obesity has become the principal nutritional disorder of developed communities. Akinpelu, Atinmo, and Palmer (1984) emphasize the extent of the problem by considering obesity to be of national significance, while Glore, Layman, and Bechtel (1984) remark that "obesity is one of the major nutritional problems of modern man".

Obesity is increasingly observed in children particularly in those from developed countries (Christakis, Miridjanian, Nath et al, 1968; Stunkard, D'Aquili, Fox, and Fillion, 1972), and thus the problem is no longer confined to the traditionally obese middle aged individual. However, within this group the prevalence of obesity continues to rise. At the extreme of affluence, in the United States, records for 1971-1974 show that, within the age group 25-44 years, 15.3 and 20.7 per cent of males and females respectively were obese, (Bray, 1979). In a recent study, incorporating 953 subjects from an English community, 21 per cent of males and 22 per cent of females were considered obese (Dawes, 1984).

Obesity is not only a cosmetic problem, it may also have associations with other major diseases. An early study by Joslin (1923) highlights this point, indicating that obesity is the main factor predisposing to type II diabetes. Later studies have since reinforced this association, (Anonymous, 1983; West, 1974). In addition obesity has been shown to increase the risk of CHD (Prior, 1974) and gout (Prior, Beaglehole, Davidson and Salmond, 1978).

However, a recent follow-up study (Larsson, Svärdsudd, Welin et al, 1984) has shown, that for middle aged men, the distribution of adipose tissue is a better indicator than degree of obesity.

Keys (1980) states that the association between relative body weight and premature death due to CHD has been greatly exaggerated. Keys puts forward, with considerable evidence, that, for the middle aged man an average relative weight, or marginally above, offers the best protection against early death. Small departures on either side of the average relative weight incur small increases in risk; only excessive departures leading to a significant danger.

Albanase, Wein and Carroll (1984) cite a report that suggests associations may exist between obesity and 23 known diseases which account for 10 to 15 per cent of the mortality rate in the United States. The above associations did not include those that exist for CHD, stroke, or diabetes.

2.3.3 DIABETES MELLITUS.

Diabetes Mellitus is the most frequently diagnosed endocrine disorder. In the United States it is estimated that $3\frac{1}{2}$ - 4% of people suffer from the disease (Mitchell, Rynbergen, Anderson and Dibble; 1976). In Britain over 1 per cent of the population are thought to be affected, but only 50 per cent of cases are recognised (Davidson et al, 1975).

Observations by WHO (from Mitchell et al, 1976) have shown that the incidence of diabetes has generally

increased throughout the world. This is particularly noticeable in countries that are becoming affluent. As noted above an association between diabetes and obesity is thought to exist. Evidence in the literature largely supports this association, (Ostfeld, Shekelle, Tufo, et al, 1971; Feldman, Sender and Siegelau, 1969; Joslin, Dublin and Marks, 1936; Luft, Cerasi and Anderson, 1968), although a recent study failed to find a constant association (Kohrs and Tobben, 1982). Diabetes is, additionally, recognised as an important risk factor predisposing to the development of CHD (Brusis and McGandy, 1971).

2.3.4 GOUT.

Traditionally gout was a disease that confined itself to the rich and overindulgent. More recently it has been shown that the incidence of gout increases with rising prosperity, and that the incidence in the affluent West is from 1 to 2 per cent of the adult population (Mertz, 1971). Males are more often affected than females.

Apart from the production of extremely painful attacks of gouty arthritis, and the increased risk of uric acid formation in the urinary tract, gout also has associations with other diseases. Hyperuricaemia is itself a risk factor for CHD (Brusis and McGandy, 1971) and is also associated with the risk factors, hypertension, diabetes, and hyperlipoproteinaemia (Mertz, 1973).

2.4 SUGAR INTAKE AND AFFLUENT DISEASE.

Sections 2.2 and 2.3 have identified separate reasons for investigating man's physiological responses to fructose. In the first case fructose is now more abundant in the diet and in the second this has occurred during a period when disease patterns have changed. By further examination and combination of these factors a further important reason and a direction for physiological study can be achieved. For this purpose this section reviews literature that deals with the possible links between increased dietary sugar and CHD. Whether or not the changes highlighted above have occurred in isolation are examined in this way.

By far the most important degenerative disease to emerge is CHD and for this reason receives primary attention at this stage. However, the interrelationships between affluent diseases is of crucial importance and plays a key role in consideration of later experimental designs where use is made of the biochemical lesions underlying these diseases.

Links that may exist between the level of dietary sugar and CHD has been dealt with in four sections. Section 1 examines gross statistics, section 2 sugar intake of individuals with CHD, section 3 the effect of different carbohydrates on the internal environment, and section 4 the views of experts and expert committees on the association between sugar and CHD.

Section 3 forms the main bulk of the discussion and essentially examines the effects of different carbohydrates on various blood constituents. Considerations of cholesterol carriage in the blood and as a CHD risk factor are necessarily included in this section. This particular area receives focussed attention and is used as the foundation for the experimental studies conducted later in this thesis.

2.4.1 NATIONAL STATISTICS.

Yudkin (1957) investigated the relationship between the sugar intake of individuals within different prosperous populations and the mortality rate from CHD. The findings of this study demonstrated a general association between sugar intake and CHD although not all data was in agreement. Examination of national statistics, although providing useful information and direction, can give rise to confusing or even erroneous conclusions. These could be drawn as a result of the multitude of interacting, uncontrolled and possibly unaccounted factors.

Concerning the above study Yudkin points out that varying methodologies adopted by different nations can also influence the results. He also adds that the intake of one component of the diet under investigation may vary in accordance with another, and it may be this other component that is connected with disease development.

In further criticism poorer nations to those studied by Yudkin exist that record high sugar intakes (Food and Agriculture Organisation, 1969) yet do not have a high CHD mortality rate (WHO, 1969; Stocks, 1969).

Data from a study examining the possible relationship between sugar intake and CHD in the United Kingdom indicated that increased sugar intake was associated with a rise in the incidence of CHD (Ashton, 1965). However, a closer correlation was found between fat and total caloric intake. Ashton (1965) also points out that this type of statistical comparison is particularly naive as similar associations can be recorded between an increase in CHD and television and vehicle licenses.

2.4.2 SUGAR INTAKE OF INDIVIDUALS WITH AFFLUENT DISEASE.

To investigate further the possible associations between sugar intake and degenerative diseases Yudkin and Roddy, (1964) compared the sugar intake of individuals who had suffered a recent first attack of myocardial infarction, or who suffered from peripheral arterial disease (PAD) with apparently healthy subjects. Sugar intake was assessed by dietary questionnaire. For individuals suffering from degenerative disease the assessment of sugar intake was made within three weeks from the time of diagnosis of myocardial infarction, or before or soon after commencement of treatment for PAD. The timing of dietary assessment is important in studies of this nature since dietary habits often change as a result of the diagnosis

and treatment of disease (Yudkin and Roddy, 1966).

Patients in both the disease groups studied by Yudkin and Roddy (1964) recorded significantly higher sugar intakes than the apparently healthy control group. The mean sugar intakes per day for the myocardial infarct group, the PAD group, and controls were 132g, 141g, and 77g respectively. Thus a possible association between high sugar intake and degenerative disease has been demonstrated using a retrospective study.

The above study has met with criticism. Others conducting similar studies have failed to find an association between high sugar intake and degenerative disease. Additionally the reliability of the questionnaire technique used for the assessment of sugar intake has been questioned.

Little, Shanoff, Csima et al (1965), Papp, Padilla and Johnson (1965), Paul, Macmillan, McKean and Park (1968), Finegan, Hickney, Maurer and Mulcahy, (1968), Finegan, Hickney, Maurer and Mulcahy, (1969), Howell and Wilson (1969), Burns-Cox, Doll and Ball (1969), Elwood, Waters, Moore and Sweetman (1970), and a working party of the Medical Research Council (1970) have investigated, in a similar manner to Yudkin and Roddy (1964) the possible associations between sugar intake and CHD. Results from these studies have demonstrated either a small or no difference between the sugar intakes of the control subjects and those suffering from CHD.

Yudkin and Roddy (1966) have responded to their critics and have attempted to explain the different findings recorded by other workers. Concerning the validity of the questionnaire technique Yudkin and Roddy (1966) have compared the assessment of sugar intake with results obtained by a 7 day dietary recording. Using 23 patients the mean sugar intake using the questionnaire technique was found to be 71g compared with 69g using the 7 day recording.

The assessment of dietary sugar intake by Little et al (1965) and Papp et al (1965) took place from between 3 to 6 months after diagnosis of myocardial infarction. Yudkin and Roddy (1966) have clearly demonstrated that sugar intake changes in response to the awareness of the presence of disease and have criticised the above studies on this basis. Although differences in techniques can be used to explain the observed discrepancy between these studies the later investigations by Burns-Cox (1969) and the Medical Research Council (1970) used the same techniques as Yudkin and Roddy (1964). Additionally the authors of these studies, in the light of available evidence, conclude that sugar is unlikely to be an important factor leading to CHD. Thus using retrospective studies researchers have again disagreed over the importance of sugar as an agent increasing the likelihood of degenerative disease.

2.4.3 DIETARY STUDIES AND CHD RISK FACTOR CHOLESTEROL.

Another approach to examine the possible involvement of high sugar intakes with degenerative disease development is to explore the data obtained from prospective studies. Although such studies have failed to directly implicate sugar the emergence of risk factors for CHD are of particular interest. The main risk factors that have been identified in over 20 prospective studies in 14 countries are, the presence of a high fasting serum cholesterol concentration, high blood pressure, and cigarette smoking (Truswell, 1985). Truswell (1985) adds that members of expert committees, set up for the purpose of reducing the incidence of CHD, from Australia, Canada, France, Netherlands, New Zealand, Scandinavia, United Kingdom, United States of America and West Germany have all recommended a reduction in the level of serum cholesterol as a protective measure. Where a lowering of serum cholesterol has been achieved a reduction in CHD incidence and progression of existing CHD has been recorded (Oliver, 1984; Nikkila, Viikinkaski, Valle and Frick, 1984). Thus serum cholesterol and its manipulation by drugs and diet has attained a key position in the investigation of CHD. Other risk factors have been identified, and have been mentioned, but those cited above have demonstrated the strongest links with CHD. For purposes of this discussion the fasting serum concentration of cholesterol and related metabolites are of particular relevance. Numerous studies have been

conducted to examine the effect of dietary change on this risk factor.

In order to clarify future discussions the carriage of cholesterol in the blood is considered at this stage. This is an essential inclusion since many studies deal with the various fractions of cholesterol and with certain components of cholesterol-containing structures.

Essentially blood cholesterol is found in 4 different macromolecules that consist of protein, phospholipid, and triglyceride. The cholesterol-containing macromolecules are called, chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL). These macromolecules serve to solubilize lipids and facilitate the transfer of lipids from the blood to the cells. The lipoproteins differ in their protein, phospholipid, cholesterol, and triglyceride composition. This allows their separation by ultracentrifugation (due to density differences) and by electrophoresis (due to macromolecular charge differences). The various compositions of the lipoproteins are illustrated in table 2.4.1 (Stafford, 1976).

Chylomicrons are synthesised in the intestinal cells largely from fats absorbed in the form of micelles from the intestinal lumen. The triglyceride component of the chylomicron is provided entirely by the diet.

TABLE 2.4.1 COMPOSITION OF THE LIPOPROTEINS.

	TG	PRO	PHOS	CHOL
	(%)	(%)	(%)	(%)
Chylomicrons	83	2	7	9
VLDL	50	9	18	22
LDL	10	21	22	47
HDL	8	50	22	19

TG Triglycerides

PRO Protein and carbohydrates.

PHOS Phospholipid

CHOL Cholesterol

The function of the chylomicron is to transport dietary fat from the intestinal cells via the lacteals to the blood and finally to the sites where triglyceride is utilized. The main sites of chylomicron-triglyceride removal are in the capillary beds of adipose tissue and muscle. However, any capillary bed containing lipoprotein lipase is capable of this function.

Following removal of the majority of chylomicron-triglyceride by lipoprotein lipase the resultant chylomicron fragment is transported to the liver. The remnant particle is then converted to LDL, glycerol, free fatty acids, and cholesterol. The removal of chylomicron-triglycerides from circulation is rapid, their half-life being less than one hour (Nestel, 1964).

Very low density lipoproteins are the next least dense lipid carrying macromolecules. VLDL is synthesised in the liver and effectively serves to transport triglyceride of endogenous origin (Skipski, 1972). The triglyceride contained in the VLDL is again removed by tissues containing lipoprotein lipase.

The persistence of VLDL in the blood is short and they are broken down within a few hours (Davidson et al, 1975). In man the degradation of VLDL leads to the formation of LDL (Bilheimer, Eisenberg and Levy, 1972; Eisenberg, Bilheimer and Levy, 1972; Eisenberg, Bilheimer, Levy and Lindgren, 1973). In fasting blood the concentration of

VLDL is directly related to endogenous triglyceride production.

LDL is the main carrier of cholesterol in the blood and is degraded slowly over a period of 2.25 - 3.58 days (Langer, Strober, and Levy, 1972). The concentration of LDL in fasting blood is correlated with total cholesterol and can also be considered a measure of long term triglyceride production since LDL is derived from the triglyceride carrier VLDL. LDL is commonly termed β -lipoprotein.

The precise function of the remaining, most dense lipoprotein, HDL is unknown but this macromolecule does participate in protein exchanges with chylomicrons on entry to the blood and on their degradation (Scow, Blanchette-Mackie, Hamosh and Evans, 1973; Simmonds, 1972).

Thus in blood cholesterol is represented in several fractions which can be related to the concentration of certain other metabolites. The discussion continues with dietary studies set up to examine the influence of different carbohydrates on various blood lipids.

In a cross over design using 7 subjects Macdonald and Braithwaite (1964) investigated the effects of high sucrose and starch diets on serum lipid levels. (Other variables were also recorded in this study). Subjects were fed experimental diets for 25 days followed by

a return to their typical diets for 25 days before taking part in the final 25 day period of the study. During each experimental period the daily diet contained 500g of sucrose or raw maize starch as the carbohydrate component.

In comparison to the serum lipid concentration on a free-choice diet lipid levels fell during starch feeding and rose during sucrose feeding. A rise in the glyceride fraction accounted for the increased total lipid concentration recorded on the high sucrose diet. An increased glyceride concentration will be reflected in a raised concentration of VLDL and LDL. Macdonald and Braithwaite (1964) suggest that the metabolic handling of sucrose and starch may be different. This finding would not be unexpected considering the digestion products of sucrose and starch. The glucose component of sucrose might be dealt with in an identical manner to digested starch, the fructose portion being the cause of the different metabolic response.

The dietary change that was implemented in the above study involved the digestion of an amount of sugar or starch providing approximately 2000 kcal per day. This is an extremely high inclusion and must be borne in mind when interpreting the results, eg., the above study provides a valuable comparison between high starch and sucrose exchanges but does not indicate what would happen at low level exchanges.

In a later study by Macdonald (1965) the serum lipid profile was investigated while subjects ingested carbohydrates over a 5 day period. The level of carbohydrate feeding was set at 7.5g/kg BW per day and again is at a high level. The carbohydrates on test were maize starch, liquid glucose (BPC), sucrose, maltose and glucose.

During the feeding of sucrose the concentration of cholesterol remained unchanged whilst all other carbohydrates resulted in a reduction in serum cholesterol. Serum glycerides, free sterols, and free fatty acids rose significantly whilst subjects ingested the high sucrose diet. Other carbohydrates either had no effect or resulted in a lowering of the serum concentration of this fraction. Total serum lipids were unaltered during sucrose and starch feeding whilst the other carbohydrates on test resulted in a reduction in serum lipid concentration.

Macdonald (1965) points out that a change in the serum lipid pattern would be expected on the virtually fat-free diet consumed during the test periods. Additionally he adds that the significance of the glyceride response observed during sucrose feeding is increased bearing in mind the low lipid intake and the serum lipid responses recorded during the feeding of the other test carbohydrates.

Again the main difference in digestion products between

the test carbohydrates is that fructose would be present after hydrolysis of sucrose. In all other cases glucose would be the resultant monosaccharide.

Further evidence into the influence of the type of carbohydrate fed on serum cholesterol concentration comes from the studies of Winitz, Graff and Seedman, (1964) during their development of chemical diets for space travel. These researchers fed a pure chemical diet to 18 volunteers for a period of 19 weeks. The project was divided into 3 phases during which time the carbohydrate source was manipulated.

During phase 1 all the carbohydrate fed was glucose, during phase 2 75% was provided by glucose and 25% by sucrose, and during phase 3 subjects returned to a diet that contained glucose as the sole carbohydrate source. Phase 1, 2, and 3 covered the periods 0-4 weeks, 5-7 weeks, and 8-19 weeks respectively.

During phase 1 a drop in serum cholesterol concentration from 227 mg% (base-line value) to 160 mg% (week 4) was observed. When sucrose partially substituted for glucose in the diet a rise to 208 mg% occurred. A return to glucose as the sole carbohydrate source produced a lowering of serum cholesterol to 151 mg% by the end of week 19. All changes that had occurred by the end of each phase were significantly different from the corresponding values at the beginning of each phase.

The diet in the above study was again high in carbohydrate and low in fat. The rise in serum cholesterol concentration that occurred during phase 2 approached the pre-study base-line values. This is in close agreement with the earlier reported findings of Macdonald (1965). In the case of this study the fraction of cholesterol that changed as a result of the dietary regimens cannot be deduced since cholesterol only was determined. However, fructose must again be implicated as the cause of the different metabolic response by virtue of the digestion products of sucrose.

In a review concerning dietary carbohydrates and serum cholesterol compiled by Grande (1967) a number of studies are cited that are of relevance to this discussion. Keys, Anderson and Grande (1960) conducted a study that compared the serum cholesterol levels during the feeding of diets containing either complex carbohydrates or a mixture of sucrose and lactose.

The different sources of test-carbohydrate contributed approximately 17% towards the total calorific intake. Each diet was fed at two levels of fat. The diet containing the complex carbohydrates resulted in a serum cholesterol concentration 17 mg% lower than that recorded on the diet containing simple carbohydrates. The small difference in cholesterol concentrations was similar at both fat levels and statistically significant. The inclusion of lactose in the simple sugar diet should be

borne in mind as a confounding variable when considering the cause of the observed cholesterol differences. However, a serum cholesterol difference was observed when the contribution of sucrose in the carbohydrate test portion of the diet was considerably lower in comparison to studies cited previously. This is an important point to note when extrapolating experimental diets to "real-life" situations.

Anderson, Grande, Matsumoto and Keys, (1963) studied the effects of different simple sugars in an exchange experiment. The sugars investigated were sucrose, glucose and a mixture of glucose and lactose. Sucrose and glucose were fed at a level of 233g per day, the sugar mixture containing 129g of glucose and 104g of lactose. The subjects were 12 men and the study lasted for three weeks. The diet containing sucrose produced the highest cholesterol concentration, the mean difference between the cholesterol levels on the other sugars being 5 mg%. This small change was statistically insignificant. No difference was detected between the mean cholesterol concentrations recorded during the feeding of the glucose diet and the glucose/lactose diet.

The findings of the above study have led the authors to conclude that, in normolipemic individuals the substitution of glucose or a mixture of glucose and lactose with sucrose, at a level of approximately 30% of the total caloric intake, does not result in a significant elevation

of serum cholesterol concentration.

Shamma'a and Al-Khalidi (1963) have recorded similar findings. Using diets containing 50% of the total caloric intake as glucose, sucrose, lactose, or galactose fed over a period of 16-25 days the authors failed to demonstrate a significant difference between the serum cholesterol levels when the different sugars were fed.

Grande (1967) in conclusion of the review suggests that the effect of dietary carbohydrates on the serum cholesterol level is limited and can only partly explain the difference recorded within populations. Identification of all components of the diet that influence serum cholesterol concentration is of importance. Only when armed with this knowledge will it become possible to successfully regulate serum cholesterol concentrations by dietary means.

In a study conducted by Macdonald, Coles, Brice and Jourdan (1970), designed to investigate effects of food intake frequency, diets high in sucrose were fed during which time serum total cholesterol and triglyceride concentrations were monitored. Eleven male subjects took part in the study which included ingestion, as part of the diet, of 7 g/kg BW per day of sucrose for 18 days. Subjects participated in both sections of the study which involved either "nibbling" or "gorging" their daily rations.

During the gorging regimen the percentage increase in serum triglyceride concentration was significant on days 3 and 7. On day 18 serum triglycerides were elevated by approximately 12 per cent over base-line values. Nibbling produced a significant percentage rise in serum triglyceride levels on days 14 and 18. A regression analysis yielded a significant positive correlation for percentage change in triglyceride concentration regressed on time. At the end of the nibbling period the percentage change in triglyceride concentration was approximately 28 per cent above the base-line value. The total serum cholesterol concentration was significantly decreased during both gorging and nibbling periods of the study.

Since VLDL is the main carrier of endogenous triglyceride (Skipski, 1972) the above result indicates an elevation of VLDL. The degradation of VLDL leads to the formation of LDL, (Bilheimer et al, 1972; Eisenberg et al, 1972; Eisenberg et al 1973) the main carrier of serum cholesterol (Stafford, 1976). Thus the greater formation of VLDL observed above would yield a greater serum concentration of LDL and therefore elevate serum cholesterol. However, serum cholesterol levels were observed to fall. An explanation for this occurrence is that although LDL concentration is boosted via this route (increased endogenous triglyceride synthesis) other pathways of LDL formation (e.g. from dietary fat) are reduced. The reduction in dietary fat (as occurred in the above study)

would lead to a reduction in chylomicron formation, the degradation of which must be considered a source of LDL (Eisenberg and Levy, 1975). The net effect of this situation (where elevated endogenous triglyceride formation occurs at the same time as a reduction in chylomicron formation) would be to lessen the serum cholesterol reducing effect of dietary fat reduction.

However, an alternative explanation that must be considered is that LDL levels do increase and the concentration of another cholesterol-carrying macromolecule is reduced to an extent that a fall in total serum cholesterol is observed. Thus in this case the lipoprotein profile would have been disturbed. These possible explanations are of great methodological importance since lipoprotein disturbance may pass undetected by examination of serum cholesterol alone.

In a later study by Macdonald (1978a) the effect of high sucrose and glucose diets on the blood cholesterol carrying macromolecule HDL have been examined in conjunction with total cholesterol and triglyceride levels. Two female and 7 male subjects (20 to 28 years of age) participated in this study which required individuals to ingest 6g of sucrose or glucose per kg BW per day for 19 days. Subjects received both diets.

A significant fall, with respect to pre-study base-line values, was recorded for the concentration of HDL

cholesterol during the feeding of both sugars. On the high sucrose diet a minimum cholesterol value was recorded on day 5, when the mean concentration was 11 mg% below the pre-study level. From this point the cholesterol concentration rose slowly to a maximum value of 18 mg% above the base-line level on day 19 of the study. A similar pattern was observed on the glucose diet except that the initial fall in concentration (-25 mg%) was steeper on this diet and the subsequent rise failed to reach the base-line value. The concentration of serum triglycerides was increased significantly during the sucrose diet whereas this fraction was lowered significantly whilst subjects received the high glucose diet.

In common during the feeding of both sugars the contribution of HDL cholesterol to the total cholesterol level was reduced in comparison to base-line values. This was the only similar lipoprotein disturbance recorded for glucose and sucrose. Reduction of HDL would serve initially to limit any observable rise in total cholesterol resulting from the elevation of other lipoprotein fractions. For sucrose feeding an elevation of VLDL was observed which would add to the level of total cholesterol by virtue of the cholesterol component of this macromolecule. In addition, and perhaps of more importance, the rapid breakdown of elevated VLDL would yield a greater quantity of the more blood-persistent LDL macromolecule. The steady rise of blood cholesterol after day 5 of sucrose feeding demonstrates the increased contribution of VLDL and LDL to the total cholesterol concentration. Since

HDL is regarded as protective and LDL as injurious with respect to CHD development (Truswell, 1985) these changes clearly represent a detrimental lipoprotein disturbance.

Fears, Glenny, Tredger and Lindsay (1981) have investigated further the influence of a high sucrose diet on the various cholesterol fractions, including HDL-cholesterol. Eleven male subjects (18 to 35 years of age) were incorporated into the above study, which required individuals to ingest sucrose at a level providing 60 per cent of their total energy requirements for 5 days. Estimations of the serum concentration of; total cholesterol, triglycerides, HDL-cholesterol and HDL-triglycerides were carried out during the study. Levels of serum VLDL and LDL cholesterol and triglycerides were calculated using a pre-study-verified method.

The concentration of serum triglycerides rose significantly over the period of high sucrose feeding. The authors point out that this increase was mainly accounted for in the VLDL-triglyceride fraction. This clearly indicates a rise in the synthesis of endogenous triglyceride. Levels of both HDL and LDL-cholesterol fell significantly over the course of the study, during which time only a slight non-significant drop in the total cholesterol occurred.

The high sucrose/low fat diet provided would be expected to result in a slight fall in total cholesterol unless

sucrose, or a constituent of sucrose has cholesterol elevating properties. A severe restriction of dietary fat would lead to a reduction in the amount of LDL formed from chylomicron degradation i.e., less fat derived LDL would appear in circulation. In earlier reported studies of a similar design base-line cholesterol levels have been maintained or even exceeded thus indicating that sucrose exhibits cholesterol promoting activity. In the case of high sucrose feeding significant triglyceride elevations are usually observed. The origin of the triglyceride is indicated from the above study to be the liver since the VLDL-triglyceride fraction was increased.

As highlighted previously VLDL contributes to the maintenance of total serum cholesterol, but more importantly, as regards long term effects, this would lead to a greater formation of LDL. In the above study LDL concentration fell significantly but clearly this fall was limited by an increase in carbohydrate-derived LDL formation. In the study conducted by Macdonald (1978a) a similar fall in total cholesterol concentration was observed over the first 5 days when the high sucrose diet was fed. However, from day 5 and throughout the remainder of the study total cholesterol levels rose. A possible explanation for these observations is that the initial fall in total cholesterol is caused by a greater rate of fat-derived LDL removal than carbohydrate-derived LDL formation. After 5 days the contribution of carbohydrate-derived LDL exceeds the

loss occurring as a result of dietary fat removal and total cholesterol levels rise.

In diets that have been observed to elevate serum triglyceride and cholesterol concentrations the primary difference between the digestion products of the carbohydrates fed is that fructose is liberated from sucrose. That fructose might be the responsible component of the sucrose molecule for initiating the altered lipid profiles has been investigated by Macdonald (1966).

Macdonald (1966) incorporated 5 young men, 6 pre-menopausal, and 3 post-menopausal women into a study that involved the feeding of 3 different mixtures of carbohydrates over periods of 5 days. The various carbohydrate mixtures were fed at a level of 7.5 g/kg BW per day and were as follows; 40% fructose and 60% corn starch, 40% glucose and 60% corn starch, and 40% fructose and 60% glucose.

A significant decrease in the serum concentration of cholesterol was observed in the post-menopausal group for all diets. Male cholesterol values dropped but the changes failed to reach significance. Pre-menopausal women recorded similar cholesterol levels on all the diets fed. Significant increases in the level of glycerides were observed for males and females of the post-menopausal group when receiving the fructose/starch and fructose/glucose diets. No significant glyceride changes were

observed in the pre-menopausal women, although higher levels were recorded on the two diets containing fructose.

Serum glyceride concentrations were significantly higher in males and post-menopausal females when fed the fructose/starch diet in comparison to values recorded on the glucose/starch diet. Thus, it can be interpreted that, under the conditions of this study, fructose results in the increase in the glyceride levels in these groups. That pre and post-menopausal women respond differently suggests that cyclic oestrogenic hormones exert an influence on lipid metabolism. Evidence from studies using baboons supports this suggestion (Coltart and Macdonald, 1971).

Lees (1965) has suggested that the different metabolic effects of starch and sucrose observed in earlier studies may be due to the poor absorption of the raw starches fed. Lees (1965) has used cooked wheat and rice starch to investigate this. Subjects received diets of either a high level of cooked wheat and rice starch, or sucrose in a cross-over design for periods of 4 to 14 days. The level of inclusion of the different carbohydrates accounted for 90% of the total calories. The serum concentration of cholesterol and glycerides were monitored throughout the dietary periods.

No significant differences were observed between the cholesterol and glyceride responses when subjects received

either the cooked starch or the sucrose diets. Serum cholesterol concentration rose by mean values of 120mg % and 119mg % when the starch and sucrose diets were fed respectively. The corresponding mean increases for serum glyceride levels were 109mg % and 88mg %.

The inclusion of 90% of the total calories as carbohydrate in the above diets may have been sufficiently high to cause a different metabolic response to that observed in other studies where raw starches have been fed. Additionally the subjects that were included in the study were 6 young adult females and 1 young adult male. Therefore, the finding that starch and sucrose produced similar effects on the lipids investigated could have been due to the influence of the pre-menopausal hormone pattern on lipid metabolism as observed by Macdonald (1966). Poor absorption of raw starch would result in the production of watery stools. This complaint has not been reported in earlier studies except when high levels of fructose have been fed alone.

A contribution to this particular area of investigation i.e. carbohydrate induced changes in serum cholesterol concentration and distribution, has been achieved in many studies involving the use of laboratory animals. Studies of particular relevance to this discussion are cited below. The use of animals in such experiments offers the advantage of precise control of the environment and therefore the elimination of confounding variables, but

unfortunately the results cannot be directly extrapolated from one species to another. This cautionary note should be taken into account when interpreting the following material.

Vrána, Fábry and Kazdová, (1976) fed ad-libitum diets to groups of female rats that contained either glucose or fructose at a level providing 70 per cent of the total calories for periods of 3, 7, and 30 days. At all sampling times the concentration of serum triglycerides was greater in the fructose fed group. In a further study reported in the same article the authors fed, as the 70 per cent carbohydrate allowance, mixtures of fructose and glucose. Fructose was fed at 0, 33.3, 66.6 and 100 per cent of the sugar mixture for a period of 3 weeks. All diets containing fructose resulted in a significant increase in the level of serum triglycerides.

Thus in the above series of experiments a difference in the metabolic handling of fructose and glucose is clearly indicated, the former sugar leading to a rise in circulating triglycerides. This observation was recorded over the entire feeding period and at low levels of fructose inclusion. A similar triglyceride elevation has been observed in human subjects when fed fructose, (or inferred when fed sucrose) with one important difference. Women, or at least pre-menopausal women, are not generally susceptible to the triglyceride response elicited by fructose feeding. Presumably female

rats are not afforded the same hormonal protection to this lipid disturbance. This observation that fructose causes an elevation of serum triglycerides in female rats, has been supported by later studies conducted by Vrána and Kazdová (1982).

Diets containing 60 per cent of total calories in the form of starch, glucose or fructose have been compared for their effect on triglyceride concentrations in the rat by Merkens, Tepperman and Tepperman (1980). Diets were fed over a 6 day period, determinations of triglyceride being performed on days 3 and 6 of the study. Fructose produced significantly higher triglyceride concentrations that were detected on day 3 and were maintained to day 6 of the study. High levels of dietary glucose resulted in similar triglyceride levels to those recorded for rats receiving the high starch diet. Again a carbohydrate difference with respect to serum lipid profiles has been demonstrated. Additionally the "carrier" of glucose does not, in this case, appear to influence the triglyceride response. This is an important point to note indicating that, in the rat at least, triglyceride levels are influenced to the same degree by two forms of ingested glucose (i.e., complex or simple carbohydrate forms). When considering the dietary changes that have occurred such an observation aids in the assessment of the relative importance, with respect to lipid profiles, of various carbohydrate exchanges i.e., starch to glucose or starch to sucrose.

Further studies have been conducted using rats to examine the influence of the components of sucrose, glucose and fructose, on the concentration of serum triglycerides. Aoyama, Hattori, Yoshida and Ashida (1980) fed rats of 4 different strains for 14 days diets containing, as the carbohydrate source, either glucose or fructose at a level providing 69.9 per cent of the total calories. Serum triglycerides were significantly higher in rats of the Donryu, Wister, and Fischer strains and tended to be higher in rats of the Sprague Dawley strain when fed fructose. The above observations have been confirmed in later studies by Aoyama, Hattori, Yoshida and Ashida (1981, 1982).

Longer term studies using rats to investigate the effect of different carbohydrates on serum lipid fractions have been conducted. Høstmark, Spydevold, Lystad and Eilertsen (1982) fed diets for a period of 10 weeks containing high levels of starch, glucose, sucrose or fructose to different groups of rats. A fifth group were fed a purified stock diet. The carbohydrate component of the test diets contributed 72 per cent towards the total calorific value of the foods presented. Serum samples were obtained on week 10 and the various lipoproteins examined. In all groups the triglyceride was, as would be predicted, mostly located in the VLDL portion. The VLDL-triglyceride level was greater in the groups fed high sucrose and fructose rations than in all other groups. The values recorded for VLDL-triglycerides were;

19 mg%, 56 mg%. 93 mg%. 172 mg%, 181 mg% for the starch, stock, glucose, fructose and sucrose diets respectively.

The above results, not only demonstrate the triglyceride response induced by fructose and sucrose, but also indicate that the high level of serum triglycerides are maintained for a considerable time. The composition of VLDL and LDL were unaffected by the different dietary regimens even though their concentrations were markedly altered between the groups. Therefore, a disturbance of the composition of VLDL and LDL does not occur when diets high in fructose and sucrose are fed but simply an elevation of the entire macromolecules. Thus the relationship between VLDL and triglyceride concentration, and total cholesterol and LDL levels remains intact despite a marked alteration in overall lipid metabolism.

A later study using rats conducted by Høstmark, Spyderold, Lystad and Haug (1984) demonstrates the effect of high fructose and glucose diets on VLDL and LDL-triglycerides, cholesterol esters, protein and free cholesterol. In this study diets high in fructose and glucose were fed for 8 weeks. The proportion of energy derived from the sugars was 72 per cent of the total energy provided.

A significantly higher triglyceride concentration was observed in the fructose fed group at the end of the dietary period. This finding agrees with the earlier observations of Høstmark et al (1982). Additionally, as would be expected, the location of the majority of

triglyceride was in the VLDL and LDL fractions. It is probable, as previously mentioned, that the rise in LDL-triglyceride is a direct result of an increase in the number of VLDL particles formed. In the same paper a further study is reported that investigated the hepatic output of VLDL components during liver perfusion with fructose and glucose. The results suggest that the synthesis of all VLDL components are stimulated by fructose infusion, and that VLDL composition remains unaltered at different formation rates of triglyceride i.e., during fructose and glucose perfusion. This is an important observation considering the increased levels of cholesterol and triglyceride remaining in circulation in the form of LDL following VLDL breakdown.

Blakely, Hallfrisch, Reiser and Prather (1982) have conducted a long term trial feeding low levels of fructose to rats during which time serum levels of triglycerides and cholesterol have been monitored. Two test diets of 54 per cent carbohydrate content were fed, one diet consisted of 54 per cent pre-gelatinized corn starch and the other of 15 per cent fructose and 39 per cent pre-gelatinized corn starch. Serum triglycerides and cholesterol were recorded at 3, 7, 9, and 15 months.

Triglyceride levels rose on both diets to a maximum value at 9 months. On the starch only diet, and on the fructose starch mixture the serum triglyceride concentrations at 9 months were 181 and 203 mg% respectively. At 15 months the starch group had dropped to 161 mg%

and the starch/fructose group to 110 mg%. Only at this stage of the trial were the levels of triglyceride significantly different between the 2 groups. The pattern of a rise to a peak at 9 months and a fall at 15 months in the triglyceride concentrations recorded for both diets was reflected in the cholesterol levels which followed a similar course pattern. This probably demonstrates the influence of triglyceride formation in the liver on cholesterol concentration as outlined above.

The level of fructose included in the diet of the above study was designed to be equivalent to the typical fructose intakes in the United States. Thus this particular study does not add weight to a fructose induced raised cholesterol level, with a subsequent increase of CHD development, as a result of fructose exchanges for starch. However, as previously noted, animal studies cannot be directly related to man. This is particularly true in this case as a typical age related increase in cholesterol, as observed in man, was not recorded in this study.

To summarise at this level of investigation, i.e., comparative effects of high carbohydrate diets on the concentration of serum cholesterol components, the following points are of relevance. Not all studies conducted in man or in animals have demonstrated the same effects. Methodological differences, including level of carbohydrate inclusion, subject characteristics, and the duration of feeding probably account for these differences.

In most studies an elevation of the VLDL fraction has been recorded during the feeding of diets containing fructose. A rise in circulating VLDL indicates a stimulation of endogenous triglyceride production. In studies where triglyceride concentration has been recorded, and observed to rise, an increase in VLDL levels can be inferred since the carrier macromolecule is synthesised for the purpose of transporting endogenously synthesised triglycerides.

Serum cholesterol has shown variable responses during the feeding of high fructose diets. Maintenance, rise and fall of cholesterol levels have all been noted; at times in the same study demonstrating a time course effect.

The majority of diets that have been constructed to determine physiological effects of carbohydrates have necessitated a reduction in fat levels from the amount that would normally be derived from a free-choice diet. The lowering of the fat content of a diet will lower serum cholesterol concentration due to a reduction in the formation of chylomicrons and their cholesterol rich degradation products.

When a particular study design has resulted in a downward trend in cholesterol levels, in comparison to other test carbohydrates, fructose has resulted in the least

cholesterol reduction or even maintenance. The lowering in cholesterol concentration is promoted by a reduction in the formation of LDL derived from dietary fat intake.

In the case of fructose feeding an increase in carbohydrate-derived LDL occurs to lessen the cholesterol-lowering effect of dietary fat removal. An increase in carbohydrate-derived LDL is a direct consequence of a raised endogenous triglyceride production. This increase in triglyceride synthesis that occurs during fructose feeding necessitates the formation of VLDL which in turn, results in a rise of LDL levels.

It should be noted that the prediction of LDL concentration from cholesterol levels might at times result in an inaccurate view of lipoprotein changes. Other lipoproteins may change to affect cholesterol concentration. HDL levels have been observed to fall when diets high in sucrose are fed and therefore their contribution to the total cholesterol concentration would be lessened, thus artificially lowering the LDL estimate.

In longer term studies, during the feeding of fructose containing diets, a pattern of serum cholesterol changes has been reported. During the early stages a fall occurs to below values recorded on a previous free-choice diet. From this "low point" continued feeding of fructose

containing foods results in a rise of serum cholesterol to a value significantly higher than the free-choice cholesterol base-line.

The main influencing factors acting on the serum cholesterol concentration during the initial stages of high fructose feeding would be the reduction in fat-derived LDL and the quantity of triglyceride formed from the carbohydrate provided by the diet. Under these circumstances a "lag" period appears to exist during which time the production of carbohydrate-derived LDL is building up to eventually mask and exceed the effect of dietary fat removal. This build up is dependent upon the rate and the total amount of triglyceride released into circulation in the form of VLDL.

It would appear, assuming a constant efficiency of LDL removal irrespective of the diet, that fructose containing diets reported to exert a cholesterol raising effect, are exerting a greater positive influence on LDL concentration than the dietary fat of a free-choice diet acting via chylomicron breakdown.

During periods of metabolic disruption caused by severe dietary change the composition of the VLDL and LDL fractions has been observed to remain unaltered. This indicates that the response to a changing demand for VLDL synthesis, brought about by an alteration in triglyceride production,

is to vary the number of particles manufactured and not alter their composition. As noted previously this observation has additional importance concerning the prediction of cholesterol fractions.

Finally a sex difference has been observed between the lipid responses to diets containing high levels of fructose. Pre-menopausal women appear to be protected against the triglyceride elevation resulting from fructose feeding in comparison to males and post-menopausal women. An endocrine effect is obviously indicated.

2.4.4. OPINIONS OF EXPERTS AND EXPERT COMMITTEES.

The remaining level of investigation to be considered, in order to formulate an answer for the above section title, is the view held by experts on dietary change, the relationships with disease development, and on recommendations for future changes aimed at improving the existing diet. (Discussion will be confined to areas concerned specifically with sugar). The experts in this particular field obviously draw their conclusions from the previously discussed levels of investigation.

At an individual level a range of views on the relationship, if any, between the diet and degenerative disease are held. Some workers consider that dietary change is virtually unconnected with disease patterns, whilst others believe that the diet is a major influence in this area.

Fortunately extreme views tend to take on their appropriate values when considered by expert committees. Additionally the response of relevant societies and by large numbers of workers in this area, to questioning concerning the diet/disease debate, results in an emphasis on the general feelings and does not highlight isolated controversial thoughts. However, recent exchanges between Marks and Yudkin in *Biologist* (September 1986 and February 1987) demonstrate clearly the extreme views held by experts on the subject of sugar and disease. These exchanges border on personal criticism.

Yudkin, a prominent nutritionist, has paid particular attention to the role of sucrose in the causation of disease. Yudkin's own work covers all levels of investigation considered here. In a review by Yudkin (1972) he concludes that there is evidence, of varying strengths, to implicate, as a contributory factor, high intakes of sucrose with the increased incidence of certain diseases. The diseases that Yudkin mentions are dental caries, obesity, coronary thrombosis, diabetes, and diseases of the skin, digestive tract and joints.

Walker (1971) has also examined and reviewed the literature concerning possible links between sugar intake and disease. The conclusions drawn by this author are in sharp contrast to those by Yudkin. Walker states "Firstly, although evidence is incomplete, such evidence as is available does not significantly incriminate sugar.

Secondly, bearing in mind the multifactorial aetiology of CHD, it is questionable whether, within a given context, major incrimination of sugar is possible."

Walker (1971) has considered Yudkin's conclusions carefully and has criticised them from several angles. Firstly the population data from which Yudkin reported his original association between high levels of sugar intakes and CHD included unreliable components. These components include the difference between "real" and "actual" sugar intakes recorded on a national basis, the population composition, and the differing criteria used for the determination of the cause of death. Walker (1971) adds that a truly random sample, from the populations selected for comparison on a national basis, have not been made.

Walker's (1971) second, and a crucial criticism, relates to the lack of agreement between results obtained by other workers and those reported by Yudkin. (These results concern the sugar intakes of individuals with and without CHD; some of this data has been discussed earlier.) In addition to failing to demonstrate a firm relationship between sugar intake and CHD, these workers have also recorded vastly different sugar intakes, for both control and CHD patients, between their studies. Walker cites the conclusions reached by two study groups, Burns-Cox et al (1969) and the Medical Research Council

(1970), who conducted surveys on similar lines to those of Yudkin. These conclusions are recorded here due to their importance. Burns-Cox et al (1969) --"that the totality of data now available does not suggest that consumption of refined sugar is likely to be a major or specific factor in the production of myocardial infarction". Medical Research Council (1970) -- "the evidence in favour of a high sugar intake as a major factor in the development of myocardial infarction is extremely slender." Clearly Yudkin's views are lacking in firm support from these areas.

Walker's (1971) third major criticism concerns the use of population data over a changeable time period. Changes other than in sugar intake have occurred during the last century which would certainly confuse attempts to associate a single dietary factor with the increased incidence of a particular disease. Patterns of exercise, other dietary alterations, and general "lifestyle" changes can be included as particularly likely factors to confound results.

Trowell (1975) has also considered the role of increased sugar consumption in the causation of disease. Regarding sugar intake and CHD development Trowell draws much the same conclusions as Walker, emphasizing the lack of positive evidence incriminating sugar, arising from the various studies conducted. Concluding on the available evidence Trowell comments, "the main danger from sucrose

is the possibility of over-consumption." Development of obesity, as previously mentioned, is a significant problem in affluent societies and may have associations with other degenerative disease development. Certainly if sucrose contributes to the occurrence of obesity clearly this sugar poses a threat to the quality of life.

Considerable attention has been paid by Truswell (1981) to the possible effects of the diet on the development of CHD. Although the main consideration of Truswell's article concerns the involvement of dietary fat, other aspects are included that are of relevance to this discussion. Truswell cites the study of Norum (1978) who sent a questionnaire to 200 experts around the world asking their opinion on diet/disease matters. Of 193 experts that replied 176 considered that the present diet was not satisfactory with regard to CHD and changes could be recommended. Nine changes were mentioned; a reduction in the amount of sugar consumed was ranked number 5 in this list of modifications. Clearly from this informed sample a majority view is held that sugar in some way increases the likelihood of CHD development. If this is the case, then equally, the physiological responses to sugars, ingested in large quantities in affluent societies, should be thoroughly explored.

Another particularly interesting finding from the questionnaire study of Norum (1978) was the view of the experts on serum cholesterol concentration. Of the 193 respond-

ents 189 felt that there is an association between the level of serum cholesterol and CHD risk.

Another interesting questionnaire study concerning diet and disease has been conducted, which involved members of the Nutrition Society in the United Kingdom and Eire, (Brown, Brown and Naismith, 1977). Members were requested to offer their opinion on certain diet/disease hypotheses. Respondents had the choice of answering "yes", "no", or "undecided" to the various issues. Of the 941 members originally sent the questionnaire, 67 per cent replied. Concerning diet and CHD development 23 per cent thought that sucrose consumption was an important aetiological factor. In addition 70 per cent of these members reported that they had reduced their sucrose consumption. The authors note that the number of members rejecting the hypotheses was generally smaller than the number who were undecided. Therefore, again consideration by experts of information concerning sucrose and disease development has resulted in the acceptance, by a sizeable proportion, of the importance of sucrose and CHD development and thus indicating an important direction for research.

To complete this discussion, the views of committees that have been assembled to examine dietary change and disease patterns are of particular relevance.

Essentially the reduction of sugar in the diet, in order to directly prevent CHD, has not been recommended by any expert committee (DHSS, 1981). However, limiting sugar intake has frequently been recommended in order to assist in the prevention of obesity and therefore attempt to reduce risk in general of possibly-related degenerative disease development.

A panel originating from the Committee on Medical Aspects of Food Policy (COMA) have reported on such issues, with special emphasis on CHD and cerebrovascular disease (DHSS, 1974). One aspect of the diet, considered by the panel to be a possible risk factor, was the excessive consumption of sucrose. Of added interest is the inclusion of a raised serum concentration of triglycerides and cholesterol, and diabetes mellitus as main risk factors. The panel recommends that intakes of sucrose should be lowered if only to achieve a reduction in the incidence of obesity. This recommendation does not appear to directly implicate sugar in the causation of CHD, but rather suggests a possible link via the CHD risk factor obesity.

The avoidance of obesity is also stressed by a report published by a Joint Working Party of the Royal College of Physicians of London and the British Cardiac Society (1976). Accepting that obesity may not be such a significant risk factor as other factors e.g. a raised serum cholesterol concentration and hypertension, the Working Party recommends the correction of obesity due to its

close association with diabetes mellitus, hypertension, and hyperlipoproteinaemia, which are important CHD risk factors. The interrelationships between affluent diseases are obviously an important consideration when examining the influence of various dietary factors. In addition the physiological states associated with these diseases are of particular importance, since they can be monitored to determine a precise risk status without the actual presence of disease.

In 1983 a subgroup formed from the National Advisory Committee on Nutrition (NACNE) published a report that included recommendations for dietary changes aimed at improving health in the United Kingdom. The subgroup, headed by Professor James, was briefed to use material from existing reports arising from expert bodies to compile their report. The NACNE report has experienced a controversial history and recently much publicity. Basically the controversy reflects the attitude and power of certain British food industries that would be severely affected if the recommendations contained in the report were successfully implemented. One change the subgroup recommends is a reduction in the amount of sugar consumed. Sugar, the NACNE report recommends, should provide 10 per cent of the total caloric intake, a reduction from the present day figure of 20 per cent. This recommendation was geared to reduce the prevalence of obesity.

Reports from NACNE (1983), DHSS (1974) and (1984), and from the Joint Working Party of the Royal College of Physicians of London and the British Cardiac Society (1976) point out the contribution of sugars to the development of obesity. This could appear to detract from the importance of reducing the intake of dietary sugar, the mechanism of action of sugars acting purely through the provision of excess calories. However, when the interrelationships between various affluent diseases are considered the importance of limiting sugar intake, and thus reducing the risk of obesity, can be released. However, the involvement of sugar in the development of obesity is far from conclusive, Durnin (1986) concluding, after reviewing the relevant literature, that sugar intake did not play an important part in the development of obesity.

From a starting point of the provision of excess calories in the form of sugar to the eventual recognition of obesity, a variety of underlying physiological changes occur. Investigation of these physiological events will yield valuable information concerning the interrelationships of affluent diseases. The physiological effects of particular importance will be those that result in the modification of the overall risk of degenerative disease development.

This section completes the discussion aimed at the

formulation of an answer to the chapter title:- "Why look at the Physiological Responses to Fructose?". The following section is in the form of a summarised answer, drawing from the information raised in the preceding sections.

2.5 AN ANSWER - SUMMARY.

The contribution of fructose to the United Kingdom diet has increased and with advancing technologies in the food industries further increases in dietary fructose are anticipated. The present level of fructose intake now represents a significant addition to the body of a new energy carrier that has to be dealt with by existing physiological mechanisms. The consequences of such an addition are largely unknown and for this reason the physiological effects of this sugar should be thoroughly investigated.

The change to an affluent diet, that has included a rise in fructose consumption has occurred during a period when disease patterns have altered. Diseases such as CHD, diabetes mellitus, gout and obesity, now characteristic of the United Kingdom, are a major threat to the quality and quantity of life. It is of particular importance to examine if the increase in fructose consumption has contributed in any way to the increased prevalence of these diseases. Links between these changes have been examined and although the field is highly controversial

and contradictory indications are evident that further work is required in this area.

Investigation of gross statistics and study of the sugar intake of individuals with and without CHD has yielded the most contradictory data. This type of study method appears shrouded in difficulty both in a methodological sense and in the interpretation of results obtained. Expert committees examining possible links between diet and disease have not recognised sugar as having a direct involvement with degenerative disease prevalence except through obesity development. As a result committees typically recommend the reduction of sugar intake in order to assist in the prevention of obesity.

However, the emergence of CHD-risk factors from prospective studies has provided an ideal opportunity to examine the effects of individual dietary components on those risk factors in an experimental situation. There is now clear evidence from dietary studies that fructose (and sucrose) influences the metabolism of the CHD risk factor cholesterol in a different manner to both starch and glucose. The mechanisms that produce these differences are poorly understood. Further development in this area is required in order to examine the precise mode of action of fructose and to determine the extent to which this involvement is likely to impair the internal environment. Thus a need to examine man's physiological responses to fructose has been identified along with a precise direction to follow.

PART THREE

MAN'S PHYSIOLOGICAL RESPONSES TO FRUCTOSE.

PART 3 MAN'S PHYSIOLOGICAL RESPONSE TO FRUCTOSE.

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3.1 INTRODUCTION.

Studies cited in the previous section fall into three broad areas, investigation of international statistics, examination of the diets of individuals with disease, and the effects of experimental diets on various physiological parameters. The studies in this section fall into the last category.

The vast majority of human studies reported have involved the provision of large quantities of sucrose, glucose and starch in highly controlled diets, usually to male subjects, although there are exceptions. From these studies physiological differences evoked by the different carbohydrates have been observed. These observations have been of great value and contribute significantly to the understanding of the influence of type of carbohydrate on the internal environment with respect to disease development. Experimental work in this thesis uses this information as a foundation to explore further the comparative physiological effects of carbohydrates. Studies in this section primarily involve the addition of low levels of fructose and glucose to the free-choice diets of young males and females. The following discussion outlines the reasons for this design.

In section two it was put forward that recent dietary change has resulted in fructose making a significant contribution to the diet. This has occurred as a result of exchanges within the carbohydrate category of glucose (or glucose yielding sources) for fructose (or fructose

yielding sources). At present sucrose provides the majority of dietary fructose, although technological advances and marketing ploys may alter this balance. Most, but not all, previous studies have concentrated on the comparison of the physiological effects of starch and sucrose. This is a logical initial step since sucrose has increased in the diet at the expense, within the carbohydrate section, of starch. However, this exchange, in biological terms is confused by definitions. In a developmental sequence, one carbohydrate has been exchanged for another (starch by sucrose), one sugar has been exchanged for another (glucose by fructose) or more precisely the structure of one energy source has been replaced by another. For this reason, and as a consequence of the importance of structure in physiological systems, the physiological effects of glucose and fructose have been compared in the following studies.

Previous studies have typically employed large increases in sugar intake and have been criticised on this basis. However, Yudkin (1972) has defended the use of high sugar levels in experimental diets and certainly results from such studies provide valuable information on the influence of carbohydrates on the internal environment. A logical extension from this work would be to reduce the level of sugar inclusion in experimental diets. By so doing the results will be more directly applicable to a greater proportion of the population. The following studies, that have involved an increase in sugar intake, have

increased sugar intake by 1g/kgBW/day. The addition of 1g of sugar/kg BW/day has been described as a low level increase. It is important to note that this statement is only accurate when this addition is compared with sugar levels used in studies reported earlier. An addition of 1g of sugar/kg BW/day should produce on average a dietary sugar level that is close to the sugar intake of individuals within the population that record the highest levels of sugar intake (Rugg-Gunn, 1986). Thus the diets fed in the following studies must be considered high sugar diets.

Most studies cited have investigated the physiological effects of carbohydrates on males. There are important exceptions and when female groups have been included different physiological responses have been revealed. In most cases the practical purpose of work in this area is to examine possible links between carbohydrate intake and physiological responses that are thought to be conducive to degenerative disease development. For CHD the most susceptible groups are men and post-menopausal women. Thus again it would seem logical to initially concentrate research efforts on these susceptible groups.

Pre-menopausal women have been examined (Macdonald, 1966) and some physiological responses to high sugar diets have been shown to be different to males and post-menopausal women. In addition further work by Coltart and

Macdonald (1971) with baboons has indicated that certain physiological response differences between the sexes are due to oestrogens. Thus the bias towards the examination of male subjects in this area appears largely justified. However, ideally a female group should be included especially when physiological parameters not previously investigated in detail are included in the design. This inclusion serves to provide an additional "control group" that enables comparisons to be made between the physiological responses of a CHD-protected group and a CHD-susceptible group. From such examination the underlying physiological mechanisms that offer protection against degenerative disease development may be revealed. In the following studies (with the exception of the final section) male and female groups have been included.

Precise control of experimental diets has enabled researchers to demonstrate different physiological properties of certain carbohydrates (namely, starch, glucose, fructose, and sucrose). This represents the first step towards identifying the effects of specific carbohydrates in "real" dietary situations on the internal environment. Although obviously essential in this elucidation experimental diets have various drawbacks that limit their usefulness in prediction "outside the laboratory". The demonstration, by precise dietary manipulation, that sucrose elevates triglyceride levels in comparison to

starch does not indicate what would result if carbohydrate levels change when the background diet is highly variable and capable of change as a result of carbohydrate alteration.

A study where the exchange of sucrose and starch takes place asks the initial research question, what are the physiological differences between these carbohydrates? The addition of carbohydrate to a free-choice diet asks the next research question, can the above changes in the internal environment be observed when the diet is highly variable and free to change? The second research question relates approximately to a real-life situation when either an existing or an entirely new component of the diet increases its overall contribution to the diet. Under such circumstances a variety of changes including, alteration of the background diet, the stimulation of a physiological mechanism geared to maintain bodyweight during periods of caloric excess and bodyweight gain, are possible. For any case the resultant changes may act to mask the physiological effects of the added carbohydrates that, although occurring, are rendered insignificant in terms of observable modifications of the internal environment. In studies that follow the physiological effects of fructose and glucose added to free-choice diets are examined. In addition, in the final experimental section, the effects of these additions to the free-choice diets are explored.

The experimental section has been divided into five sections, the first section deals with experimental

techniques and the remainder cover the studies conducted. The first study examines blood absorption profiles of orally administered fructose and glucose. This investigation is designed to reveal the initial differences in the internal environment that occur in terms of "sugars present" in circulation that initiate future metabolic events. Studies 2, 3, and 4 essentially have the same design in that physiological measurements are examined before, during and after periods of increased dietary fructose or glucose. The physiological measurements selected include indicators of the quality of the internal environment with respect to degenerative disease development, factors closely related to these indicators, and control mechanisms that act to preserve the consistency of the internal environment. Study 2 covers metabolic and study 3 hormonal effects as revealed by examination of blood samples. Study 4 examines the urinary output of "free" cortisol and in addition the effect of glucose and fructose additions on the composition of the free-choice diet.

3.2 EXPERIMENTAL TECHNIQUES.

This section contains relevant information on all measurement techniques employed in the following series of studies. Each technique was carefully selected to satisfy requirements for, laboratory safety, delivery time, shelf life, equipment expenditure, and per assay

cost, time and reliability. In order to achieve the above selection criteria the author vigorously appraised the techniques before incorporation into any study. This is an obvious and necessary step to ensure that reliable results are obtained and valuable samples are not wasted.

A total of twelve techniques were used which are detailed below. This section has been divided into "kit" and "non-kit" estimations. Kit estimations refer to commercially available assay methods and non-kit estimations to methods that were "set-up" from information available in the literature.

All assays in the preliminary stages and during the later studies were performed by the author and therefore problems associated with between observer error were not experienced. In all studies assays were carried out in duplicate except for the determination of coagulation time which was performed in quadruplicate.

3.2.1. NON-KIT ESTIMATIONS.

3.2.1.1. FRUCTOSE DETERMINATION/ROE'S TECHNIQUE.

Although kits are available for fructose determination these are designed for estimation of fructose in foods and are not recommended for use with body fluids. A search of the literature indicated that Roe's technique

(1937) (RT), or a modification of this method, was in most frequent use for the quantitative estimation of fructose in body fluids. As a result of this finding RT was examined and modified in order to satisfy the requirements of this study.

RT for the determination of fructose in blood and urine originally appeared in the literature in 1934 and is a modification of a procedure developed by Seliwanoff (1887). Basically fructose is dehydrated by hot concentrated hydrochloric acid to yield a derivative of furan (furfural). The furfural condenses with resorcinol to produce a characteristic cherry red colour, the intensity of which is proportional to the fructose concentration. Roe's modification achieved an improved colour intensity in comparison with the original Seliwanoff technique. This was achieved by using 30% hydrochloric acid instead of 12 per cent and by preparing the resorcinol in 95 per cent ethanol. Both of these modifications improve the reaction-dehydration conditions and ethanol also acts as a solvent and colour stabiliser for furfural.

Investigation and modification of RT for use in future studies was conducted in seven separate experimental stages. Brief accounts covering the basic aims and conclusions of each experimental stage are reported below.

Stage 1.

Standard solutions corresponding to 25 (1.4), 50 (2.8), and 100 mg% (5.6 mmol/l) fructose were prepared in saturated benzoic acid and run through RT in quadruplicate on two separate occasions. The standard concentrations prepared were identical to those used by Roe (1934) and, although beyond the range intended for future use, served the purpose of, determining the wavelength of maximum absorbance and assessment of the ease of use and reliability of the technique.

The wavelength of maximum absorption was located at 480 n.m. which is in agreement with the method of Kulka (1955), a modification of RT. The quality of the absorbance measurements recorded for the fructose standards used and the ease of handling of the technique justified further development.

Stage 2.

Standards corresponding to concentrations of 2 (0.1), 4 (0.2), 8 (0.4), and 16 mg% (0.8 mmol/l) fructose were prepared and run through RT in quadruplicate on two separate occasions. The aims of this stage were to determine whether or not the sensitivity of the technique was sufficient to detect low fructose concentrations, examine the reliability of the technique over this range,

and to examine the relationship between fructose concentration and absorbence.

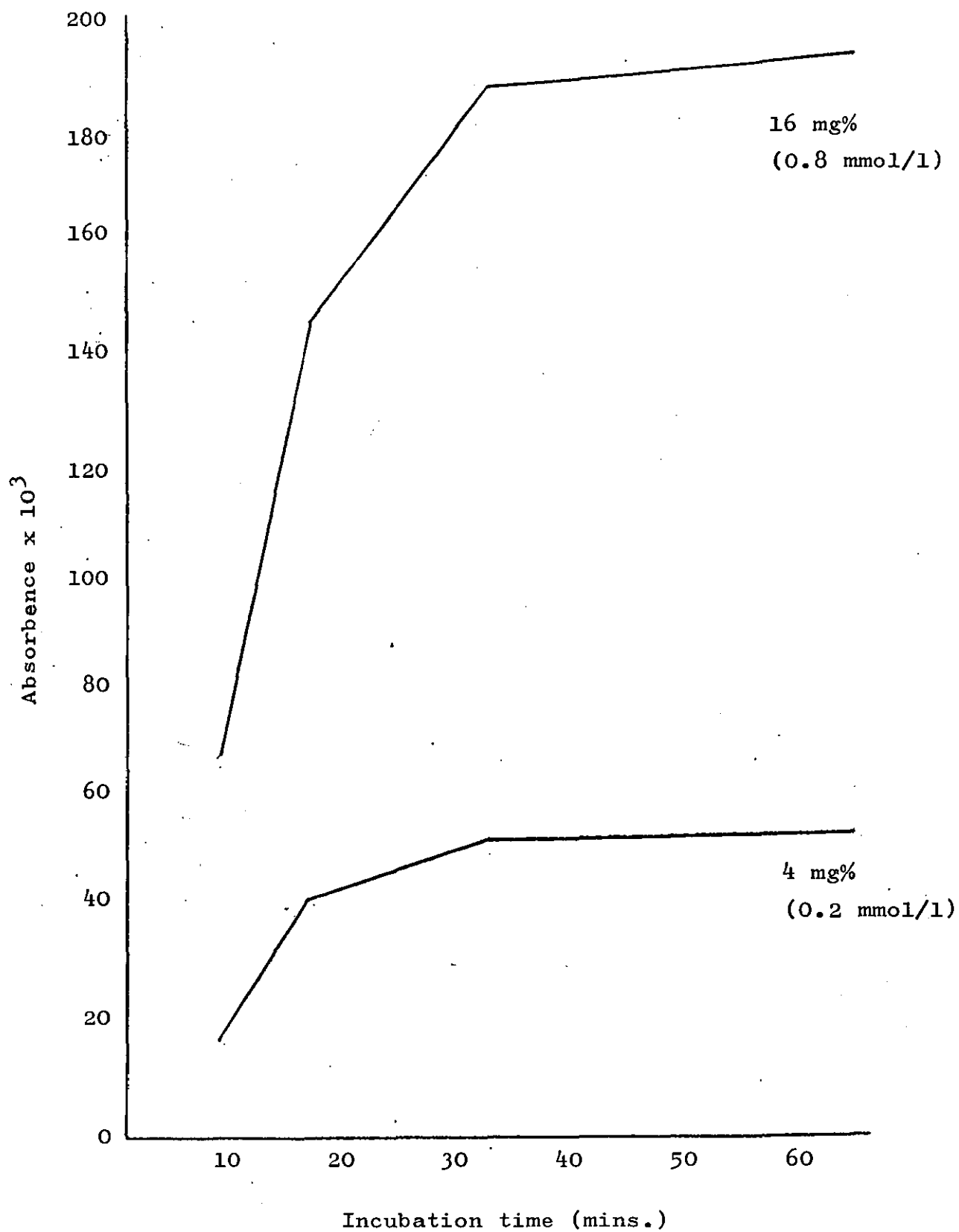
RT produced sufficient colour development for the spectrophotometric detection of low fructose concentrations. However, for concentrations below 2 mg% a greater colour development would be desirable. Test-re-test reliability study provided excellent results, the standard error of measurement SMES being 0.0006 absorbance units. The relationship between fructose concentration and absorbance demonstrated a significant linear trend ($r = 1.00$).

Stage 3.

Fructose standards corresponding to 4 (0.2) and 16 mg% (0.8 mmol/l) were run through RT using, for separate paired samples, incubation times of 8, 16, 32, and 64 minutes. The aim of stage 3 was to investigate the possibility of improving the colour development by increasing incubation time. Results are illustrated in figure 3.2.1.

Colour intensity was found to increase with incubation time and approached a maximum value at approximately 35 minutes. An incubation time of 35 minutes was subsequently used for fructose determinations. This is a modification on RT which suggests an 8 minute incubation time.

Figure 3.2.1. EFFECT OF INCUBATION TIME ON
COLOUR DEVELOPMENT.



Stage 4.

Standards corresponding to fructose concentrations of 4 (0.2) and 16 mg% (0.8 mmol/l) were run through RT using, for separate sample pairs, hydrochloric acid concentrations of 25.0, 30.0 and 35.4%. The purpose of this stage was to determine whether or not colour development could be improved by varying the concentration of hydrochloric acid. Results are illustrated in Figure 3.2.2.

The maximum colour development occurred for both fructose concentrations at a hydrochloric acid concentration of 30%. Thus the strength of hydrochloric acid recommended by Roe (1934) results in greatest colour development in comparison to other concentrations examined.

Stage 5.

Two series of fructose concentrations consisting of 2 (0.1), 4 (0.2), 8 (0.4) and 16 mg% (0.8 mmol/l) were run through RT, one series with the addition of glucose to yield a concentration of 100 mg% glucose (5.6 mmol/l), the other series without glucose addition. The purpose of this stage was to observe the extent of glucose interference with colour development. Results are illustrated in figure 3.2.3.

The presence of glucose at a level of 100 mg% significantly ($t = \pm 355.172$, $df = 3$, $p < 0.001$) increased the absorbance values. Thus results indicate that in the presence of glucose a correction must be applied to determine the precise fructose concentration.

Stage 6.

A series of fructose standards containing 1.0 (0.05), 2.0 (0.1), 4.0 (0.2), 8.0 (0.4), and 16 mg% (0.8 mmol/l) were prepared in glucose concentrations ranging from 50 mg% (2.8 mmol/l) to 150 mg% (8.3 mmol/l) at 25 mg% (1.4 mmol/l) intervals. The complete series was run through RT on four separate occasions. The purpose of this stage was to generate data in order to produce a set of regression equations for the prediction of fructose concentration corrected for the presence of glucose. Interpolation between each successive line for a constant glucose concentration enables the prediction of fructose concentration corrected at 5 mg% (0.3 mmol/l) glucose intervals over the range 50 - 150 mg% (2.8 - 8.3 mmol/l). The equation $F = - \left(\frac{a - A}{b} \right)$ can be used to predict fructose concentration $[F](\text{mg}\%)$ where A = absorbance reading, and a and b take on the appropriate values for the corresponding glucose concentration as illustrated in table 3.2.1.

Figure 3.2.2. EFFECT OF HYDROCHLORIC ACID STRENGTH
ON COLOUR DEVELOPMENT.

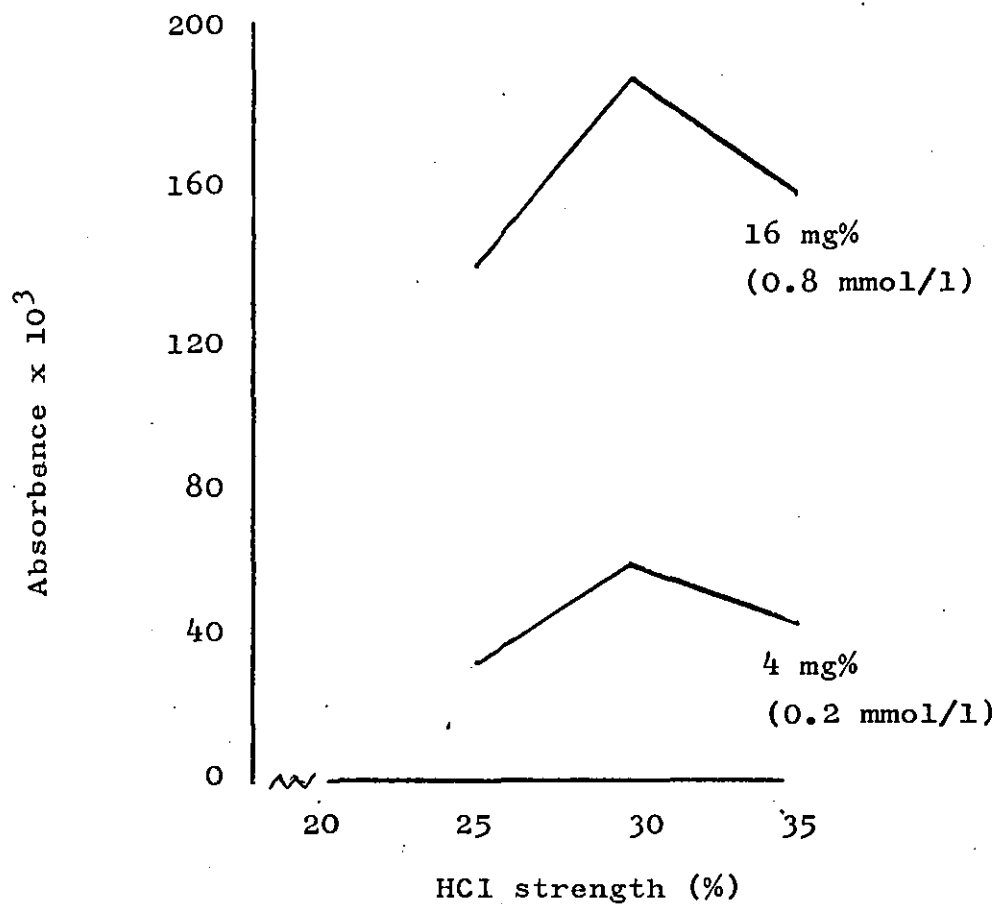


Figure 3.2.3. EFFECT OF GLUCOSE ON COLOUR DEVELOPMENT.

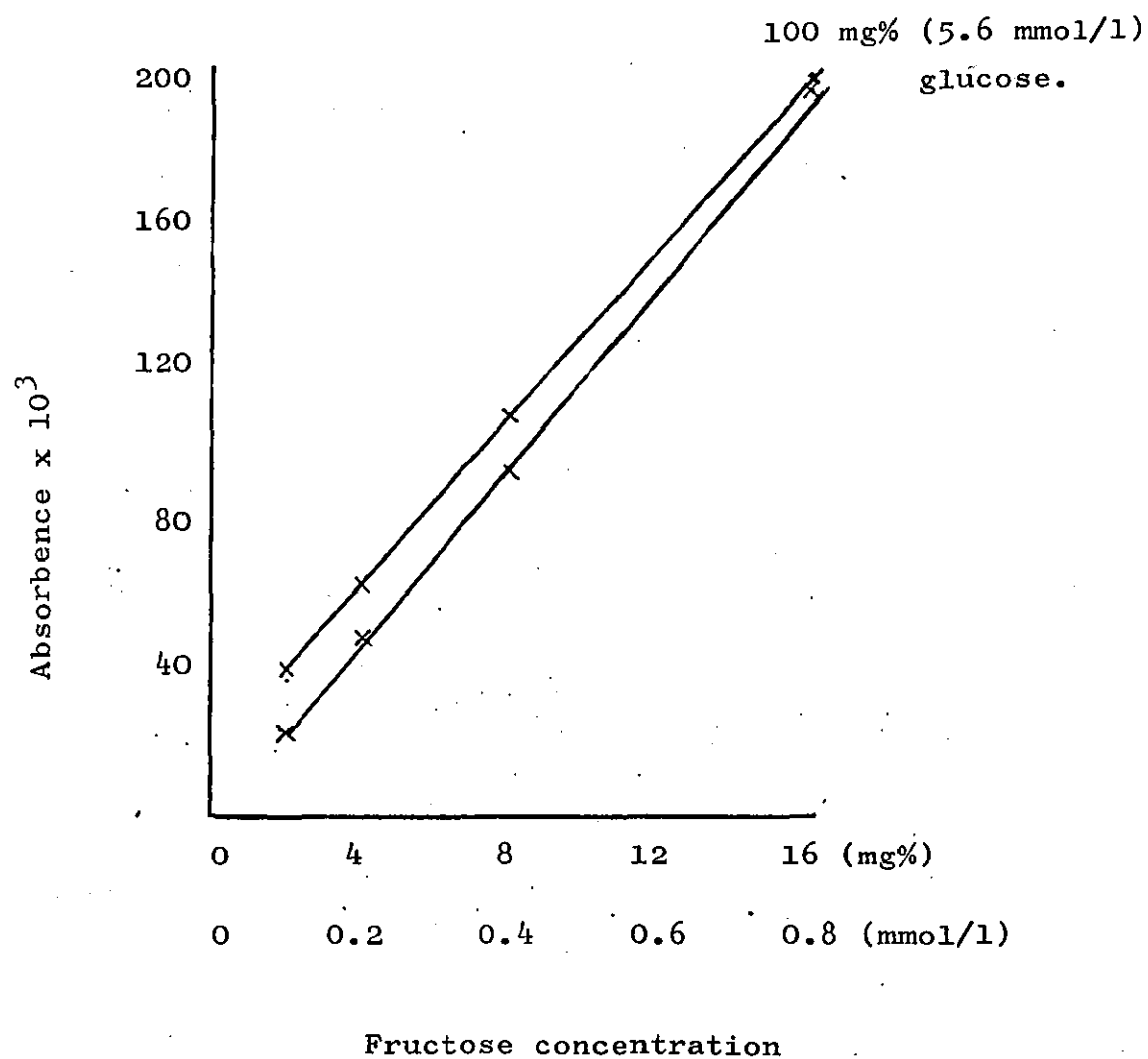


Table 3.2.1. VALUES FOR "a" AND "b" AT 5 mg % GLUCOSE
INTERVALS FOR FRUCTOSE PREDICTION IN THE
PRESENCE OF GLUCOSE.

Glucose concentration		a	b
(mg%)	(mmol/l)		
50	2.8	0.0109	0.0100
		0.0117	0.0099
60	3.3	0.0124	0.0099
		0.0132	0.0099
70	3.9	0.0140	0.0098
		0.0148	0.0097
80	4.4	0.0155	0.0097
		0.0163	0.0097
90	5.0	0.0171	0.0096
		0.0179	0.0096
100	5.6	0.0187	0.0095
		0.0194	0.0095
110	6.1	0.0202	0.0095
		0.0210	0.0094
130	7.2	0.0218	0.0094
		0.0226	0.0093
140	7.8	0.0233	0.0093
		0.0241	0.0093
150	8.3	0.0249	0.0092
		0.0257	0.0092
160	8.9	0.0265	0.0092

Stage 7.

Additions of fructose in 0.2 ml solution were made to 9.8 ml of whole blood to produce a series of increases in fructose concentration of 2 (0.1), 4 (0.2), 8 (0.4), and 16 mg% (0.8 mmol/l). Estimations of fructose concentrations were made before and after fructose additions. Glucose concentration was determined prior to fructose estimations using a glucose oxidase/peroxidase method marketed in kit form by Merck.

The purpose of this stage was to examine the recovery of fructose after addition to whole blood in the presence of glucose. The method described below was used for this stage and for all later blood fructose determinations.

ROE'S TECHNIQUE - MODIFIED.Deproteinisation.

Pipette into centrifuge tubes.

0.5 ml whole blood)	
)	
3.5 ml distilled water)	Mix thoroughly then add

0.5 ml 10% Zinc sulphate.

0.5 ml 0.5N Sodium hydroxide.

Mix then centrifuge at 3000 rpm for 5 minutes. Use 2 ml of the clear supernatant for fructose determination as follows;

Fructose determination.

Pipette into boiling tubes.

	Sample	Blank
Supernatant	2 ml	-
Saturated benzoic acid	-	2 ml
*0.1% alcoholic resorcinol	2 ml	2 ml
30% hydrochloric acid	6 ml	6 ml

(*0.1% resorcinol in ethanol absolute).

Mix well, incubate at $73^{\circ}\text{C} \pm 1$ for 35 minutes, then cool

under running tap water for 5 minutes. Read absorbance at 480 n.m. and calculate fructose concentration using appropriate regression equation.

Results for the series of fructose additions are listed in table 3.2.2.

Table 3.2.2. RECOVERIES OF FRUCTOSE ADDITIONS TO WHOLE BLOOD.

Addition (mg%)	Fructose (mg%)		(%) recovery
	Before	After	
2	0.6	2.4	92
4	0.2	4.3	102
8	0.7	8.6	99
16	0.3	15.9	98

Recoveries of fructose added to whole blood determined by a modified RT after correction for glucose concentration were excellent. Thus the modifications have achieved a reliable technique for the estimation of blood fructose concentration in the presence of glucose.

3.2.1.2. BLOOD COAGULATION TIME.

Coagulation time was estimated using the method of Lee and White (1966). This procedure requires strict

standardisation and was carried out as follows:

Estimations were carried out on 1 ml quantities of blood added to pre-heated (37°C) glass tubes, 8 mm x 50 mm. In order for this volume to be measured accurately the glass tubes were firstly scored with a file to indicate this level. Before use, on every occasion, the sample tubes were thoroughly washed in sulphuric acid, rinsed with distilled water and then dried. Dry clean tubes were placed in a water bath at 37°C for at least 30 minutes to warm before a determination was conducted. Tubes were held in a container that allowed manual tilting and in addition prevented movement due to the water bath agitator.

Blood was drawn by venepuncture into a 10 ml syringe and the timing of coagulation begun as soon as blood entered the syringe. This was achieved by subject cooperation, the subject starting a stop watch on instruction. 1 ml samples of blood were then added to the warmed tubes. Blood was always delivered down the centre of the tubes and not along the tube wall.

At 90 seconds after blood had entered the syringe and at 30 seconds intervals thereafter until coagulation had occurred the tubes were tilted gently to approximately 45° . This tilt angle enabled a slight flow of unclotted blood to occur. Once this flow had been observed the

tubes were returned to the upright position. The end-point is easily recognised when the blood surface remains at right angles to the tube wall and ceases to flow.

Following end-point recognition in any sample the tube was removed from the container and tilted gently through $90-135^{\circ}$ to confirm coagulation. Blood flow at this stage indicated an erroneous determination of coagulation time which resulted in the discarding of that particular sample time. A mean value of all correctly determined blood coagulation times was calculated.

Problems were occasionally encountered when blood failed to flow smoothly in the tubes during tilting the blood creating a large bulb-like meniscus. Coagulation times were not estimated from tubes in which this occurred. A short study was conducted to determine the reliability of this technique.

Seven subjects attended the test station on one occasion, after an overnight fast, where two estimations of blood coagulation time were performed as detailed above. One estimation (based on the value derived from four tubes) was performed after the subject had been resting for 1 hour and the second determination after 2 hours rest. An assumption was made that blood coagulation time would not vary during this period and that observed variations would be due to the technique. Results are illustrated in Table 3.2.3. The standard error of measurement was

calculated from this data and found to be 0.29 minutes.

Table 3.2.3. BLOOD COAGULATION TIMES FOR RELIABILITY
STUDY.

Mean Coagulation Times			
Mins.			
		1 hour	2 hours
Subject	1.	5.9	6.0
	2.	6.2	6.4
	3.	5.8	6.1
	4.	5.4	6.4
	5.	6.4	6.0
	6.	7.0	7.1
	7.	6.5	6.3

3.2.2. KIT ESTIMATIONS.

The detailed instructions for all methods are listed in the appendix.

All kits were tested for reliability by taking duplicate samples through the technique on two occasions.

Samples to provide standards for this purpose were prepared from, freeze-dried serum, as manufactured stabilized series of concentrations, or made up in the laboratory in a medium specified by the kit instructions. Table 3.2.4. contains the results of these reliability studies, references for the specific techniques used, and the medium in which the standards were prepared.

Table 3.2.4. RELIABILITY AND KIT TECHNIQUE DATA.

<u>Measurement</u>	<u>Standard Medium</u>	<u>Method</u>	<u>SMES</u>
Glucose	Freeze-dried serum	Trinder (1969)	2 mg%
NEFA	Stearic acid in chloroform	Duncombe (1964)	0.01mEq/l
β .lipoproteins	Freeze-dried serum	Burstein and Samaille (1958), Watson (1960)	5 mg%
Urate	Freeze-dried serum	Kageyama (1971)	0.04 mg%

Triglycerides	Freeze-dried serum	Eggstein 1.9 mg% (1966) Eggstein and Kreutz Schmidt (1968)
Cholesterol esters	cholesterol in stabilised solution	Seidel et al 1.8 mg% (1981) Stahler et al (1977) Trinder (1969)
Insulin	Freeze-dried serum	RIA Amersham 2.9 Iu/l
Cortisol	Freeze-dried Serum	RIA Amersham 0.5 µg /100 ml
Thyroxine (T ₄)	Freeze-dried serum	RIA Amersham 0.6 µg /100 ml

3.3 ABSORPTION PROFILES OF FRUCTOSE AND GLUCOSE IN YOUNG ADULTS.

Basically this section examines blood absorption profiles of orally administered fructose and glucose in young healthy adults. This study provides precise comparative information (between sugars and sexes) on the initial physiological differences promoted by these sugars and permits the recognition of the "driving force" behind future metabolic events. The reader is reminded that the central theme of this thesis concerns dietary change and its effect on the internal environment with special reference to affluent disease. This is an important point to consider when examining the author's interpretation of this study.

Before the experimental treatment of absorption profiles a section has been included which examines the basic factors involved with the production of a blood sugar profile.

3.3.1. MAJOR DETERMINANTS OF BLOOD SUGAR ABSORPTION PROFILES.

The blood profile of any orally administered sugar is primarily determined by the following: delivery of the sugar to the sites of absorption, the mode of transport at absorption sites, and the physiological events that determine the time course of the removal of the sugar from circulation.

The large majority of an oral fructose and glucose load is absorbed in the distal duodenum and jejunum (Borgstrom, Dahlquist, Lundh and Sjöval, 1957; Cook, 1969), where the rate of absorption is proportional to the amount of monosaccharide present (Holdsworth and Dawson, 1964). Delivery to these sites of absorption is dependent upon the rate of gastric emptying, an event that clearly influences the resultant blood sugar profile.

Gastric emptying is primarily governed by the volume of food delivered to the stomach and the effect this has on the various nerve systems present (Hally, Lloyd and Scratcherd, 1976). Basically the larger the volume of food present in the stomach the greater is the excitability of the stomach musculature. Additional mechanisms present in the duodenum, sensitive to acidity, lipid concentration, and osmolarity exert an effect on the rate of gastric emptying. As lipid concentration, acidity and osmolarity increase a retarding effect on gastric emptying is initiated via these receptors (Hunt and Knox, 1968).

At the site of absorption, the small intestine, fructose and glucose are transported through the epithelial cells (Csáky, 1964), a significant barrier to water soluble molecules, to the bloodstream. Different specialised mechanisms for fructose and glucose operate to facilitate this transport.

In common both systems involve a carrier that is associated with the epithelial cells that is, capable of recognising the luminal sugar, forming a complex with it, and transporting the sugar through the cell to the bloodstream. In the case of intestinal carriers function is geared for one directional transport only i.e., from intestinal lumen to the bloodstream.

The carrier mechanism involved with intestinal glucose transport is a sodium dependent system and for fructose absorption a sodium independent system (Hopfer, 1975). These systems are termed active-mediated and passive-mediated respectively. Active-mediated absorption of glucose is more rapid than passive-mediated transport of fructose but fructose absorption is relatively efficient and will approach the rate of glucose at higher luminal concentrations (Holdsworth and Dawson, 1965).

The proportion of fructose absorbed metabolically unaltered into the portal system is similar to the quantity of unchanged glucose transferred following glucose loading (Holdsworth and Dawson, 1965). Cook (1969) estimates this proportion to be from 80 to 90%, the intestine accounting for less than 10% of fructose metabolism.

Fructose and glucose are taken up rapidly by the liver (Cahill, Ashmore, Earlo and Zottu, 1958; Steele, 1966), glucose at a faster rate than fructose. Transport from the extracellular compartment continues until the concentration in both compartments are equal (Baur and Heldt, 1977). Although glucose enters the hepatocytes more rapidly fructose continues to be taken up for a longer period. A probable explanation for this observation is a result of the relative rates of metabolism of fructose and glucose, the metabolism of fructose occurring at a greater rate via the fructokinase pathway than glucose via the hexokinase pathway.

The liver is the most important site of fructose metabolism. During a prolonged intravenous fructose infusion over 75 per cent of the total uptake was accounted for by hepatocytes (Mäenpää, Raivio and Kekomäki, 1968; Wolfe and Leuthardt, 1953). In the absence of insulin glucose metabolism in the liver would approach that observed for fructose, the extrahepatic tissues being unable to remove and therefore utilise circulating glucose (Wick, Sherrill and Drury, 1953). In the non-diabetic subject glucose is rapidly taken up by adipose tissue and muscle as well as by the liver. The uptake of fructose in adipose tissue and muscle, and therefore resultant fructose metabolism in these tissues, is very small, (Froech, 1972).

Wick et al (1953) using rabbits with their kidneys and

liver removed observed that fructose metabolism, during fructose infusion, only accounted for about 5 per cent of the total ongoing metabolism. In these studies Wick also demonstrated the slow transference of fructose from extracellular to intracellular compartments even in the presence of insulin. This is an important difference between the sugars fructose and glucose i.e., the action of insulin on their uptake.

Erythrocyte fructose metabolism provides a contribution to the extrahepatic fructose metabolism by the production of lactate which can be readily taken up and metabolised by other tissues (Long and Horsfall, 1932). The kidney also has the potential to metabolise fructose by virtue of the presence of fructokinase. However, this organ, along with the small intestine, is only of secondary importance with respect to fructose metabolism in comparison to the liver.

An additional removal route from circulation of fructose and glucose following loading occurs when the blood concentration of these sugars exceeds their renal threshold. Under such circumstances the sugars will appear in the urine and will represent another factor influencing the blood sugar profile.

Weichselbaum, Elman and Lund (1950) have monitored the urinary losses of fructose and glucose occurring during

their intravenous administration. Of the total quantity of fructose infused, that produced a peak blood fructose concentration of 60 mg% (3.3 mmol/l), 5.8 per cent was recovered from the urine. During the infusion of glucose the corresponding figures were a peak blood level of glucose of 348 mg% (19.3 mmol/l) and a 25 per cent loss of the total infused material recovered in the urine. Although this clearly demonstrates the influence of the "urinary pathway" on the blood sugar profiles the peak blood concentrations of both sugars during their infusion were extremely high. Following oral loading of fructose and glucose such high blood sugar concentrations would not be achieved in typical healthy subjects.

The urinary output of fructose following oral sugar loads has been investigated in two studies by Ranade (1975). In the first study subjects received 50g of fructose after which time collections of urine were made at 30 minute intervals and the concentration of fructose determined. The peak urinary fructose concentration was found to be 14 mg% (0.7 mmol/l), the corresponding blood maximum being 3.6 mg% (0.2 mmol/l). The second study involved the oral administration of 100g of hydrolysed sucrose. The mean quantity of fructose recovered from the urine in the 3 hour period following loading was found to be 54 mg (3.0 mmol), approximately 0.1 per cent of the administered fructose. Thus following an

oral fructose load only a small contribution is made by urinary losses to the removal of fructose from circulation.

In summary, both fructose and glucose blood profiles are basically dependent on three factors; gastric emptying, absorption from the intestinal lumen, and removal from circulation. However, aspects of the precise physiological treatment of fructose and glucose are quite different in these areas. Fructose transport across the intestinal lumen is achieved by passive-mediated and glucose by active-mediated transport. The liver, and adipose tissue and muscle in the presence of insulin are the main tissues responsible for the removal of absorbed glucose. The removal of fructose from circulation is achieved mainly by the liver, the contribution of adipose tissue and muscle being minimal.

The following section involves the investigation of the blood sugar profiles in young adults following oral fructose and glucose. For either sugar the absorption profile represents the interaction of the physiological processes outlined above.

3.3.2. EXPERIMENTAL DESIGN.

Six male and six female subjects took part in this study. All subjects received both an oral fructose and glucose load, a period of at least four weeks separating the two

loadings. Blood concentrations of glucose and fructose together and glucose alone were monitored following fructose and glucose loading respectively. Detailed comparisons were made of the resultant sugar profiles between sexes and sugars.

3.3.2.1. SUBJECT DETAILS.

The twelve subjects that volunteered to participate in this study were all within the age group 19-25 years, non-obese, and apparently in good health. Subjects were provided with a detailed plan of the proposed study which included precise information covering their participation and several relevant questions regarding their health. (A full copy of this subject handout is contained in the appendix.) The purpose of including questions on health was to eliminate potential subjects that might have been at risk as a result of sugar loading or blood sampling.

3.3.2.2. SUBJECT PARTICIPATION.

Subjects were instructed to arrive at the test station in a fasting state, free of food for not less than 12 hours and of alcohol for 24 hours. After a rest period of 30 minutes on a medical couch the following procedure was adopted:

Time	-5 mins.	Fasting blood sample withdrawn.					
	0 "	Sugar load administered.					
	15 "	1st post-load blood sample withdrawn.					
	30 "	2nd	"	"	"	"	"
	60 "	3rd	"	"	"	"	"
	90 "	4th	"	"	"	"	"
	135 "	5th	"	"	"	"	"
	140 "	Subjects free to leave.					

Before leaving the test station a provisional appointment four weeks later was made with each subject for conducting the second part of the study. The procedure followed at the second session was exactly the same as that outlined above except that the other sugar load was administered on this occasion.

3.3.2.3. SUGAR LOADING.

Three male and three female subjects received the fructose load first while the remainder of the group were given the glucose load first. Fructose and glucose loads were prepared as 25 per cent solutions in tap water. Volumes containing 1g sugar/kg BW were administered on each of the two study days. All solutions were freshly prepared on every test day.

Subjects were requested to drink the loads as quickly as possible which was achieved, despite the extremely

sweet nature of the 25 per cent sugar solutions, in less than one minute. A loading of 1g of fructose or glucose/ kg BW provides approximately 10 per cent of the daily recommended energy intake.

3.3.2.4. BLOOD COLLECTION AND ANALYSIS.

All blood samples were taken by the author using venepuncture, the collection sites in all cases being the superficial veins of the cubital fossa. 2.5 ml blood samples were taken at the appropriate times and transferred to fluoride oxalate sample tubes. Samples were stored at $\pm 4^{\circ}\text{C}$ for later analysis which on all occasions took place on the same day as blood collections.

All blood analyses were performed in duplicate by the author, glucose was determined by a glucose-oxidase method and fructose by a modification of Roe's technique. (Both techniques have been previously described in this thesis.) Blood samples taken following fructose loading were analysed for fructose and glucose concentration and after glucose loading for glucose concentration only.

3.3.2.5. STATISTICAL ANALYSIS.

The changes in blood fructose and glucose concentration, and the comparisons of male and female responses following oral sugar loading have been analysed using standard

analysis of variance techniques. The first step of each sugar profile analysis has involved the male and female comparisons. The purpose of initiating each profile analysis with this comparison is to permit pooling of male and female data should a non-significant result occur. The following analyses have been conducted:

- a) Comparison of blood glucose concentrations following oral glucose in males and females.
- b) Blood glucose concentrations following oral glucose in males and females.
- c) Comparison of blood fructose concentrations following oral fructose in males and females.
- d) Blood fructose concentrations following oral fructose in males and females.
- e) Comparison of blood glucose concentrations following oral fructose in males and females.
- f) Blood glucose concentrations following oral fructose in males and females.

Comparisons between male and female absorption curves are concerned with the identification of differences between the sexes of the concentration of a sugar at a specific time e.g., the difference between male and female levels at 0, 15, 30, 60, 90, and 135 minutes.

The examination within a sex, or using pooled data, deals with the differences in the blood concentration with respect to time e.g., the differences in blood sugar levels at 0, 15, 30, 60, 90, and 135 minutes.

Analysis of variance summary tables are contained in the appendix - results section. A statistically significant result has been accepted when $p \leq 0.05$, an F value of significance being indicated by asterisk symbol. For all significant results a detailed comparison of means has been conducted using the Newman-Keuls method (Winer, 1971).

3.3.3. RESULTS.

All fasting blood glucose levels were within the typical limits (54.0 mg% - 90 mg%, 3-5 mmol/l; Lee and Laycock, 1978). No significant differences were shown between the sexes in the blood levels of fructose and glucose at any of the blood collection times. Therefore, in the following discussion the responses noted refer to pooled data i.e., that occurring in both sexes, unless otherwise stated.

Graphs have been prepared (figure 3.3.1) illustrating the changes in blood concentrations of fructose and glucose following oral loading with fructose and glucose, for each subject, the mean values obtained for males and females, and the overall mean values of male and female pooled data. Each graph contains the three profiles investigated; glucose following glucose, fructose following fructose, and glucose following fructose. These profiles are represented by ---,

_____, and ----- lines respectively. Male and female subjects have been numbered 1-12 on figure 3.3.1. These numbers relate directly to the subject numbers recorded in the appendix-results section. The mean profiles for males and females, and the overall mean profile include the standard error of the mean at each measurement time.

The tables containing summary data for each subject (contained in the appendix - results section) include "actual values" and changes from the respective baseline concentrations.

Statistical information, to identify the sampling times when concentrations are significantly different, has been summarised on the pooled male and female graph. The statistical summary notation adopted indicates that the concentrations are significantly different at the sampling times not connected by a line. The order in which sampling times have been ranked (i.e. from left to right) indicate times of decreasing glucose levels. The key to the summary is as follows: G after G = blood glucose concentration after oral glucose, G after F = blood glucose concentration after oral fructose and F after F = blood fructose concentration after oral fructose.

Analysis of variance summary tables and detailed comparison of means are contained in the appendix-results section.

Figure 3.3.1. FRUCTOSE AND GLUCOSE ABSORPTION PROFILES.

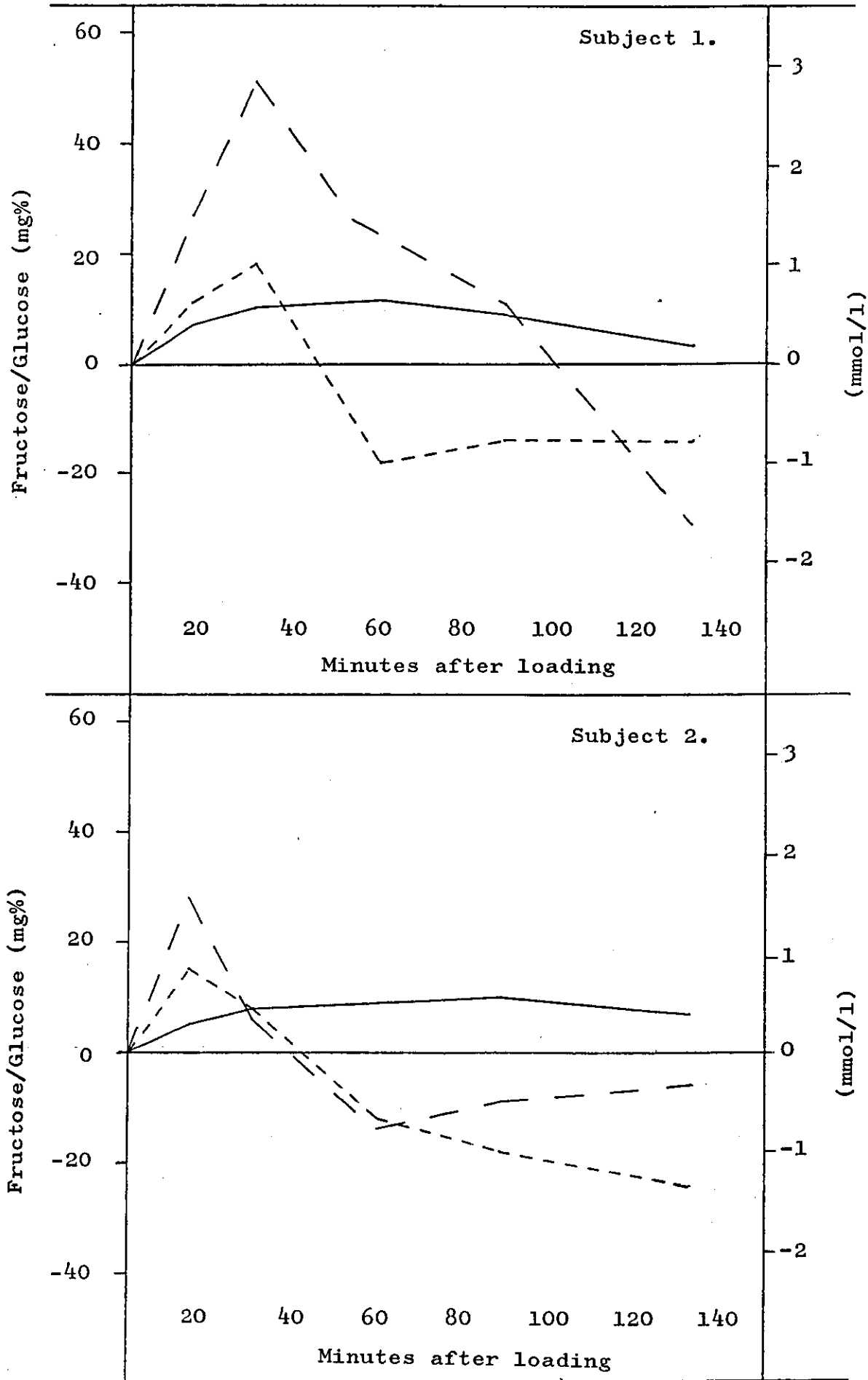


Figure 3.3.1. FRUCTOSE AND GLUCOSE ABSORPTION PROFILES.

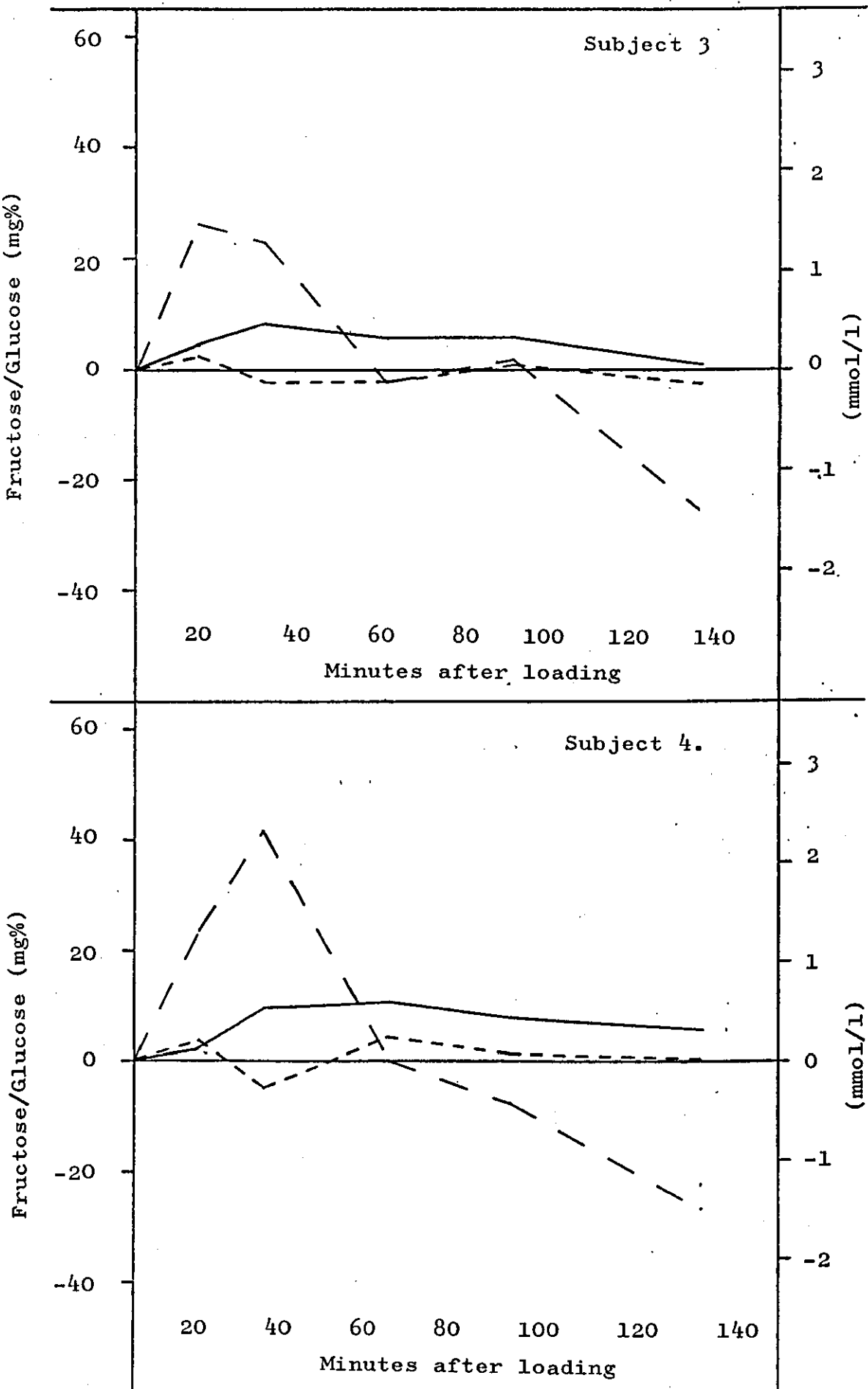


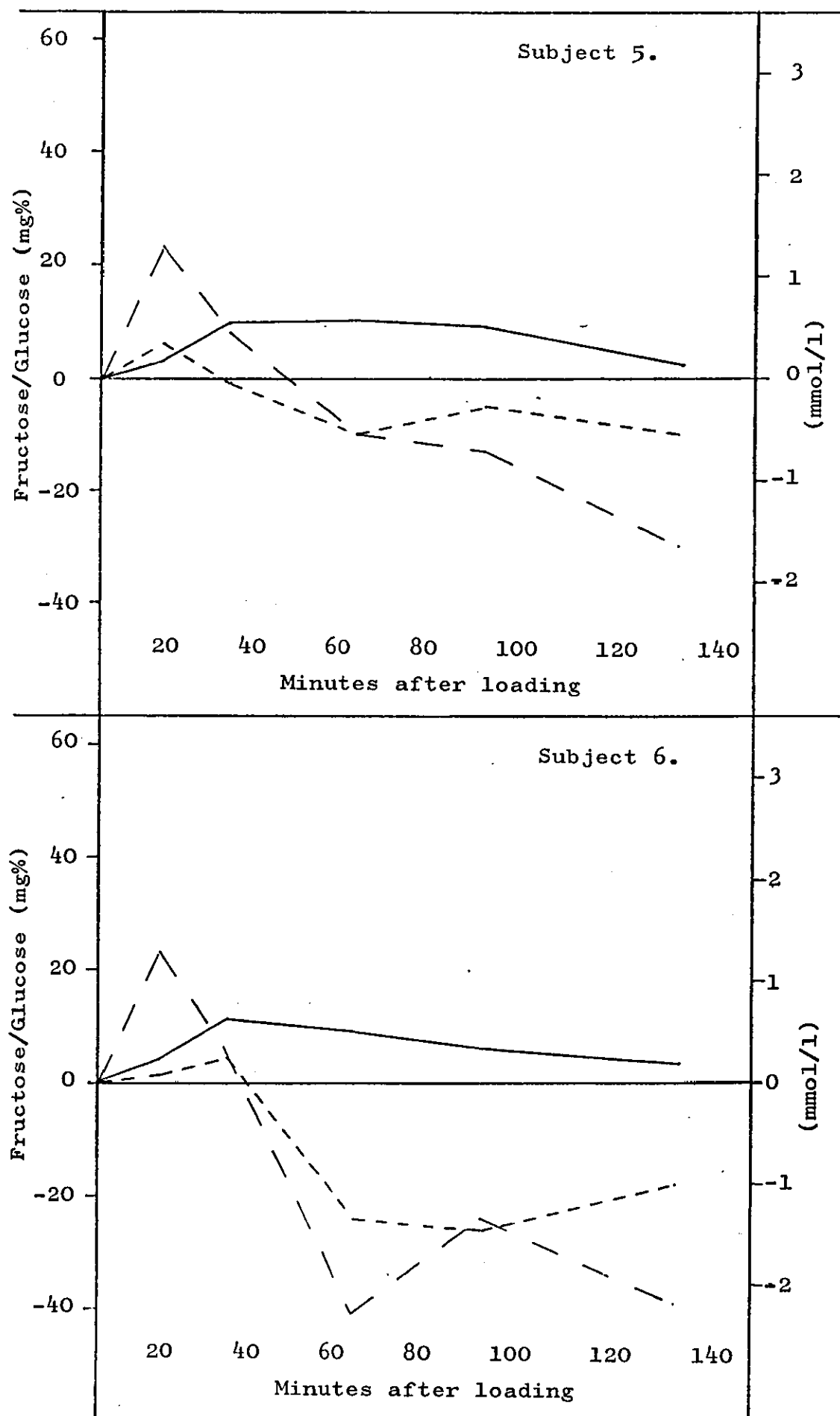
Figure 3.3.1. FRUCTOSE AND GLUCOSE ABSORPTION PROFILES.

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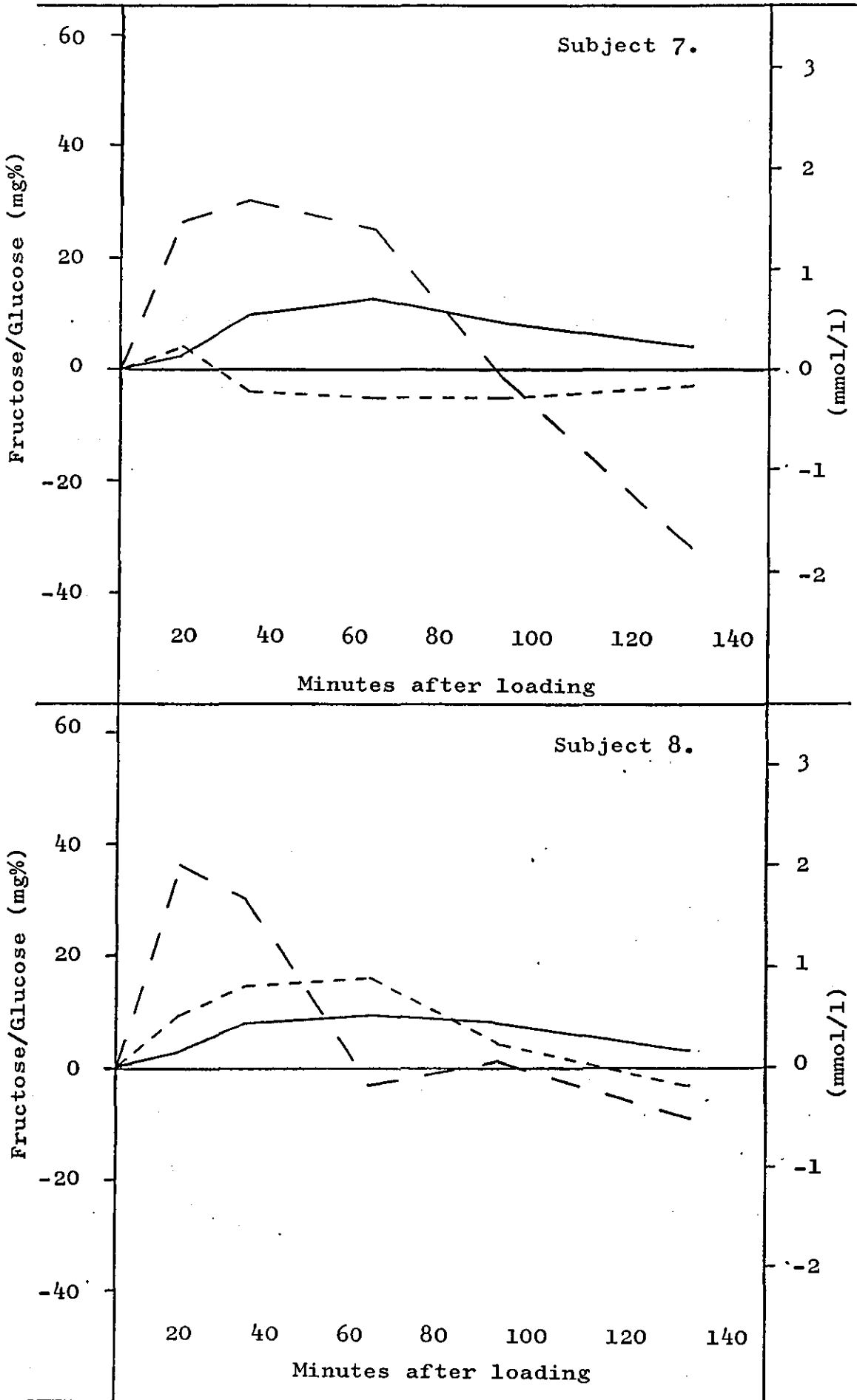


Figure 3.3.1. FRUCTOSE AND GLUCOSE ABSORPTION PROFILES.

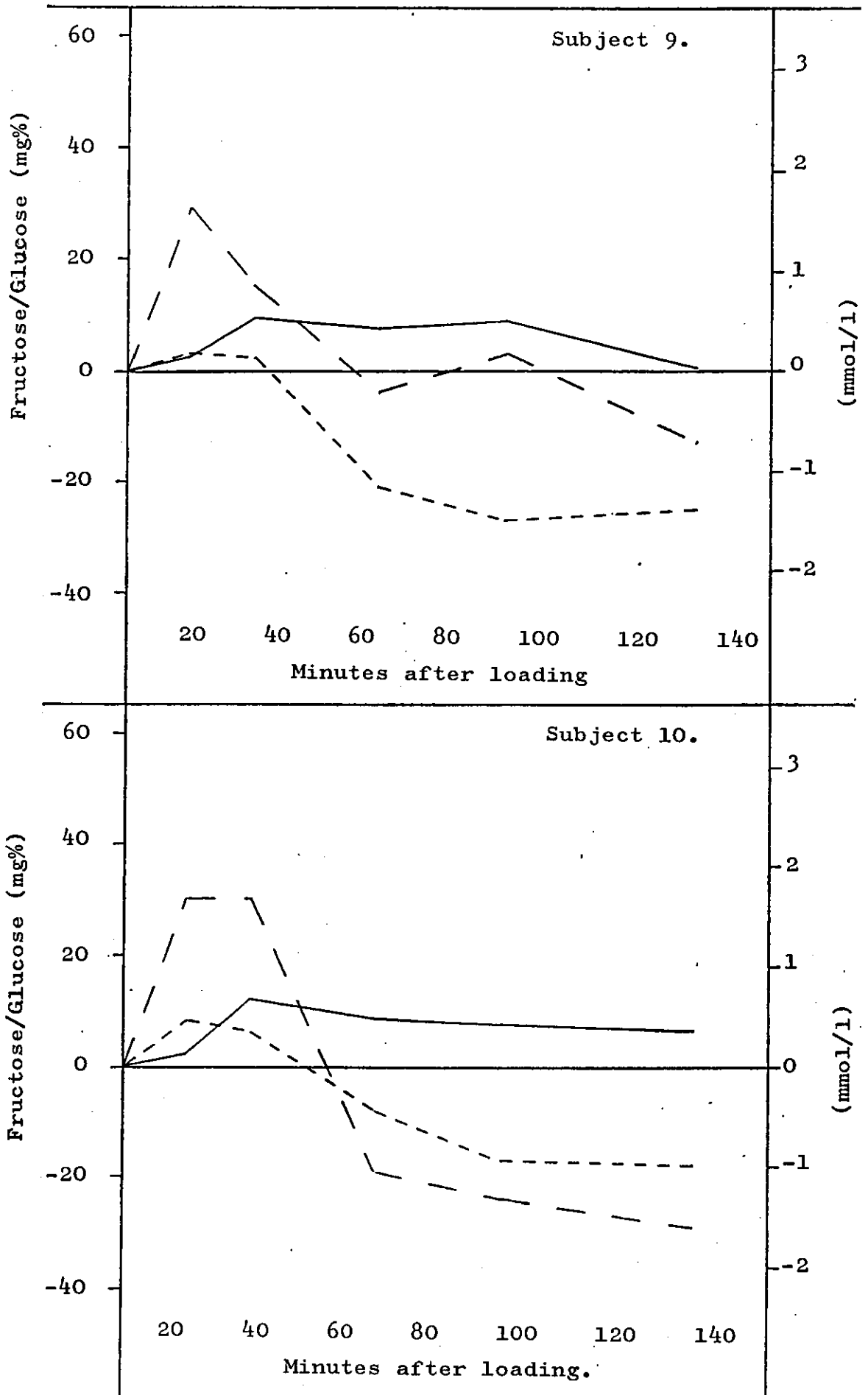


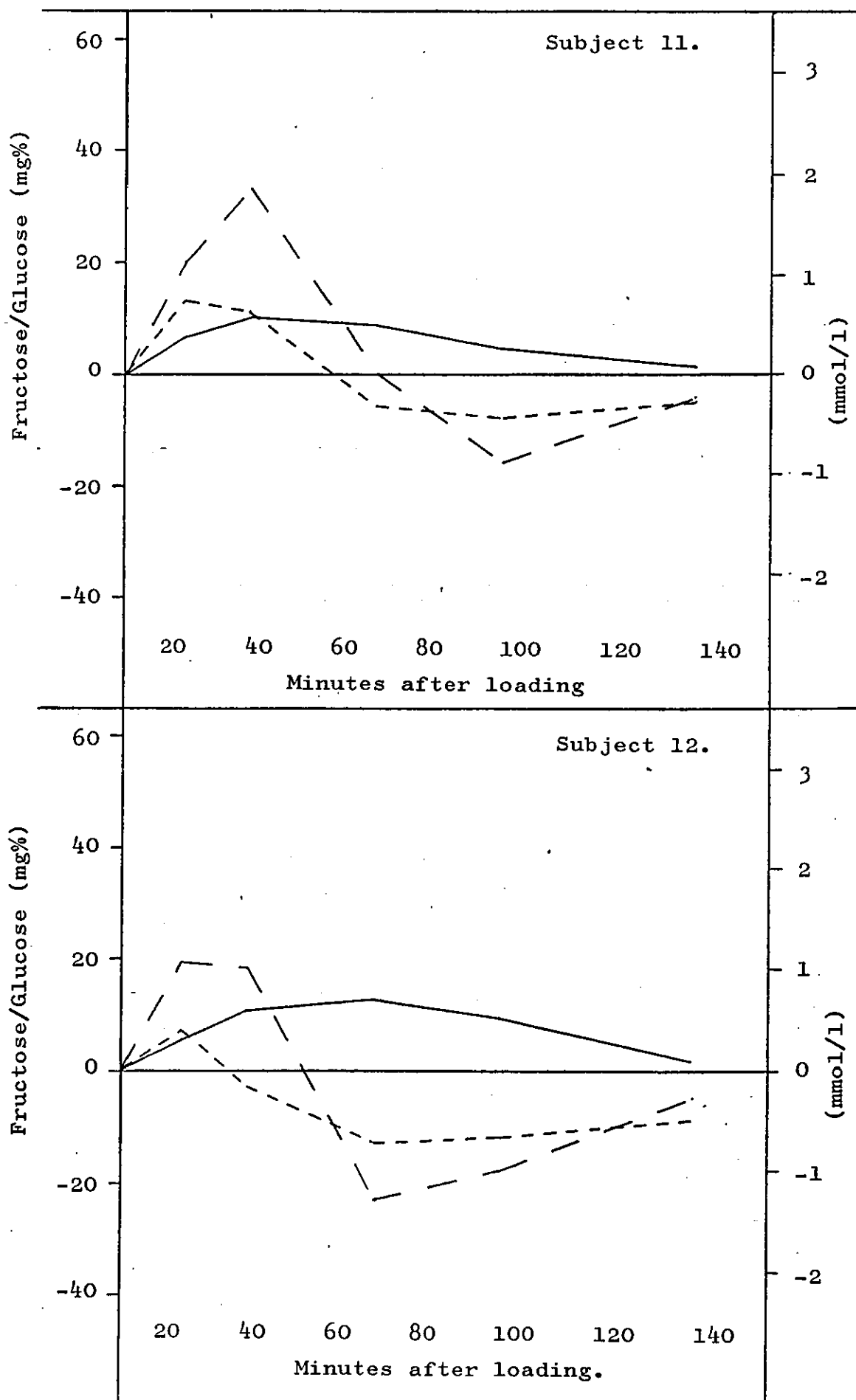
Figure 3.3.1. FRUCTOSE AND GLUCOSE ABSORPTION PROFILES.

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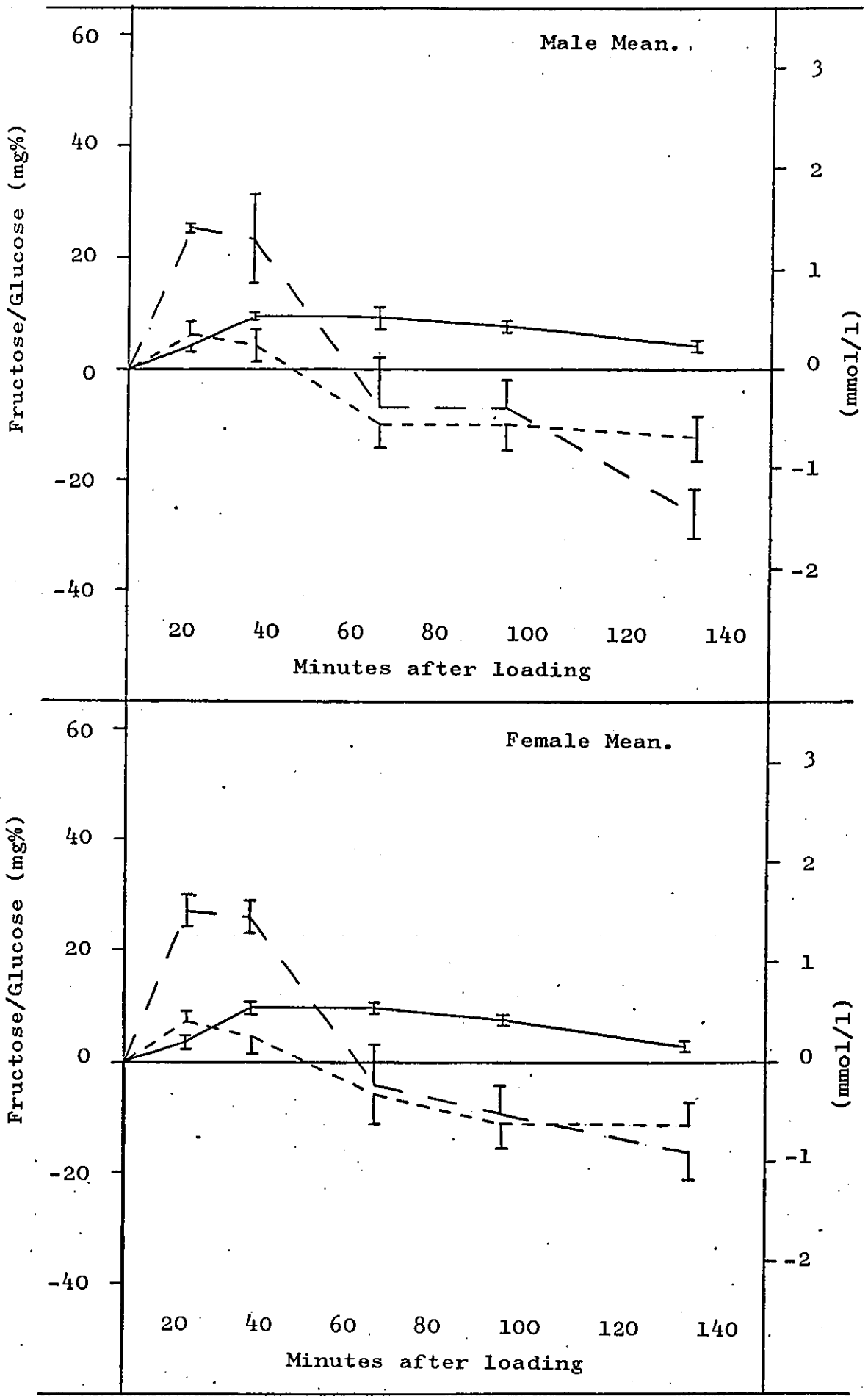
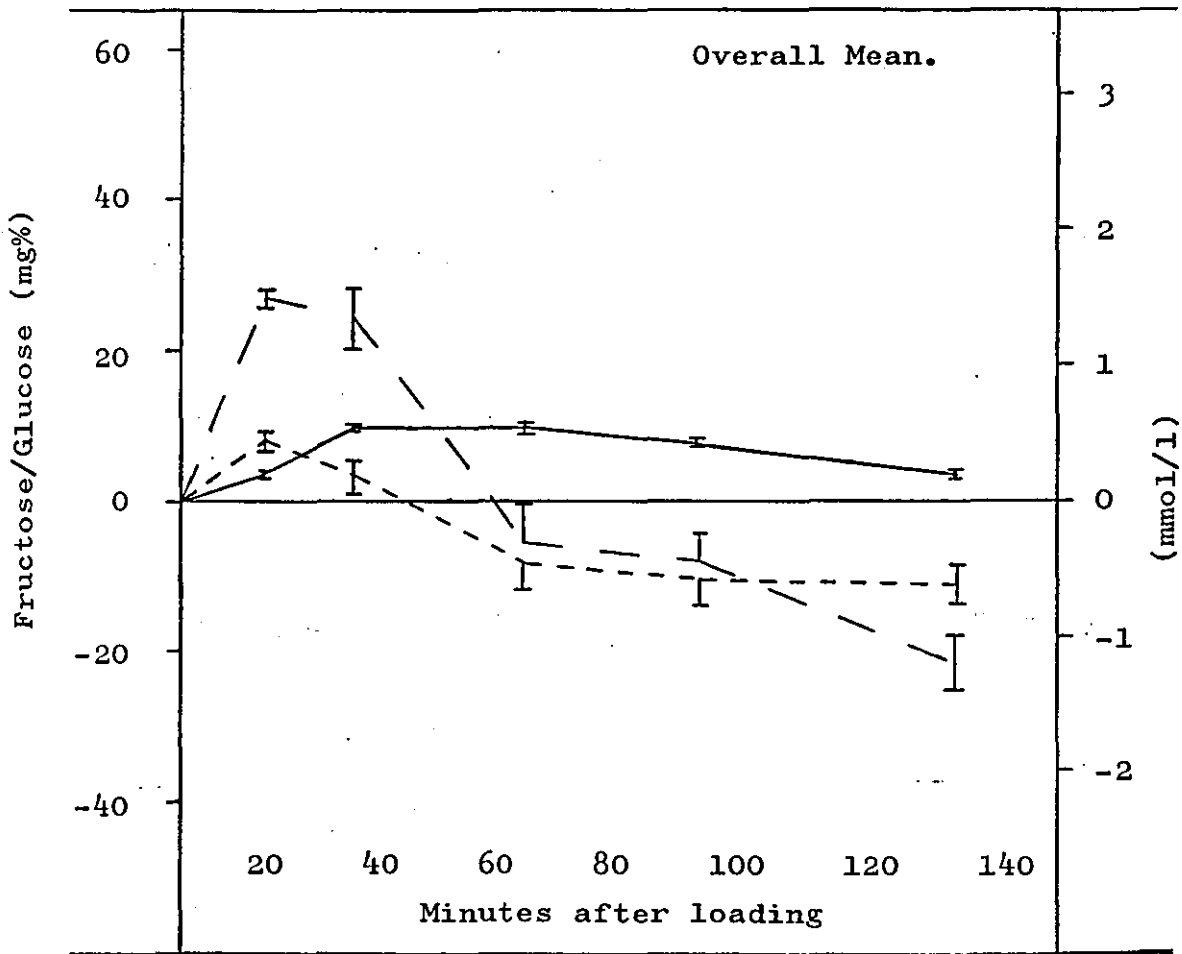


Figure 3.3.1. FRUCTOSE AND GLUCOSE ABSORPTION PROFILES.



Blood sample 15 30 0 60 90 135
G after G

Blood sample 15 30 0 60 90 135
G after F

Blood sample 30 60 90 15 135 0
F after F

3.3.4 DISCUSSION OF RESULTS.

3.3.4.1 BLOOD GLUCOSE RESPONSE TO ORAL GLUCOSE.

Following oral glucose significant changes in blood levels of glucose were observed. At 15 and 30 minutes the concentration of blood glucose was significantly greater than at all other times. No significant difference was shown between the blood glucose levels determined at 0, 60, and 90 minutes, all of which were significantly greater than the blood glucose level at 135 minutes.

The above results illustrate the typical features of the blood glucose response to oral glucose administration in a non-diabetic individual. (Routine tests of this nature are used as an aid in the diagnosis of diabetes mellitus and agromegaly (hypersecretion of somatotrophin)).

The sharp rise in blood glucose following oral administration illustrates the rapid absorption of glucose from the intestinal lumen. The precise position of the peak glucose value varies between subjects and is probably due to the interaction of several factors including subject levels of dietary glucose and the sensitivity of the pancreatic β -cells to a raised glucose concentration.

The rapid rise in blood glucose level is followed by an equally rapid decline that is caused by the action of insulin. The release of insulin from the pancreas is stimulated by a rise in blood glucose concentration. Insulin acts to lower circulating glucose by facilitating the transport of glucose from the blood to the peripheral tissues including the heart, skeletal muscle and adipose tissue. Again variable responses have been observed in the above study and an additional factor in this case, concerning the different rates of glucose removal, would be the varying sensitivities between subjects of the target tissues to insulin.

Similar absorption studies have been conducted by Macdonald, Keyser and Pacy, (1978), Dodds, Fairweather, Miller and Rose (1959), Swan, Davidson and Albrink (1966) and Rosselin, Claude, Eschwege et al (1971), and are useful here for comparative purposes. A part of Macdonald et al's (1978b) study involved the oral administration of 1g/kgBW of glucose to adult male subjects. Blood glucose concentration was determined at 0, 15, 30, 60, and 90 minutes.

In comparison to this study a more marked glucose peak of +48 mg% (2.7 mmol/l) over the base-line value was recorded by Macdonald et al (1978b) which occurred at 30 minutes. The increase over fasting levels persisted

throughout the Macdonald et al's study, blood glucose being elevated by +4 mg% (0.2 mmol/l) at 90 minutes. The greatest discrepancy between the two sets of data is observed at 60 minutes where the subjects used by Macdonald et al (1978b) had markedly elevated glucose levels, +28 mg% (1.6 mmol/l) in comparison to a return to near base-line values in this study. Generally the shape of the glucose profile observed in this study was more "compressed" than that observed by Macdonald et al (1978b) but essentially demonstrates the same key points that have been discussed.

Dodds et al (1959) administered 50 g of glucose to four female and one male subject and monitored the blood glucose response at 0, 14, 50, 60, and 90 minutes. The highest glucose level was at 30 minutes, a value of 35 mg% (1.9 mmol/l) above the base-line concentration. The precise position of the peak occurred between 14 and 30 minutes, the glucose values at these times being 107 (5.9) and 108 mg% (6.0 mmol/l) respectively. These values are in close agreement to those observed in this study although the maximum glucose level recorded by Dodds et al was 10 mg% (0.56 mmol/l) higher.

After 30 minutes both blood glucose responses demonstrate a downward trend although the rate of fall in this study of 0.97 mg%/min (0.05 mmol/l/min) was greater than that recorded by Dodds et al (1959) 0.47 mg%/min (0.03 mmol/l/min). At 90 minutes both sets of data illustrate blood

glucose levels that are below their respective baseline values. For Dodds et al (1959) and for this study the glucose concentrations were -9 mg% (0.5 mmol), and -10 mg% (0.6 mmol/l) down on baseline values respectively.

Essentially both blood glucose concentrations demonstrate a similar pattern, the major difference occurring at 60 minutes where the glucose level remained elevated in the Dodds et al (1959) study and was below baseline values in this study. A similar discrepancy was noted with Macdonald et al (1978b) data.

Part of the study conducted by Swan et al (1966) required young male subjects (n=9) to ingest 100g of glucose after which time blood glucose levels were monitored. These authors followed the response for 8 hours post ingestion, their recordings made at 0, 30, 60, and 120 minutes, being of use here for comparison.

The highest level of blood glucose was recorded at 30 minutes, a value 56 mg% (3.1 mmol/l) above the baseline value. This rise above the base-line glucose is similar to that reported by Macdonald et al (1978b) but approximately double that recorded in this study. At 60 and 120 minutes blood glucose levels determined by Swan et al (1966) remained elevated above base-line values, the increases being 39 mg% (2.2 mmol/l) and 9 mg% (0.5 mmol/l)

respectively. In this study glucose concentrations had returned to near baseline values at 60 minutes and were below this at 90 and 135 minutes.

The study conducted by Rosselin et al (1971) involved the determination of blood glucose concentration, before and two hours after the oral administration of 75g of glucose in 721 male subjects. Although only two "time" measurements are included the large number of subjects examined by Rosselin et al (1972) (unfortunately all males), gives a clear indication of the highly variable response that occurs, following oral glucose. This is an important consideration to be borne in mind when interpreting the differences that have been highlighted above between mean figures.

The blood glucose concentrations recorded by Rosselin et al (1971) before and 2 hours after 75g of oral glucose were 101.4 mg% (5.6 mmol/l), SD=12.2 (0.70), and 101.1 mg% (5.6 mmol/l) SD=33.8 (1.9) respectively. These results on a large sample clearly demonstrate the wide range of responses that exist.

Although differences in peak position, magnitude, and persistence of elevated blood glucose have been described, the findings of this study are in agreement with previously reported work, and thus illustrate typical blood glucose responses to oral glucose. Differences are due to many interacting factors which may include; body composition,

typical dietary sugar intake, typical diet in general, habitual activity, pre-loading hormonal balance, individual variation in gastric emptying, intestinal transport and tissue uptake, and other anatomic variability. In addition the studies compared above, with the exception of Macdonald et al (1978b), had different experimental designs to this study and this would obviously contribute to the differences described.

3.3.4.2. BLOOD FRUCTOSE RESPONSE TO ORAL FRUCTOSE.

Following oral fructose, significant changes in blood levels of fructose occurred. All post loading fructose levels were significantly greater than the fasting value. The levels at 30, 60 and 90 minutes were significantly higher than the concentration recorded at 15 and 135 minutes.

In comparison to the glucose levels following glucose the blood fructose concentration demonstrated a slower rise to a peak value. Taking into account the rise in blood glucose concentration following fructose administration the increase in blood fructose during the first 15 minutes occurred at approximately half the rate of glucose appearance following glucose. This may represent a "true" difference in intestinal absorption rates, a difference in the rate of hepatic removal of fructose and glucose or a combination of these factors.

The peak increase in sugars following fructose, i.e., maximum glucose and fructose increases, was approximately half the maximum increase in glucose concentration following glucose loading. The peak blood fructose level occurred between 30 and 60 minutes in comparison to the blood glucose peak which occurred between 15 and 30 minutes.

An elevation in the level of blood fructose persisted for the entire duration of the trial. In comparison, blood glucose levels following glucose remained elevated for approximately 60 minutes only. As previously mentioned fructose does not stimulate insulin secretion and even in the presence of insulin is not taken up by the peripheral tissues at a faster rate. The most important site of fructose removal is the liver and the shape of the fructose blood profile is therefore an indication of the liver uptake of fructose.

In part of a study conducted by Macdonald et al (1978b) adult male subjects were given oral fructose at a level of 1g/kg BW. The concentration of blood fructose (and glucose) before, and at 15, 30, 60, and 90 minutes after fructose loading was determined.

Essentially the fructose profile recorded by Macdonald et al (1978b) closely resembles the curve determined in this study, although slight differences are in evidence.

The peak value recorded in this study of 9.9 mg% (0.6 mmol/l) was observed at 30 and 60 minutes and in the study of Macdonald et al (1978b) a peak of 9.5 mg% (0.5 mmol/l) was noted at 60 minutes. It is most likely that a "true" peak occurred in both groups of subjects between 30 and 60 minutes.

After 60 minutes the rate of clearance of fructose from the blood occurred at faster rates in the subjects of Macdonald et al (1978b) than in this study, the respective values being 0.09 mg%/min (5 μ mol/l) and 0.06 mg%/min (3 μ mol/l). This fructose clearance discrepancy obviously results in a higher fructose level at 90 minutes in this study than recorded by Macdonald et al (1978b).

Similar methodologies were adopted in this study and that conducted by Macdonald et al (1978b). However, in the former study whole blood was used (preserved immediately in fluoride oxalate) and in the latter serum. The metabolism of red blood cells would cease immediately on addition to fluoride oxalate. In the preparation of serum glycolysis would proceed for a short-time until serum separation and preservation were achieved. Perhaps this accounts for the discrepancies discussed above.

3.3.4.3. BLOOD GLUCOSE RESPONSE TO ORAL FRUCTOSE.

Significant differences were observed in the concentration of blood glucose following fructose administration. No significant differences were shown between the baseline blood glucose concentration and the levels recorded at 15, 30, and 60 minutes. Glucose levels at 15 and 30 minutes were significantly greater than those recorded at 60, 90 and 135 minutes. Baseline glucose was significantly greater than the levels at 90 and 135 minutes.

The glucose profile following oral fructose demonstrates similar features to the blood glucose curve following glucose administration the typical characteristics being a rise to a peak value at 15 minutes and a decline to a concentration below the baseline. However, the extent of the glucose fluctuation is less marked after fructose than after glucose. The increase to the peak glucose and the reduction in blood glucose was approximately one third and one half respectively of the changes observed following glucose loading. The previously cited work of Macdonald et al (1978b) can be used for comparison with this study of the blood glucose concentration changes following oral fructose.

The overall shape of the profile recorded by Macdonald et al (1978b) and in this study are in close agreement and demonstrate the same key features previously discussed.

However, certain slight differences are apparent and will be reported.

The fasting blood glucose concentration was lower in the group of Macdonald et al (68 mg%, 3.8 mmol/l) than for subjects in this study (81 mg%, 4.5 mmol/l).

Although both fasting values are within typical limits this difference may have exerted an effect on the shape of the curves. In both studies a maximum blood glucose concentration occurred after 15 minutes, values for the study of Macdonald et al (1978b) and for this study being 84 (4.7) and 88 mg% (4.9 mmol/l) respectively. Although the subjects of Macdonald et al (1978b) recorded a slightly lower peak glucose concentration, clearly a greater increase in blood glucose occurred.

At 60 and 90 minutes the blood glucose concentrations observed in both studies were below their respective base-line values, although the decrease in blood glucose in this study over the last 60 minutes occurred at a greater rate. The respective decreases in blood glucose over the last 60 minutes for the study of Macdonald et al (1978b) and for this study were 8 (0.4) and 14 mg% (0.8 mmol/l).

3.3.5 GENERAL DISCUSSION.

Due to the different incidence of degenerative disease in males and pre-menopausal females, and their different

internal environments, it is of particular importance to note that the changes in male and female blood sugars following oral loading with fructose and glucose, were not significantly different. The addition of fructose and glucose to the internal environment will stimulate a series of metabolic events that, for males and females, may or may not be equivalent. The blood sugar profiles recorded in this study provide an indication that, initially, the physiological processes are acting in a similar way for males and females.

The absorption profiles of fructose and glucose differ in several respects. Differences range from what can be considered obvious, e.g., blood fructose is elevated following fructose but not after glucose, to the actual nature of the profiles produced by the sugars. All differences are of considerable importance to the development of future studies examining the physiological responses to fructose with emphasis on disease development.

It has been put forward that recent dietary change has resulted in a reduction in the amount of glucose and an increase in the amount of fructose absorbed into circulation. Within the theme of this thesis the simple observation that blood levels of fructose increase following oral fructose and not after glucose is of importance and should not be neglected.

Substantial increases of blood fructose would not occur on diets low in fructose or sucrose. That a blood fructose profile exists at all represents a major change in the internal environment that has occurred only in recent dietary history. On a physiological level a new "raw material" has become abundant, and although an isomer of "blood sugar" glucose, fructose must be dealt with by existing physiological systems that are primarily geared for glucose.

The simple observation of a change in blood fructose levels represents the initial internal environmental change from which future metabolic consequences are governed. It should not be supposed that fructose will be metabolised in a similar way to glucose in a system where structure is of paramount importance.

The differences in the shape of the fructose and glucose curves are an indication of the different interactions of the physiological processes concerned with intestinal absorption and the clearance of the sugars from the blood. Glucose is absorbed from the intestinal lumen and taken up by tissues rapidly. As previously mentioned the main sites of glucose uptake are the liver, and under the influence of insulin, adipose tissue and skeletal muscle. Fructose is absorbed from the intestinal lumen and taken up by the tissues relatively slowly in comparison to glucose. The main site of fructose uptake is the liver,

and as indicated by the fructose profile, removal is slow via this route in comparison to the removal of glucose by the liver, adipose tissue and muscle.

This initial investigation draws attention to the major acute changes in blood sugar levels that occur following fructose and glucose ingestion. Major differences between the internal environments produced by fructose and glucose result after this administration. In addition the distribution of fructose and glucose throughout the body is different as a result of this physiological handling.

Future studies that have been conducted extend from the profiles of fructose and glucose in the blood to an examination of the influence of these changes on particular physiological events. The events that are considered of interest to this thesis are those that reflect a change in the quality of the internal environment with respect to degenerative disease development.

3.3.6 CONCLUSION.

Males and females given oral fructose and glucose loads (1g/kg BW) produced similar glucose profiles following fructose and glucose, and similar fructose profiles following fructose. Glucose profiles following glucose were characterised by a rapid rise to a peak value and

an equally rapid fall to levels below base-line values. Oral fructose resulted in changes in blood glucose levels that corresponded to those observed following glucose although changes were less pronounced. Blood fructose levels following oral fructose were significantly elevated for the entire duration of the test.

In comparison to the blood glucose profile following glucose blood fructose levels following fructose increased at a slower rate to a lower peak value and thereafter declined at a slower rate. Therefore the physiological mechanisms operating to produce the characteristic blood glucose profile following glucose do not function in response to fructose administration. This is an important difference in physiological response between glucose and fructose. This difference probably occurs as the result of the inability of fructose to stimulate insulin secretion and, even in the presence of insulin, to be acted on by the mechanisms that are enhanced by insulin. These mechanisms are geared to transport glucose from the blood to the tissues.

Clearly in view of the dietary substitution of glucose, sources, by fructose, the internal environment changes. The environment is altered, not only in the type of blood sugars present, but in a way that is not characteristic of that produced by additions of the replaced sugar glucose.

3.4. METABOLIC EFFECTS OF FRUCTOSE AND GLUCOSE IN YOUNG ADULTS.

This experimental section covers the examination of the short-term metabolic effects of fructose and glucose additions to the typical diets of young adults. The effects selected for study are those that can be considered metabolic indicators of degenerative disease susceptibility although certain additional measurements have been included. This study takes the form of those discussed in part 2 section 4.3. although there are important differences in experimental design.

Basically studies in part 2 section 4.3 involved the daily administration of large quantities of the sugar under investigation and the precise control of the subjects diet. This study involves the addition of comparatively low levels of fructose and glucose (1g sugar/kg BW) to the unaltered free-choice diets of the participants. The addition of low levels of fructose and glucose to a free-choice diet creates a more realistic dietary change with the minimum of other dietary

interference. Although sugar additions in this study are comparatively lower than in other studies cited previously the diets resulting from the addition of 1g sugar/kg BW should still be considered high in sugar. The addition of sugar in this study is intended to significantly increase the sugar intake of the subjects over base-line levels but not to a degree that would be considered extreme.

In this study the period of dietary sugar increase lasts for seven days after which time the metabolic indicators recorded are compared with their respective base-line values. That metabolic changes occurring during the period of dietary sugar increase can act to influence future metabolic events is examined by the recording of the metabolic indicators seven days after the subjects have returned to their typical free-choice diets.

The aims of this study are to compare the metabolic effects occurring during and after increases in dietary fructose and glucose, in males and females.

Before the experimental design is considered in detail the physiological parameters measured in this study are introduced and explanations for their inclusion discussed. These measurements include the determination of the fasting blood concentration of cholesterol esters, triglycerides, LDL, NEFA, uric acid, and glucose, and the determination of bodyweight and blood coagulation time.

3.4.1 METABOLIC AND PHYSIOLOGICAL PARAMETERS INVESTIGATED.

This section serves to "introduce" the constituents and a property of blood that have been examined. Basically the measurements include biochemical predictors of degenerative disease and indicators of general metabolic disturbance. Detail is restricted to include only information that is of relevance to the considerations of this thesis.

3.4.1.1 BLOOD GLUCOSE.

Blood glucose, like NEFA, represents a source of rapidly available energy and also, the concentration indicates a balance between mobilisation and utilisation by the various tissues of the body. The level of glucose in the blood is critical and is regulated by a hormonal balance that includes the interaction of insulin, glucagon, noradrenalin, adrenalin and cortisol. The principal hormones of glucose regulation can be considered to be insulin and glucagon, although the other hormones listed are also of considerable importance.

The concentration of glucose in fasting blood, and the levels recorded during a glucose tolerance test, are of considerable significance in the diagnosis of diabetes mellitus. Apart from the obvious importance of the detection of diabetes mellitus, this disease itself is associated with the increased likelihood of the develop-

ment of other degenerative diseases.

Diabetes mellitus causes severe disturbances in metabolism that includes a disruption of blood glucose regulation. However, without the presence of frank diabetes mellitus it is possible that for any individual, glucose homeostasis can be disturbed by a dietary regimen in a diabetic direction, and although their glucose and insulin levels remain within typical ranges for the population they are no longer typical for that individual.

One effect of oral glucose and fructose additions is an acute change in blood glucose concentration. Repeated blood glucose disturbances, as created in this study, may result in a longer term disruption of glucose metabolism as reflected in the fasting value. This longer term disruption could result either directly e.g., increased endocrine pancreatic stimulation, or indirectly via a physiological consequence of altered blood fructose and glucose levels.

3.4.1.2. BLOOD NON-ESTERIFIED FATTY ACIDS.

The concentration of NEFA in fasting blood represents the fraction of blood lipid that is available for immediate use as energy. In this case NEFA can be viewed in a similar way to glucose except that NEFA, or degradation products, are not utilised by nervous

tissue unless under conditions of prolonged starvation. The fasting level of NEFA, like glucose, represents the balance between storage and mobilisation from the tissues. The control of the fasting level of NEFA is governed by the interaction of adrenalin, noradrenalin, insulin, glucagon, somatotrophin and thyroxine. Thus under controlled fasting conditions the blood concentration of NEFA reflects the functional balance of the above hormones, an alteration in NEFA levels indicating a change in the hormonal environment. Of particular importance to this study is the relationship between NEFA concentration and insulin, a raised fasting NEFA level indicating insulin lack or a reduction in insulin action (Wahlqvist, Relf, Myers and Lo, 1984).

Rapid infusions of fructose or glucose results in an immediate change in the blood concentration of NEFA (Forster, 1974). Administration of either sugar leads initially to a fall in NEFA levels followed by a slow return (approximately three hours) to fasting values. The typical blood NEFA profiles following fructose and glucose demonstrate a different pattern. Basically following glucose the fall of NEFA levels occurs at a greater rate than after fructose infusion. The elevated levels of insulin that occur as a result of glucose administration and the stimulating effect of insulin on NEFA uptake by adipose tissue could account for this difference.

In this study the effect of raising fructose and glucose levels in the diet over a period of seven days on fasting NEFA levels has been examined. The dietary change that occurs results in the addition of more energy in the form of sugar and demands that the existing physiological mechanisms maintain the balance between energy stores and immediately available energy. A change in NEFA concentration will indicate that the sugar additions have resulted in a disturbance in the control of rapidly available energy in circulation.

3.4.1.3 BLOOD CHOLESTEROL ESTERS, TRIGLYCERIDES AND LOW DENSITY LIPOPROTEINS.

The carriage and importance of blood cholesterol levels, with respect to degenerative disease, have been discussed earlier in this thesis. This study examines the effect of short-term low level increases in dietary fructose and glucose on the fasting blood concentration of cholesterol esters, triglycerides and LDL.

Fasting triglyceride concentrations have been determined as an indicator of the level of their endogenous production and therefore of the levels of VLDL in circulation. The recording of fasting LDL concentrations provides important information concerning the effects of dietary change on cholesterol carriage. LDL is a degradation product of VLDL and can therefore be used to examine the

influence of VLDL changes (if they are detected) on LDL concentrations. The slow degradation of LDL also serves as a guide to pre-existing VLDL synthesis rates; for example, raised VLDL levels that might occur as an immediate effect of sugar intake would not be detected following fasting, however, this acute effect could influence LDL levels, a change that would be detected after fasting.

LDL levels are used to determine total blood cholesterol since approximately 75% of circulating cholesterol is associated with this fraction. However, disturbances in metabolism could result in an elevated LDL concentration without an overall increase in total cholesterol e.g. as a result of cholesterol movement from one fraction to another, or by the more efficient removal of cholesterol from another fraction. To investigate this possible occurrence cholesterol esters have been determined as a predictor of total cholesterol. Cholesterol ester determination will allow the investigation of cholesterol distribution to be conducted. This is an important consideration bearing in mind the different involvement of the various cholesterol fractions in terms of degenerative disease risk.

3.4.1.4 BLOOD URIC ACID.

Uric acid is the final degradation product of the purine

bases adenine and guanine. In the typical pH range of blood uric acid is found mainly in the form of sodium urate. The concentration of blood urate is dependent upon the level of urate formation and the rate of kidney excretion. Blood urate determinations are of importance in the diagnosis of gout, hyperuricaemia being a biochemical lesion of gout. The diagnosis of gout not only permits the commencement of treatment for this disorder but, due to the associations of gout with degenerative disease, indicates an increased risk of degenerative disease development.

An increase in serum urate concentration has been recorded after intravenous and oral fructose loading in children and adults (Perheentupa and Raivio, 1967; Sahebji and Scalettar, 1971; Stirpe, Corte, Bennetti et al, 1970; Forster, Meyer and Ziege, 1970). However, the level of fructose administration required to produce a raised serum urate level appears to be of importance, intravenous infusions below 0.5g fructose/kg BW/hour failing to produce a significant elevation of serum urate (Kogut, Roe, Ng and Donnell, 1975; Heuckenkamp and Zöllner, 1971).

Adults fed diets high in sucrose for six weeks have recorded higher fasting urate concentrations in comparison to a diet equally high in starch (Solyst, Michaelis, Reiser, et al 1980). This study permits a comparison

of the effects of the constituents of sucrose on the fasting blood concentration of urate when these sugars are added to the diet at relatively low levels. It is interesting to note that glucose loading unlike fructose does not, in acute-response studies, result in an elevation of blood urate levels (Forster et al, 1970).

3.4.1.5 BLOOD COAGULATION.

The relevance of blood coagulation to this thesis lies in the finding that coronary infarcts are in most cases caused by thrombosis in atherosclerotic coronary arteries. Thus the increased coaguability of blood can be viewed as a shift to an internal environment that increases the likelihood of coronary thrombosis.

In a study conducted by Szanto and Yudkin (1969) subjects fed a diet high in sucrose, that developed hyperinsulinism, also demonstrated an increase in the adhesiveness of their platelets. These susceptible individuals demonstrated adverse physiological responses with respect to the likelihood of degenerative disease development and its precipitation. Thrombogenesis is disturbed in diabetic individuals, a higher level of fibrinogen and reduced fibrinolysis being recorded (Ganda, 1980). In addition a recent study (Meade, Brozovic, Chakrabarti et al, 1986) has shown that

high levels of factor VII coagulant activity and of plasma fibrinogen are associated with an increased risk of a first major CHD event. The association between the occurrence of the first major CHD event was found to be greater for a raised fibrinogen level than blood cholesterol elevation.

3.4.2 EXPERIMENTAL DESIGN.

Young adults participated in this study designed to investigate the effects of a short-term increase of either fructose or glucose (1g/kg BW/day for 7 days) on the fasting blood concentration of cholesterol esters, triglycerides, LDL, NEFA, glucose, and uric acid and on blood coagulation. Blood determinations were conducted for the base-line, after the period of raised sugar intake, and seven days after the subjects had returned to their typical diets. Comparisons between base-line values, sugar effects, and sexes have been conducted.

3.4.2.1 SUBJECT DETAILS.

Sixteen male and twelve female subjects completed this study. All subjects were within the age range 19-25 years, non-obese and apparently in good health. Subjects were provided with full details of the study (see appendix) and the extent of their required cooperation

was emphasised. Individuals that might have been at risk, had they taken part, were detected and then excluded from the study, by adding relevant questions regarding their health in exactly the same manner as in the absorption study.

3.4.2.2 SUBJECT PARTICIPATION.

Volunteers received either an increase in dietary fructose or glucose (1g/kg BW per day for 7 days) and were divided into the following groups:

Group 1. Male, fructose (10 subjects).

Group 2. Male, glucose (6 subjects).

Group 3. Female, fructose (6 subjects).

Group 4. Female, glucose (6 subjects).

The entire study lasted for 22 days, the schedule followed by each subject over this period is detailed below:

Day 1-7 (Week 1) Pre-sugar increase period. Recording of typical dietary sugar levels.

Day 8-14 (Week 2) Sugar increase period. Recording of dietary sugar levels. Day 8 first bodyweight determination and blood collection.

Day 15-22 (Week 3) Post-sugar increase period. Day 15-21 recording of dietary sugar levels. Day 15 and 22, second and third bodyweight determination and blood collection respectively.

On days of bodyweight determination and blood collection (days 8, 15, and 22) subjects were instructed to arrive at the test station in a fasting state, free of food for not less than 12 hours and of alcohol for 24 hours. After bodyweight measurement subjects rested on a medical couch for 30 minutes before blood sampling. For each subject all blood samples were drawn at exactly the same time on each occasion (9.30 am to 10 am in all cases).

3.4.2.3 TYPICAL DIETS AND INCREASED SUGAR ALLOWANCE.

Subjects were instructed to maintain their typical diets and lifestyle as precisely as possible throughout the study. During the period of increased sugar intake the "background" diet may have been altered in order to preserve a constant bodyweight. Alternatively, an increased caloric consumption would have occurred resulting in either an increase in bodyweight or a compensating physiological response geared to maintain bodyweight. Recording of dietary "free" sugar was achieved by the completion of forms provided that listed foods with high or moderate levels of sugar.

Subjects were required to complete the forms by indicating type of food and quantity consumed. A blank "free-sugar" form is illustrated in the appendix. (The investigation of changes that occur in a free-choice diet during periods of increased sugar intake is the subject of a later study).

3.4.2.4 STATISTICAL ANALYSIS.

All primary statistical comparisons have been achieved by the use of standard analysis of variance techniques. Statistical significance has been accepted where $p \leq 0.05$ and is denoted in summary tables by an asterisk symbol. Where significant differences have occurred a detailed comparison of means, using the Newman-Keuls (from Winer, 1971) method, has been conducted. The following statistical comparisons have been conducted.

- a) Between base-line (day 8) determinations.
- b) Within group 1 between "free-sugar" levels recorded during weeks 1, 2, and 3.
- c) Within group 2 between "free-sugar" levels recorded during weeks 1, 2, and 3.
- d) Within group 3 between "free-sugar" levels recorded during weeks 1, 2, and 3.
- e) Within group 4 between "free-sugar" levels recorded during weeks 1, 2, and 3.
- f) Within group 1 between determinations of day 8, 15, and 22.
- g) " " 2 " " " " " " "
- h) " " 3 " " " " " " "

i) Within group 4 between determinations of day 8, 15, and 22.

Data for within group analyses has been transformed for each subject and for each variable by summing the determinations on days 8, 15, and 22 and calculating the percentage contribution of each day value to this total, e.g. for subject 1 blood triglycerides;
 day 8 = 94mg% (1.06 mmol/l), day 15 = 136 mg% (1.54 mmol/l), day 22 = 89 mg% (1.01 mmol/l).
 % contribution day 8 = 29.47%, day 15 = 42.63%,
 day 22 = 27.90%.

All statistical summary data are contained in the appendix.

The appropriate quantity of fructose or glucose (1g/kg BW) was provided in seven sealed plastic bags for the period of increased sugar intake. Subjects were requested to dissolve the contents of one package on each day of the sugar increase period in warm water and ingest the solution within five minutes, before 10 am.

3.4.2.5 BLOOD COLLECTION AND ANALYSIS.

All blood samples were collected by venepuncture from the superficial veins of the cubital fossa. On each occasion 12 mls of blood were withdrawn and the following analyses conducted:

On whole blood - coagulation time, glucose (fluoride oxalate preservation).

On serum-cholesterol esters, triglycerides, LDL, NEFA, and uric acid.

All blood collections and analyses were conducted by the author. With the exception of blood coagulation time, duplicate determinations were performed. For coagulation time quadruplicate estimates were obtained. Techniques employed for the above analyses have been discussed previously (Section 3.2).

3.4.3. RESULTS.

Tables containing data summaries and transformation, statistical analyses and detailed comparison of means are listed in the appendix. Figure 3.4.1 and figure 3.4.2. have been included in the results section to illustrate and summarise the results. Figure 3.4.1 contains information concerning dietary sugar intake over the trial periods and figure 3.4.2 the metabolic effects that were observed. Both figures illustrate the mean value \pm SEM. For the metabolic effects figure, week 1, 2, and 3 correspond to blood samples drawn on days 8, 15, and 22 respectively.

Statistical summaries are included on all figures. Weeks not connected by a line illustrate determinations which are significantly different in terms of metabolic effect, or in the case of figure 3.4.1, dietary sugar intake. On the statistical summaries data are ranked

highest value on the left hand side of the page.

The concentration of all the blood constituents examined in fasting blood and blood coagulation time were within the typical range for the techniques employed. Comparison of the baseline data between groups demonstrated significant differences in bodyweight and uric acid concentration. No significant differences were shown between the bodyweights of group 1 and 2 both of which were significantly greater than those recorded for groups 3 and 4. This is an expected finding.

No significant differences were shown between the baseline concentrations of uric acid in groups 1 and 2 both of which were significantly greater than the fasting levels recorded for groups 3 and 4. This data is in agreement with the findings of Acheson (1969) and is thought to be due to oestrogenic activity of the female. In addition it is possible that a greater level of protein synthesis, that would occur for bodyweight maintenance in the male, might result in a higher level of purine turnover and subsequently uric acid formation.

Significant differences were recorded between the levels of sugars ingested during the various stages of the study. In all cases no significant differences were observed in the quantities of sugar ingested before or after the

sugar increase period, both stages recording significantly lower levels of sugar intake than during the period of increased sugar intake.

Figure 3.4.1 DIETARY SUGAR INTAKE-MALE DATA.

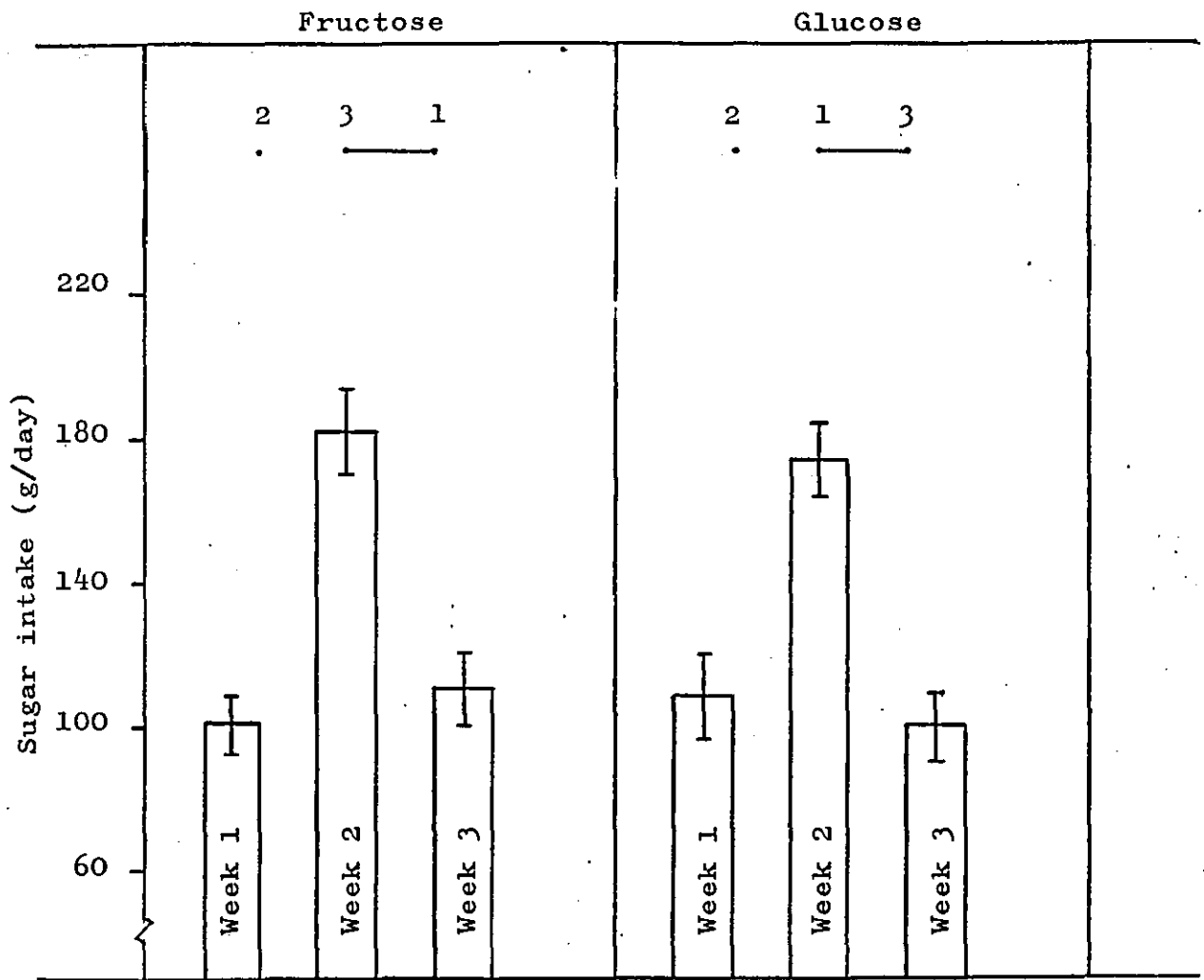


Figure 3.4.1 DIETARY SUGAR INTAKE-FEMALE DATA.

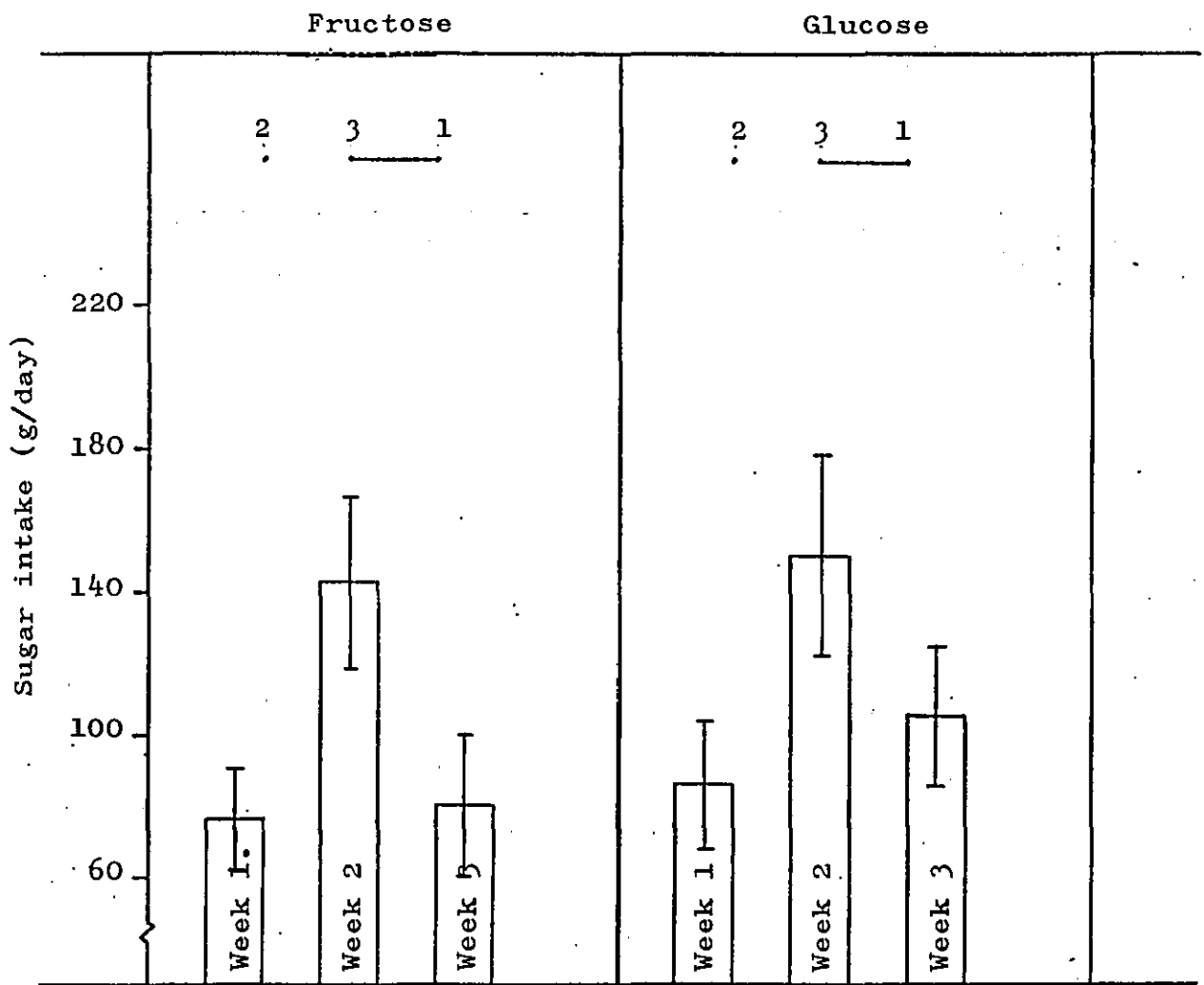


Figure 3.4.2 METABOLIC EFFECTS - MALE DATA.

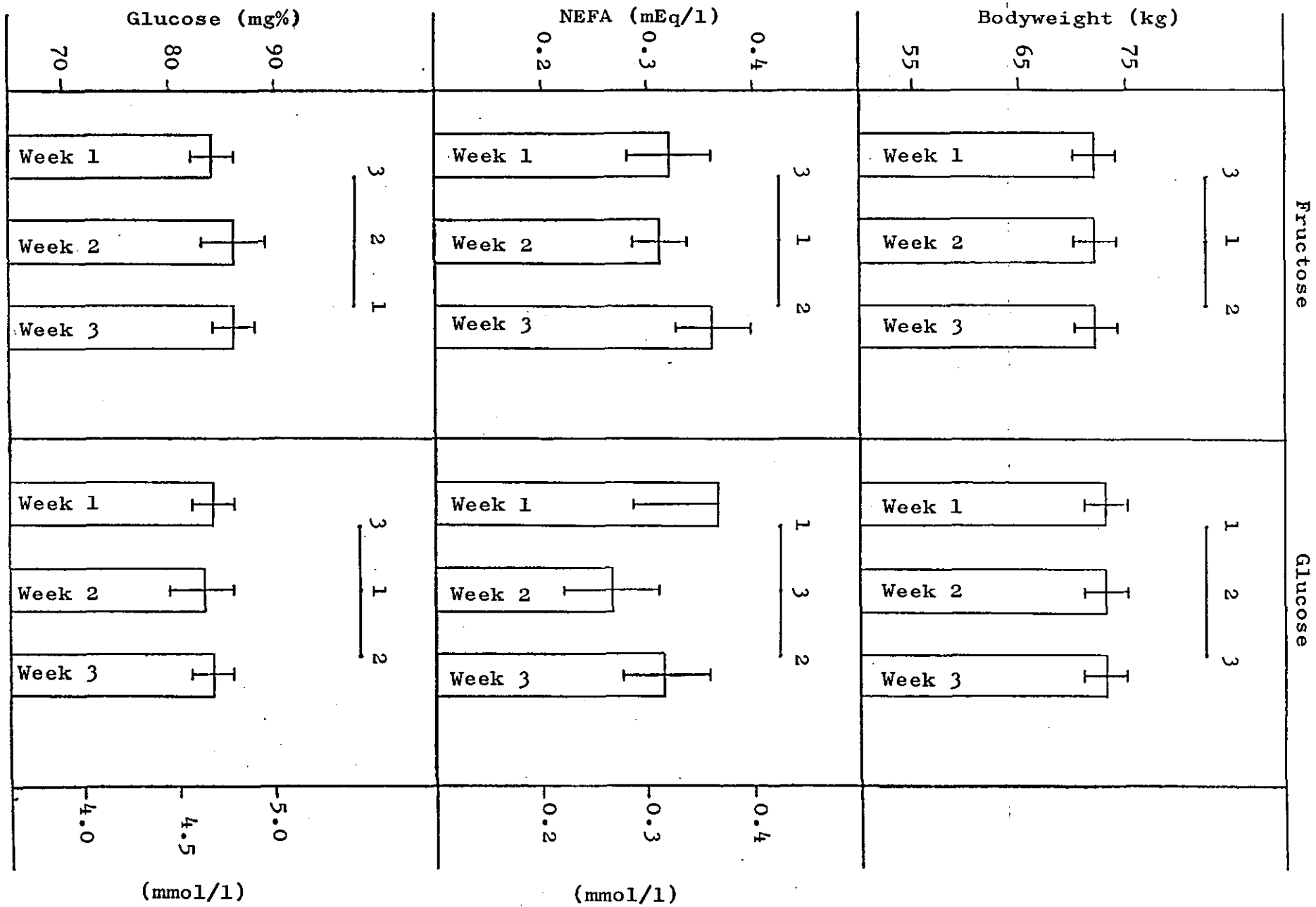


Figure 3.4.2 METABOLIC EFFECTS - FEMALE DATA.

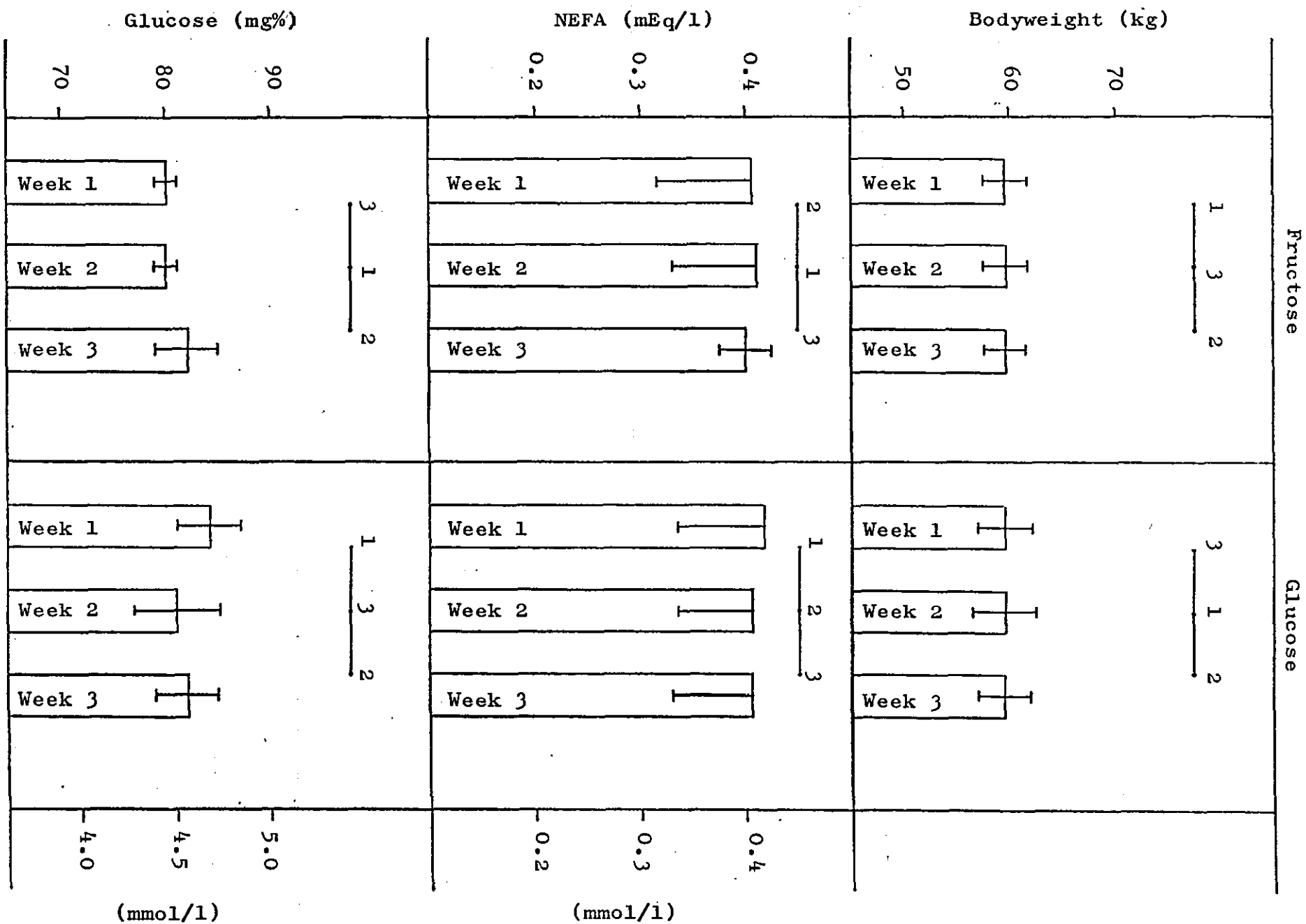


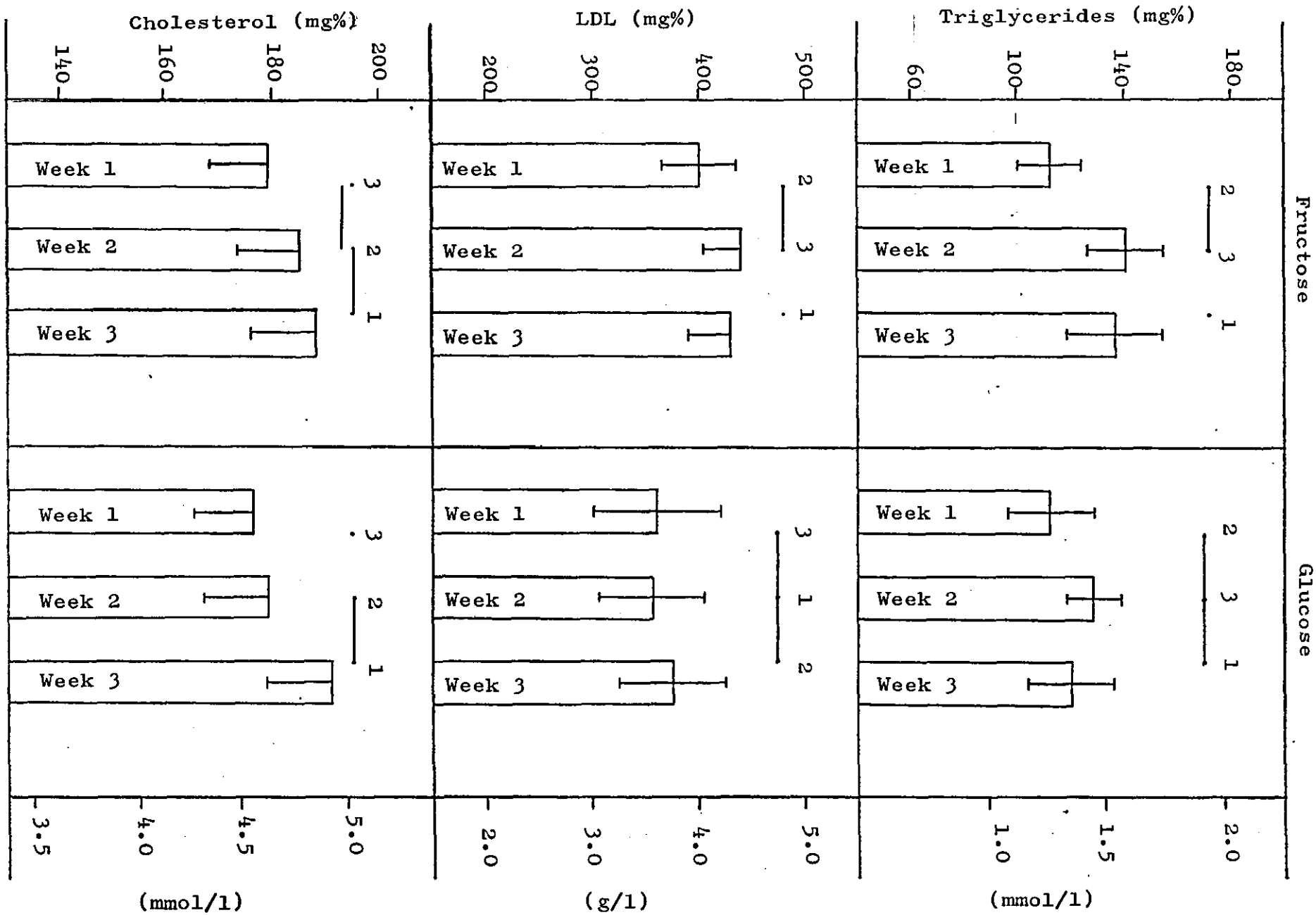
Figure 3.4.2 METABOLIC EFFECTS - MALE DATA.

Figure 3.4.2 METABOLIC EFFECTS - FEMALE DATA.

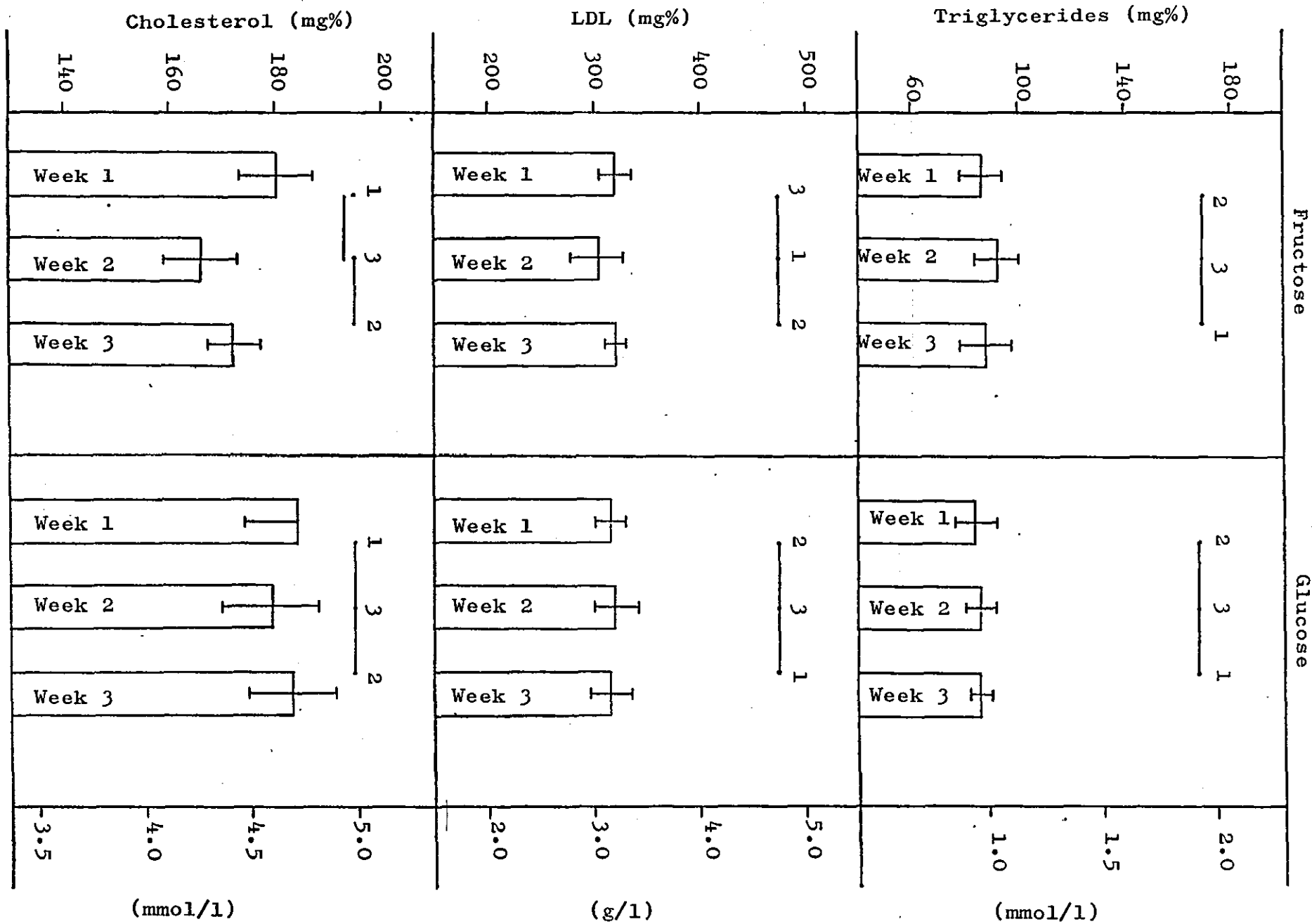


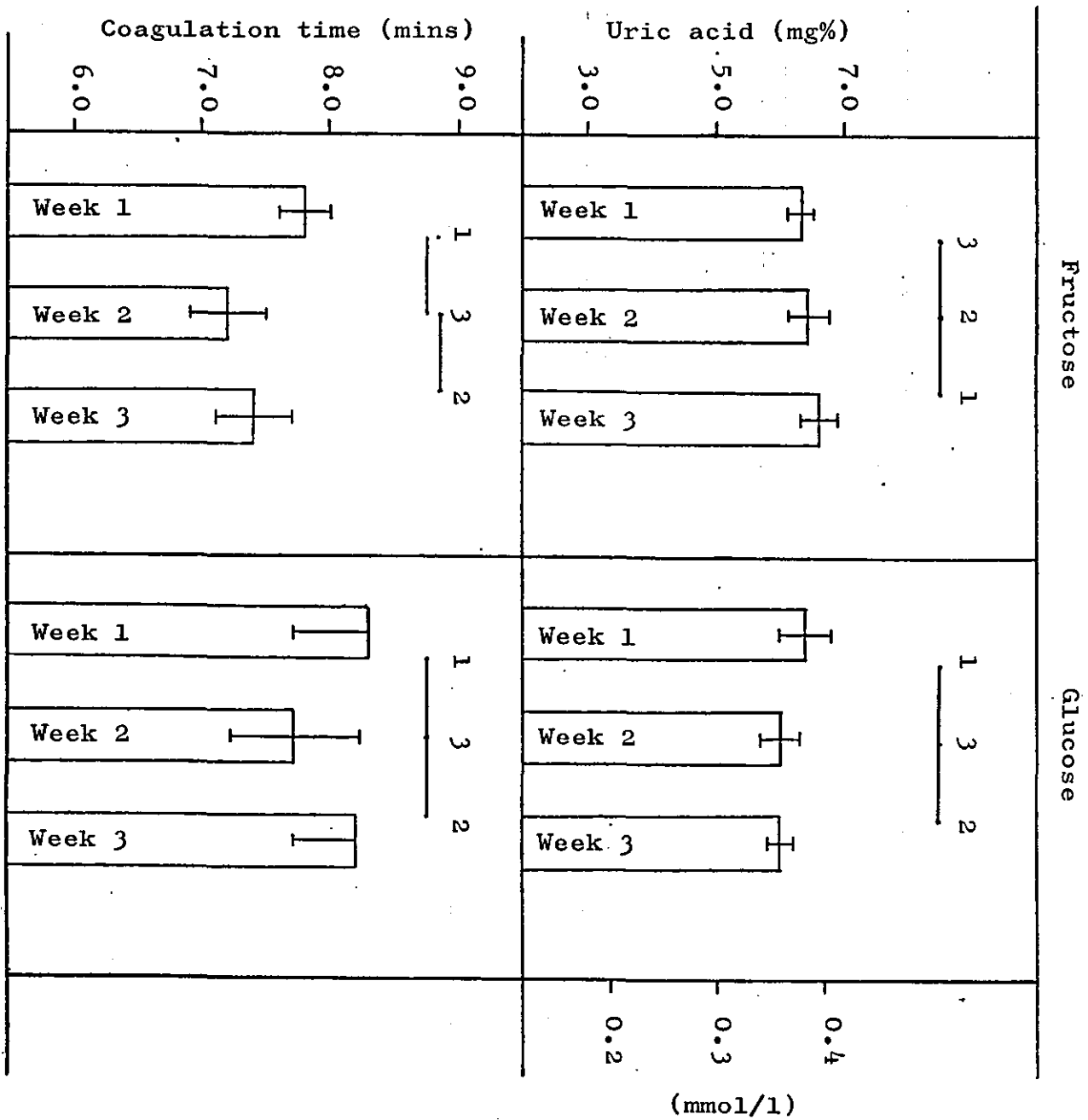
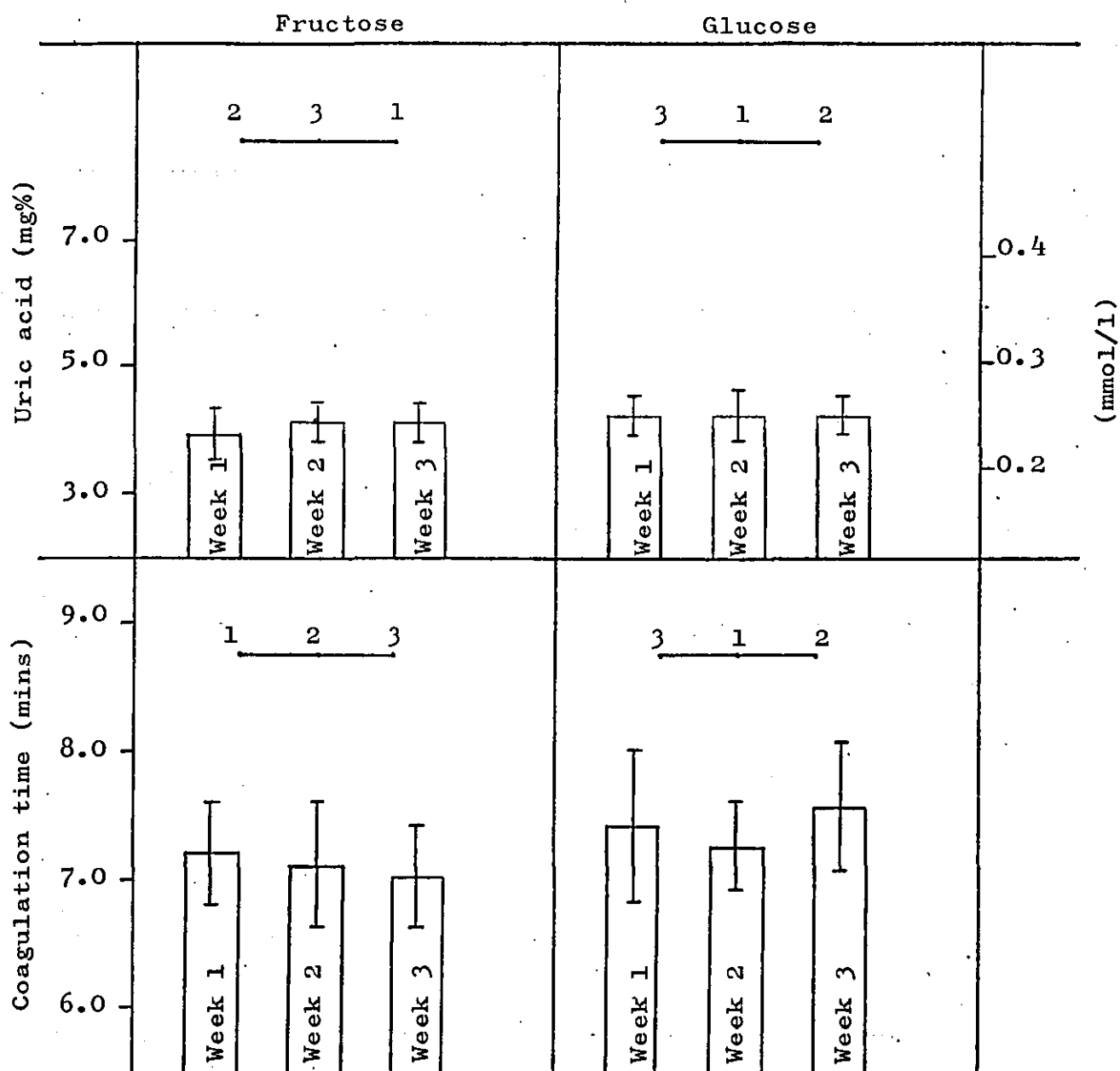
Figure 3.4.2 METABOLIC EFFECTS - MALE DATA.

Figure 3.4.2 METABOLIC EFFECTS - FEMALE DATA..

3.4.4 DISCUSSION.

The following discussion considers each variable monitored throughout the trial.

3.4.4.1 BODYWEIGHT.

For all groups no significant differences were observed between the bodyweights recorded on days 8, 15 and 22. Although additional calories were provided in the form of sugar during week two, compensatory dietary or physiological mechanisms must have occurred to achieve bodyweight maintenance.

3.4.4.2 BLOOD NEFA.

For all groups no significant differences were observed between the blood concentration of NEFA on days 8, 15, and 22. The repeated acute disturbance of NEFA levels and of the mechanisms controlling NEFA mobilisation and storage that occurred in this study does not result in an alteration of NEFA concentration. However, the alteration of energy source might be reflected in other lipid metabolism. Using NEFA values as an indicator of a change in insulin level or action these results suggest that no disturbance occurred as a consequence of the dietary changes. For this purpose NEFA might not be an appropriate indicator under this experimental design

due to the numerous factors that can act on NEFA levels. In addition elevation of fasting NEFA concentration may only be a feature of frank diabetes mellitus and small movements within the typical range masked by other factors acting on NEFA metabolism.

3.4.4.3 BLOOD GLUCOSE.

For all groups no significant differences were observed between the blood concentration of glucose on days 8, 15, and 22.

The maintenance of blood glucose levels within fine limits is an essential requirement and physiological mechanisms operate to achieve this. During this study these mechanisms were subjected to varying degrees of stimulation and this was not reflected in any changes in blood glucose concentration. However, increased physiological demand, with respect to glucose level maintenance, would have occurred and this may have long term detrimental effects. That glucose maintenance is achieved does not indicate that the overall quality of the regulatory mechanisms have also been preserved.

The same argument can be put forward for glucose as has been reported above for NEFA in that glucose may not be, in isolation, an appropriate indicator of underlying

hormonal disturbance due to the large number of factors acting on glucose metabolism and the "mild" conditions of this study. Thus any mild disturbance caused by the dietary changes of this study are not reflected in changes of blood glucose or NEFA, but it must be borne in mind that alterations to regulatory mechanisms may have occurred to achieve this. These alterations might best be viewed using a more dynamic physiological measurement.

3.4.4.4 BLOOD TRIGLYCERIDES.

A significant difference was observed between the blood triglyceride concentrations recorded on days 8, 15, and 22 for group 1. For all other groups no significant differences were determined.

Group 1. No significant difference was shown between the blood triglyceride concentrations observed on days 22 and 15 both of which were significantly greater than the level recorded on day 8.

The triglyceride concentration determined in fasting blood is an indicator of endogenous triglyceride synthesis and a predictor of VLDL levels. In this study the male fructose group recorded an increase in triglyceride levels after the period of increased fructose intake which persisted when individuals returned to their

typical diets.

Fructose entering the blood stream is rapidly taken up by the liver and the majority of fructose metabolism occurs at this site. Fructose, unlike glucose, does not stimulate insulin secretion nor does it benefit, in terms of insulin-assisted entry to adipose tissue and muscle, when insulin levels are elevated. These important differences between the physiological actions of fructose and glucose can account for the elevation in VLDL levels that occur in males after short term increases in fructose and not after glucose.

The metabolism of fructose in the liver will initially be directed towards restoration of the glycogen content of this organ and general participation in the production of energy by entry into the glycolytic pathway via fructose - 1 - phosphate and dihydroxyactone phosphate. After saturation in this metabolic direction fructose metabolism will be geared to triglyceride production via dihydroxyacetone phosphate and acetyl CoA. Hepatic synthesis of triglyceride necessitates a transport function and this is provided by the formation of VLDL macromolecules. This particular type of lipoprotein macromolecule serves the purpose of transporting triglycerides from the liver to sites, such as adipose tissue and muscle, that are rich in lipoprotein lipase. At these sites the triglyceride is hydrolysed, absorbed

into the tissue and metabolised.

Glucose transformation to triglyceride following glucose loading largely bypasses the need for blood-triglyceride transport from the liver. This is mainly due to the actions of insulin which enhances the uptake of glucose by peripheral tissues where it can be directly metabolised to triglyceride if necessary. Major tissues involved with glucose uptake following glucose loading, and subsequent insulin release, are adipose tissue and muscle. In this way the need for triglyceride transport from the liver to peripheral sites is lessened. Therefore in comparison to triglyceride synthesis following fructose a lesser proportion occurs in the liver after glucose loading. As a result the quantity of VLDL formed after glucose loading will be lower.

Although the above discussion can account for the greater formation of VLDL following fructose in comparison to glucose it largely fails to explain why the VLDL levels remained elevated on days 15 and 22 since VLDL is removed rapidly from circulation. A number of explanations can be advanced to account for this occurrence, which includes the rate of VLDL formation following fructose, a "training" effect, and the relative capacities for VLDL formation and removal.

The appearance of a raised blood triglyceride level following fructose is not an immediate effect, in fact a slight but significant lowering of triglyceride concentration has been observed 90 minutes after oral loading of 1g fructose per kg BW in male subjects (Macdonald et al, 1978b). In addition fructose removal from the blood has been shown to be a lengthy process (Section 3.3), fructose levels being significantly elevated $2\frac{1}{4}$ hours after loading. The first observation indicates a late appearance of VLDL and the second finding a prolonged availability of fructose to the liver for subsequent triglyceride synthesis.

On day 15 a component of the raised endogenous triglyceride may well have been due to the interaction of these factors. Thus the slow synthesis of VLDL coupled with the prolonged availability of fructose administered on day 14 may have had a direct effect on the triglyceride values recorded on day 15 i.e., the day 14 fructose load was still being "processed" and exerted an effect on day 15. However, it would be extremely unlikely that this mechanism could account for the raised triglyceride levels recorded on day 22 unless considerable quantities of triglyceride had accumulated in the liver during the increase period.

Prior to the fructose increase period the hepatic enzyme

profile would have been geared to cope with the typical levels of available triglyceride precursors. During the period of increased fructose intake considerably more hepatic triglyceride precursors are formed that would have resulted in an increased demand on the system designed for their metabolism. A predictable outcome of these events would be for the system to adapt to the new demand in order that the new abundant energy source can be distributed to storage depots. The result of this "training" effect would be that the liver has now a greater capacity for hepatic triglyceride synthesis. This modification could influence the direction and future hepatic metabolism, after the fructose increase period, a greater proportion of available triglyceride precursors being channelled along this route. This "training" effect could account for the elevation in blood triglyceride concentration observed on day 22.

An additional feature contributing to the triglyceride elevation is a failure of the peripheral sites, where VLDL removal takes place, to match the increases in hepatic triglyceride formation. During the course of the fructose increase period each sugar load would act to "top-up" the triglyceride synthetic reservoir, the formed VLDL being removed only at a limited rate during the following 24 hours. The mechanisms that regulate triglyceride removal will only achieve pre-sugar increase levels when the reservoir of additional

triglyceride precursors is exhausted both in the liver and that provided by the diet.

The above discussion can account for the observed findings in groups 1, 2, and 4. However, the female fructose group responded in a manner that requires additional explanation.

Available evidence suggests that the most likely mechanisms operating to maintain triglyceride levels in young females during periods of increased sucrose intake is their oestrogenic activity. Macdonald (1966) recorded an elevated blood glyceride level in males and post-menopausal women but not in pre-menopausal women when subjects were fed a diet with a high fructose content. Other studies using female humans and rats have demonstrated an increase in blood cholesterol concentration of about 15 to 30% following ovariectomy (Oliver and Boyd, 1959; Boyd, 1961). (The rise in cholesterol was largely the result of an elevation of LDL - a degradation product of VLDL). However, the mechanism whereby oestrogens protect against the fructose induced hypertriglyceridemia is largely unknown.

At a basic level two modes of oestrogenic protection are possible which involve either a limitation of VLDL formation or an accelerated rate of VLDL removal from

circulation. In a situation where hepatic triglyceride precursors are in abundance the most logical of these two mechanisms to operate would be an accelerated VLDL removal. In this case oestrogens may act to stimulate lipoprotein lipase activity, in a similar manner to insulin, to achieve this effect. An additional consideration which could assist with this effect is the number of lipoprotein receptors that are available. A major difference between the body composition of typical males and females is the greater quantity of adipose tissue and adipocytes present in the female body. Therefore it is highly probable that females have a larger number of lipoprotein receptors. The combined action of oestrogen and the quantity of lipoprotein receptors in pre-menopausal females will result in a greater VLDL removal capacity than in males or post-menopausal females.

3.4.4.5 BLOOD LDL.

A significant difference was observed between the blood LDL concentrations recorded on days 8, 15, and 22 for group 1. For all other groups no significant differences were determined.

Group 1. No significant differences were shown between the blood LDL concentrations recorded on days 22 and 15 both of which were significantly greater than the level

recorded on day 8.

The pattern of changes that occurred over the study in the concentration of LDL closely resembles those that were recorded for triglyceride levels. This similarity can be accounted for since LDL is a degradation product of VLDL and in this case is an indicator of prior VLDL synthesis.

The sequence of events in group 1 leading to raised LDL levels would begin with an increase of available hepatic triglyceride precursors provided by the fructose loadings. This would lead to a raised level of triglyceride synthesis with a subsequent elevation of VLDL production. VLDL persists in the blood for only a short period (≈ 4 hours), the action of lipoprotein lipase resulting in the formation of LDL which is removed at a considerably slower rate (2 - 5 days). Thus the observed elevation of LDL is a direct consequence of an increased endogenous triglyceride production.

The slow removal of LDL from circulation would certainly mean that the on-going processing of the fructose load administered on day 14 (and probably previous loadings) would influence the LDL levels recorded on day 15 i.e. no dramatic metabolic changes have occurred, simply the LDL elevation is a consequence of a greater triglyceride production. That LDL levels increase also

indicates that the mechanisms responsible for their removal do not respond rapidly to maintain the pre-fructose increase LDL concentration.

For all other groups elevations of triglycerides were not observed and therefore no drive was present to cause an increase in LDL levels.

3.4.4.6 BLOOD CHOLESTEROL ESTERS.

Significant differences were observed between the blood cholesterol ester concentrations recorded on days 8, 15, and 22 for groups 1, 2, and 3.

Group 1. The cholesterol ester concentration recorded on day 22 was significantly greater than the concentration observed on day 8. No other significant differences were shown.

Group 2. No significant differences were shown between the cholesterol ester concentration recorded on days 8 and 15, both of which were significantly lower than the concentration on day 22.

Group 3. The cholesterol ester concentration recorded on day 8 was significantly greater than the concentration on day 15. No other significant differences were shown.

The majority of blood cholesterol is located in the LDL fraction and for this reason it is common practice to estimate total cholesterol from the LDL levels. In group 1 LDL levels were significantly elevated on days 15 and 22 and by using the above prediction it would reasonably be expected that total cholesterol concentration should be also elevated at these times. However, using cholesterol ester levels as an alternative predictor of total cholesterol, a significant elevation was observed only on day 22. This result indicates that a change in the relationship between the proportions of the various lipoproteins occurred during the fructose increase period.

In order for the VLDL and LDL levels to rise at the same time as the total cholesterol concentration remains unaltered, requires a reduction in HDL values. This alteration must have occurred for group 1 by day 15. Over the period of fructose increase a demand for the constituents of the VLDL macromolecule (except triglyceride) would have been experienced due to the increased provision of hepatic triglyceride precursors. For this purpose the degradation of HDL may have been specifically geared to accommodate this change and as a consequence was removed more rapidly from circulation.

By day 22 for group 1 a predictable relationship between the macromolecule increases and total cholesterol had

been achieved. It is highly probable that a combination of increased HDL formation from LDL and a reduced rate of HDL removal can account for this observation.

The changes in the blood concentration of cholesterol esters in group 2 followed a similar pattern to that of group 1 except that for group 2 the level recorded on day 22 was also significantly greater than the day 15 value. For group 2 the cholesterol ester changes occurring over the glucose increase period are in accordance with what could be predicted from VLDL and LDL changes. However, the day 22 cholesterol ester concentration increase in group 2 cannot be accounted for by the sequence of events initiated by a rise in VLDL levels leading to elevated LDL values, with a subsequent increase in total cholesterol. In this particular case, since VLDL and LDL concentrations are unaltered at day 22, the total cholesterol elevation must be accounted for by the increased level of another cholesterol-containing fraction, namely HDL.

A possible explanation for events occurring for group 2 would be that during the period of sugar increase the mechanisms responsible for HDL removal were "relaxed" as a result of the replacement, or part replacement, of another dietary factor, or factors, by glucose. The "relaxation" could have resulted from a decreased demand

for HDL degradation products or a reduced level of HDL formation acting to lower the required HDL removal capacity. The return to typical diets would then involve the addition of increased amounts of this dietary factor to a system with a reduced capacity or demand for HDL removal. In this case the result would be an elevated blood HDL concentration which would act to increase the total cholesterol level.

The female fructose group demonstrated a different response to that observed for the male fructose group. Blood cholesterol esters for group 3 were reduced on day 15, at a time when VLDL and LDL levels were unaltered. The cholesterol ester concentration returned to pre-sugar increase values by day 22. The independent reduction in the concentration of cholesterol esters on day 15 suggests a reduction in HDL levels since the levels of the other cholesterol containing macromolecules were unaltered.

For group 3 during this study there was no indication that the sequence of events observed in group 2, initiated by triglyceride elevation leading finally to elevated LDL levels, occurred. If these changes occurred, as is highly probable, then the implications are that VLDL and LDL removal from circulation in females proceeds at a faster rate than in males. This can be either due to an increased-reserve removal capacity or as a result

of oestrogenic influences. The predicted effect on day 15 would be an elevation in their levels unless this macromolecule participates in the accelerated provision of "raw materials" for VLDL synthesis. If this is the case then day 15 recordings would demonstrate a drop in total cholesterol and by implication a reduction of HDL values. For group 3 the latter explanation appears appropriate.

The unchanged cholesterol ester concentrations recorded for group 4 is in agreement with the respective unaltered values of triglycerides and LDL determined during this study. The response of this group differed from the male glucose group in that no "rebound" cholesterol ester elevation occurred following the sugar increase period. It would appear that for the female group changes in dietary composition resulting from glucose addition and removal are well tolerated with respect to lipoprotein metabolism.

3.4.4.7 BLOOD URIC ACID.

No significant differences were shown between the blood uric acid concentrations recorded on days 8, 15, and 22 for all groups.

It has been previously reported that blood levels of uric acid are elevated following fructose but not

glucose loading. This observation is a direct consequence of the rapid metabolism of fructose in the liver. The initial stage of fructose metabolism results in increased levels of ADP and AMP, which are relatively unstable in comparison to ATP, and subsequently uric acid is formed at a faster rate. The rise in uric acid formation is not completely matched by an elevation of excretion in the urine and subsequently blood uric acid levels increase.

However, the rise in blood uric acid concentration is short lived and levels are reduced after fructose has been removed. This study demonstrates that short-term increases of fructose and glucose do not result in a persistent elevation of blood uric acid concentrations.

3.4.4.8 BLOOD COAGULATION TIME.

A significant difference was shown between the blood coagulation times recorded on days 8, 15, and 22 for group 1. No other significant differences were observed.

Group 1. The blood coagulation time recorded on day 8 was significantly greater than the value recorded on day 15. No other differences were shown.

The Lee and White technique for determining blood coagulation time provides only an overall view of blood coagulation and does not in any way indicate

the response of the many individual factors concerned. For this reason it would be extremely unwise to speculate over the precise cause of the above result until the effects of fructose on the individual components of blood coagulation are investigated; for example, fructose could achieve this result by direct action on a single clotting factor, by replacement of another dietary constituent, or as a result of a more general physiological effect. At this stage all that can be reported is that during the feeding of fructose to young male adults, a reduction in blood coagulation occurred, of imprecise cause. Similar observations were not recorded for the female fructose group or during the feeding of glucose to both male and female groups.

3.4.5 GENERAL DISCUSSION AND CONCLUSION.

This study was designed to investigate certain physiological effects of short-term low level dietary increases of fructose and glucose on young male and female adults. The physiological variables examined were specifically concerned with factors associated with the risk of degenerative disease development.

Throughout the study, changes in the physiological values for individuals within typical ranges were observed. This is an important observation and it should be appreciated that although an isolated physiological

determination may fall within the typical range for the population as a whole, this value may be abnormal for the individual under examination. Serial determinations, such as were conducted in this study, permits individual variation within the typical ranges to be observed. This in turn allows changes in the "risk level" to be recognised.

The fasting level of blood glucose and NEFA represent the main blood constituents that are available for immediate use as energy sources. The concentration of both constituents also indicate the functional balance of hormones and other metabolites that regulate their balance between mobilization and utilization. Blood glucose maintenance is essential, the breakdown of the controlling mechanism in diabetes mellitus representing a severe threat to the quality of life. Blood NEFA can, in addition, be considered a component in a metabolic cycle that yields both energy and triglyceride precursors. For example, NEFA released from adipose tissue can be resynthesised into triglyceride by the liver or used peripherally as an energy source. Disruptions of this cycle, as indicated by NEFA levels, may influence the quantity of NEFA that are available for hepatic triglyceride synthesis. This in turn would affect the level of VLDL and LDL in circulation. The concentration of these particular macromolecules are of critical importance with respect to the risk of degenerative disease development.

For all groups the dietary changes had no effects on the fasting concentration of glucose and NEFA. However, the maintenance of glucose and NEFA levels does not mean that the mechanisms controlling their concentrations were not affected by the dietary change, only that they may have reacted to accommodate such changes that occurred in the provision of energy.

Blood triglycerides, LDL, and cholesterol esters were additional lipid constituents selected for study. The importance of cholesterol and cholesterol-containing molecules as risk factors for degenerative disease development are well established. A metabolic sequence involving endogenous triglycerides and LDL has been emphasized. Cholesterol esters were determined for predictions of total cholesterol values.

The initial step in the above sequence involves the synthesis of endogenous hepatic triglycerides from available precursors. Whenever this occurs the demand for the formation of a macromolecule capable of solubilising the triglyceride in the blood stream arises. This results in the synthesis and subsequent release of VLDL into circulation. Breakdown of VLDL in capillary beds containing lipoprotein lipase results in the formation of the cholesterol rich macromolecule LDL. This effectively completes the sequence although changes in HDL levels can be estimated by manipulation of total

cholesterol, VLDL and LDL values. For some groups during the course of the study significant changes were observed in the fasting blood concentration of the above lipid fractions which have been explained by alterations of the various factors affecting the outlined metabolic sequence.

For the male fructose group the increased availability of hepatic triglyceride precursors resulted in elevated fasting blood levels of triglycerides and LDL immediately after the period of increased fructose intake (day 15) and after seven days' return to a typical diet (day 22). Elevation of total cholesterol (predicted from cholesterol esters) was recorded only after the period of return to a typical diet. By consideration of the changes that occurred by day 15 it would appear that HDL levels were reduced at this time. The demand for VLDL precursors (except for triglyceride) during this period may have accounted for this observation. In contrast to the male fructose group (group 1), no significant changes were observed in the fasting blood concentration of triglycerides or LDL in the male glucose group (group 2) and in the female fructose (group 3) and glucose groups (group 4). In comparison to both fructose groups the provision of hepatic triglyceride precursors, as a result of sugar additions, would have been lower in the glucose groups. This is a result of the different pattern of tissue uptake of

fructose and glucose following their respective loadings. The liver is the main site of fructose uptake with adipose tissue and muscle accounting for only a small quantity of fructose removal whereas for glucose the relative importance of these tissues for removal is reversed. In the case of glucose, insulin plays an important role in determining the distribution by promoting tissue uptake of glucose by adipose tissue and muscle. The extent of the extrahepatic uptake of glucose limits the amount of hepatic triglyceride precursors made available by glucose metabolism and thus the metabolic sequence; triglyceride - VLDL - LDL is not so greatly stimulated after glucose loading in comparison to fructose loading.

For group 2 the fasting level of cholesterol esters increased on return to a typical diet, this was not observed for group 4. Presumably an increase in HDL concentrations accounts for this increase since neither VLDL or LDL were elevated at this stage. An explanation for this occurrence is the possibility of the partial removal of a particular dietary constituent during the period of glucose increase that when present results in HDL formation and subsequently the demand for HDL removal. A reduction in the capacity of HDL removal and increased formation may then have resulted in the raised blood HDL levels following the replacement

of the dietary constituent restricted during the period of sugar increase.

In female subjects receiving increased dietary fructose it is highly probable that the distribution of tissue uptake of fructose is similar to that in the male and subsequently increased hepatic triglyceride precursors would have been made available. However, this study did not reveal any evidence of increased hepatic triglyceride synthesis as the fasting blood levels of triglycerides and LDL were not significantly elevated on days 15 and 22. As a result of this finding, one possible explanation of this occurrence must be that in the young female adult, fructose does not stimulate hepatic triglyceride synthesis. If this is not the case the young female adult must have the capacity to remove increased levels of VLDL and LDL from circulation. Certainly in the female it is likely that more sites with lipoprotein lipase are present in comparison to males due to the greater adiposity of females. The role of oestrogenic activity in influencing the above result must not be overlooked since the outcome, had post-menopausal women been studied, would probably have been similar to the male response (Macdonald, 1966).

The cholesterol ester concentration recorded for the female fructose group demonstrated a significant

reduction on day 15. Taking into account the maintenance of VLDL and LDL levels at this time this result indicates that HDL levels were depressed. Although not observed in this study it is probable that during this period (day 8 - 14) increased hepatic triglyceride and VLDL synthesis occurred. Components of the HDL macromolecule could act as precursors for VLDL synthesis and due to the greater demand for VLDL precursors at this time removal of HDL would have been accelerated.

For all groups the dietary changes that occurred during this study did not influence the fasting blood uric acid concentration. Acute changes in uric acid levels following fructose have been reported and this is due to the rapid hepatic metabolism of fructose. This gives rise to an increased turnover of ADP and AMP with a subsequent elevation of uric acid production. The increased uric acid production is not balanced by an elevation in uric acid excretion and therefore blood values rise. The results of this study indicate that increases in blood uric acid concentration following fructose are only temporary and that lasting disruption of uric acid metabolism does not occur.

A change in blood coagulation time was only observed in the male fructose group, a decrease occurring at day 15. No precise mode of action of fructose resulting in this effect can be identified from this study.

During the course of this study the low level (1g/kg BW/day) of sugar additions to the typical diets of young male and female adults over a short period (7 days) resulted in changes to certain physiological values that were selected to provide information on the risk of degenerative disease development. The changes observed occurred predominantly in the male fructose group and involved increases in the fasting blood concentration of triglycerides, LDL, and cholesterol esters, and a reduction in blood coagulation time. These changes, occurring within the typical limits, can be considered to be in a direction that increases the risk of degenerative disease development. Physiological changes were also observed for the male glucose and the female fructose groups. No changes were recorded in the female glucose group.

For both the male glucose and the female fructose group the single significant change observed occurred in the fasting blood concentration of cholesterol esters. In the male case it can be reasoned that after the period of 7 days return to a typical diet HDL levels were elevated. This can be considered to be a move in a protective direction with respect to degenerative disease development. For the female fructose group the reduction in cholesterol esters that occurred immediately following the period of sugar increase (day 15) can be argued to

be a temporary reduction in HDL levels and therefore an increase in risk. However, although cholesterol ester values changed significantly HDL changes have been predicted from the triglyceride, LDL and cholesterol ester concentrations and their relationships. This method of derivation of HDL values might not apply to all circumstances and alternative explanations for the events observed may be more appropriate. For example a change in cholesterol ester concentration occurring during a period of LDL and triglyceride stability may reflect a change in the proportion of the total cholesterol that is esterified.

Previously reported studies have recorded a triglyceride-elevating effect of fructose and sucrose. Those studies have all involved the ingestion of large quantities of sugars in exchange experiments that result in a general disruption of the diet. The studies conducted here have provided relatively low levels of sugar additions to free-choice diets that have been modified only by the subjects in order to achieve body weight maintenance. By this approach a realistic dietary modification, that would occur as a result of an increase of sugar in the diet, has been achieved. However, although low level sugar additions have been made the resultant diets should be considered high in sugar. Only in the male fructose group were changes in the internal environment observed

that reflected a shift to a pattern that is thought to be in a direction conducive to degenerative disease development. However, there is no indication from these studies that the physiological changes resulting over the period of high fructose intake would persist should the diet remain high in fructose.

3.5 HORMONAL EFFECTS OF FRUCTOSE AND GLUCOSE IN YOUNG ADULTS.

This experimental section follows a similar design to the previous section where certain physiological effects of fructose and glucose in young adults were examined. However, the emphasis in this section is concentrated on the effects of low levels of fructose and glucose additions (1g/kg BW) over a short period (7 days) on the fasting blood concentration of insulin, thyroxine, and cortisol. In addition blood levels of glucose, triglycerides, and LDL have again been monitored in order that any hormonal changes observed can be linked to trends in the concentration of these metabolites.

Insulin, thyroxine, and cortisol have been selected for study due to their key role in the regulation of metabolism and as a result of their known influence on the internal environment in certain disease states.

Alterations in the fasting levels of the above hormones may be caused by dietary change. This occurrence could represent the initial physiological step that either directly promotes or augments the production of an internal environment conducive to degenerative disease development. Certainly the selected hormones play a crucial part in the regulation of metabolism and warrant investigation in this particular type of study.

3.5.1 HORMONES EXAMINED.

This section "introduces" the hormones insulin, cortisol, and thyroxine in a manner relevant to the concerns of this thesis. The following discussion therefore concentrates on certain of their metabolic functions in health and disease.

3.5.1.1 BLOOD INSULIN.

Although insulin has many effects on metabolism for purposes of this discussion the most important action

of insulin is to lower blood glucose concentration. This is achieved by increased glucose uptake by the heart, adipose tissue and skeletal muscle. Within these tissues, and the liver, insulin exerts an anabolic effect which results in the storage of energy products in the form of glycogen and lipids.

The stimulation of insulin release is achieved by many factors the most important being the presence of blood glucose. Basically elevated levels of blood glucose stimulate insulin release which in turn results in an enhanced peripheral uptake of glucose and therefore a reduction in circulating glucose. In this manner the persistence of an elevated blood glucose level is inhibited. Insulin is also released when an individual is in a fasting condition when no specific stimulus is present. This basal release is also thought to be partly controlled by blood glucose.

The role of insulin in lowering blood glucose levels is essential for life. A deficiency or an impaired peripheral function of insulin results in hyperglycaemia, glycosuria, polyuria, dehydration, increased gluconeogenesis, stimulation of lipolysis, elevated blood cholesterol, ketonaemia, metabolic acidosis, coma and ultimately death if untreated. The disorder caused by insulin lack or absence is termed diabetes mellitus.

Two main types of diabetes mellitus are recognised, juvenile and adult (maturity onset). For the purposes of this thesis attention will be focussed on the adult form of the disease. Adult diabetes can usually be treated by dietary modification and does not need insulin administration. This form of the disease is of particular interest to the investigation of degenerative disease since it essentially appears that the system "wears out" prematurely, as a result of a gradual process. Therefore adult diabetes occupies a key position in degenerative disease development study due, not only to the postulated associations between diabetes and other disease, but as a result of the known influence of insulin on the internal environment.

The detection of diabetes typically occurs in the later stages of the disease development when the pancreatic β -cells can be described as being in an exhausted condition. This stage represents the end result of the attempt of β -pancreatic cells to cope over a long period of time with an increased demand for insulin secretion. From animal studies it has been postulated (Kramer and Evermann, 1982) that pancreatic β -cells have a finite capacity for regeneration and hyperplasia and that continued increased demands for insulin secretion result in β -cell degeneration and hyperglycaemia. Thus the β -cells respond in the short term in an attempt

to maintain glucose levels but at the expense of their longevity. The course of diabetes development to a "recognisable" stage (endocrine emergency) would be reflected in a gradual deterioration of the control of blood glucose level.

By the above mechanism of β -cell exhaustion an extra demand on their function must occur over a long period of time. A cause of an increased β -cell demand occurs when the peripheral actions of insulin are antagonised. In this particular situation more insulin would have to be released to lower blood glucose.

Insulin antagonists are well known and include secretions of the adrenal medulla, adrenal cortex and anterior pituitary gland (De Bodo and Altszuler, 1958). Insulin antagonism can be recognised by examination of the relationship between insulin and glucose concentrations. Evidence of insulin antagonism would be indicated if the quantity of insulin required to maintain a particular glucose level increases.

The above discussion highlights one specific route to the development of an internal environment that is, in the first instance a biochemical lesion of a degenerative disease itself, and in addition is also thought to be conducive to further disease development. This thesis is concerned with the physiological effects of fructose

with special emphasis on degenerative disease development. In this particular study the effect of increasing dietary fructose levels on insulin antagonism are examined. This will provide insights into whether or not fructose can influence factors associated with this particular route to disease development. The relevant factors here are the fasting blood levels of insulin and glucose.

3.5.1.2 BLOOD CORTISOL.

Cortisol plays a key role in the control of the internal environment. This control is achieved by action on protein, carbohydrate, and lipid metabolism which is caused by direct action on target tissues and by influencing the actions of other hormones. The main metabolic effects of cortisol, that are of particular relevance here, are increased protein catabolism, glyconeogenesis, and lipolysis, extrahepatic inhibition of amino acid uptake, and insulin antagonism. During periods of increased cortisol secretion these effects result in a raised blood glucose level, hyperlipidaemia, hypercholesterolaemia, and an increased blood insulin level (Lee and Laycock, 1978).

In a healthy subject cortisol is always present in circulation, the concentration during the day demonstrating a marked circadian rhythm. The variation in

blood cortisol throughout the day is caused by the rhythm of adrenocorticotrophic hormone (ACTH) release from the anterior pituitary which stimulates the release of cortisol from the adrenal cortex. ACTH release in turn is regulated by the levels of corticotrophin releasing factor (CRF).

An important factor that can influence an individual's normal cortisol level (via CRF-ACTH-cortisol mechanism) is emotional stress. Psychological and physiological stresses which include pain, anger, anxiety, frustration, shock, sadness, noise, surgical trauma, and hypoglycaemia, all produce an elevation in circulating cortisol. The "cortisol stress response" is essential for life. However, the preservation by cortisol, and associated physiological responses during stress, may in itself impair the overall physiological status and in turn reduce the quality and quantity of life. This is suggested by the indicated increased risk of degenerative disease development in individuals exposed to stress or in those who belong to a particular personality type (type A) that demonstrate physiological and psychological symptoms of emotional stress. (Russek, 1962; Buell and Breslow, 1960; Russek and Zohman, 1956; Wolf, 1971; Jenkins, Zyzanski, Rosenman and Cleveland, 1971; Cooper and Marshall, 1976; Cox, 1978; Rosenman, Friedman, Strauss et al, 1964; Keith, Lown and Stare,

1965; Caffrey, 1970; Rosenman, Friedman and Strauss, 1970).

For the purpose of this thesis it is of importance to appreciate the relationship between stress and degenerative diseases, in particular the internal environment produced by the actions of cortisol in times of stress, and the principal physiological mechanisms underlying these changes.

During stressful periods (when cortisol levels are raised) blood cholesterol, glucose, and insulin are elevated (Grundy and Griffin, 1959; Thomas and Murphy, 1958; Wertlake, Wilcox and Peterson, 1958; Leung and Munck, 1975). As highlighted above these physiological events can be the direct consequence of increased blood cortisol levels. The resultant internal environment is remarkable in that it is both characteristic of developing diabetes mellitus and also one associated with a greater risk of degenerative disease development. Thus particularly interesting links appear to exist between emotional stress, the release of cortisol in increased amounts, the effects of cortisol on the internal environment and with an increased incidence of degenerative disease. A contribution to the production of this environment arises from the extrahepatic antagonism of insulin by cortisol. This is of particular importance since, as previously mentioned, the continual

increase in demand for insulin secretion may result in pancreatic β -cell exhaustion. Thus cortisol can play a key role in the pathogenesis of degenerative disease.

Various origins of degenerative disease development exist that must at some stage merge to form a common pathway. For example the internal environment can be modified by direct additions of injurious substances to it, or the injurious substances can be formed as a consequence of a metabolic event resulting from a physiological need. In either case, although the origins are different, the end results are the same i.e. the presence of an injurious substance. A contributory factor in degenerative disease development may have a single or multiple point of entry, in that effects can be exerted at various levels before merging at the common pathway.

Fructose has been shown in previous studies to alter, in the short term in males, the internal environment in a direction associated with a greater risk of degenerative disease development. This has been explained at a "metabolic" level the result being the direct consequence of the tissue where fructose is predominantly metabolised. This investigation examines the possibility that fructose acts at an additional level, the influence

of prolonged blood fructose concentrations exerting an effect on cortisol secretion.

3.5.1.3 THYROXINE.

Thyroxine (T_4) provides in health, a homeostatic background for physiological requirements by actions on protein, lipid, and carbohydrate metabolism. This control is achieved by direct effects and by the influence on other hormones. In addition thyroxine regulates the oxygen consumption of many tissues, this action being a primary function of thyroxine. Disruptions of blood thyroxine levels have many metabolic consequences but can be viewed as a breakdown of the general stability of the internal environment.

Blood thyroxine levels are controlled by thyroid stimulating hormone (TSH) which is released from the anterior pituitary. Secretion of TSH from this gland is in turn regulated by thyrotrophic releasing factor synthesised in the hypothalamus. A feature of thyroxine, in accordance with its overall long term regulatory properties, is its slow half life which is about one week (Sterling and Lazarus, 1977). This provides a necessary long term control over the internal environment. The action of released thyroxine is delayed for about 72 hours, the peak occurring after 7 - 9 days.

Any shift in the level of circulating thyroxine will thus have long-term effects on many aspects of metabolism. An interesting feature of reduced thyroxine levels is a raised blood cholesterol concentration.

In the same way, as has been discussed in the previous section, a disruption either directly or indirectly, in thyroxine regulation, represents a further origin from which the internal environment can be altered in a direction favouring degenerative disease development. This study examines the effect of prolonged increases in blood fructose levels on this factor.

3.5.2 EXPERIMENTAL DESIGN.

Low level quantities (1g/kg BW) of fructose and glucose were added for a short period (7 days) to the free-choice diets of young adults. The fasting blood concentration of insulin, thyroxine, cortisol, glucose, triglycerides, and LDL were examined, before and immediately after the period of raised sugar intake, and seven days after individuals had returned to their typical diets. Comparisons between base-line values, sugar effects, and sexes have been conducted. Much of the experimental design follows an identical pattern to that described in Section 3.4.2 but for convenience parts are duplicated in this section.

3.5.2.1 SUBJECT DETAILS.

Ten male and ten female subjects completed this study. All subjects were within the age range 19 - 26 years, non-obese, and apparently in good health. Subjects were provided with full details of the study. Individuals thought to be at risk, as a result of their participation, were identified and excluded by questionnaire.

3.5.2.2 SUBJECT PARTICIPATION.

Volunteers received either an increase in dietary fructose or glucose (1g/kg BW/day for 7 days) added to their free-choice diets. Subjects were divided into the following groups:

Group 1	Male fructose)	
)	
Group 2	Male glucose)	
)	5 subjects per group
Group 3	Female fructose)	
)	
Group 4	Female glucose)	

The entire study lasted for 22 days, the schedule followed by each subject over this period is detailed below.

Day 1 - 7 Pre-sugar increase period. Recording of typical dietary sugar levels.

Day 8 - 14 Sugar increase period. Recording of dietary sugar levels. Day 8 first body weight determination and blood collection.

Day 15 - 22 Post-sugar increase period. Day 15 - 21 recording of dietary sugar level. Day 15 and 22, second and third bodyweight determination and blood collection respectively.

On days of bodyweight determination and blood collection (days 8, 15, and 22) subjects were instructed to arrive at the test station in a fasting state, free of food for not less than 12 hours and of alcohol for 24 hours. After bodyweight measurement subjects rested on a medical couch for 30 minutes before blood sampling. For each subject all blood samples were drawn at exactly the same time on each occasion (9.30 to 10 am in all cases).

3.5.2.3 TYPICAL DIETS AND INCREASED SUGAR ALLOWANCE.

Subjects were instructed to maintain their typical diets and lifestyle as precisely as possible throughout the study. Recording of dietary free-sugar was achieved by the completion of forms listing foods with high or moderate levels of sugar. Sugar was provided in appropriate amounts for the increase period and conditions of administration followed those detailed in section 3.4.2.3.

3.5.2.4 BLOOD COLLECTION AND ANALYSIS.

All blood samples were obtained by venepuncture from the superficial veins of the cubital fossa. On each occasion 10 mls of blood were withdrawn and the following analyses conducted:

On whole blood: glucose (Fluoride oxalate preservative)

On serum: triglycerides, LDL, insulin, thyroxine, and cortisol.

All blood collections and analyses were conducted by the author. All analyses were conducted in duplicate; for glucose, triglycerides, and LDL on the same day as blood collection. Samples of serum were stored at -20°C for later determination of insulin, thyroxine and cortisol. Techniques employed for the above analyses have been discussed previously (section 3.2).

3.5.2.5 STATISTICAL ANALYSIS.

Data transformation, statistical comparisons and the method of analysis have been carried out in exactly the same manner as detailed in section 3.4.2.5.

3.5.3. RESULTS.

Data summaries, analysis of variance tables and detailed comparison of means data are listed in the appendix.

Results have been illustrated in figures 3.5.1 and 3.5.2. Figure 3.5.1 summarises sugar intake data and figure 3.5.2 physiological effects data. For physiological effects data week 1, 2 and 3 correspond to blood samples drawn on days 8, 15 and 22 respectively. Figures include mean values plus or minus one standard error and a statistical summary. The method of statistical summary adopted has been described previously (section 3.4.3).

The concentration of all constituents examined in fasting blood were within the typical limits for the techniques employed. Comparison of the base-line data between groups revealed significant differences between bodyweights and blood triglyceride concentrations. No significant differences were shown between the bodyweights of group 1 and 2 both of which were significantly greater than those recorded for groups 3 and 4. Again, as in the previous study, this is an expected finding.

The triglyceride concentration recorded for group 1 was significantly greater than that determined for groups 3 and 4. No other significant differences were shown. This result is not in agreement with that observed in the previous study, although both male groups recorded higher triglyceride levels than the female groups. This result indicates that the level of endogenous triglyceride production in the male

fructose group was higher than either female group. In terms of the relative risk of degenerative disease development, group 1 can be considered to be at greater risk, although their triglyceride values fall within the typical range. The elevation of triglyceride synthesis in group 1 is not reflected in a significantly greater LDL concentration that might be expected from this finding. These results are not in agreement with those observed by Macdonald (1966) who recorded no significant difference between male and female fasting glyceride levels.

Significant differences were recorded between the levels of sugars ingested during the various stages of the study. In all cases no significant differences were observed in the quantities of sugars ingested before or after the sugar increase period; Sugar intakes during the period of increased sugar allowance were significantly elevated over the baseline levels for all groups. Again this was an expected finding and it was an intention of the study to produce this elevation of sugar intakes. For groups 1, 2, and 4 a significant reduction of sugar intake occurred over week 3 in comparison to week 2 data. Although the female fructose group demonstrated the same downward trend the extent of this reduction failed to reach significance in comparison to the level of sugar intake recorded during the increase period.

Figure 3.5.1 DIETARY SUGAR INTAKE - MALE DATA.

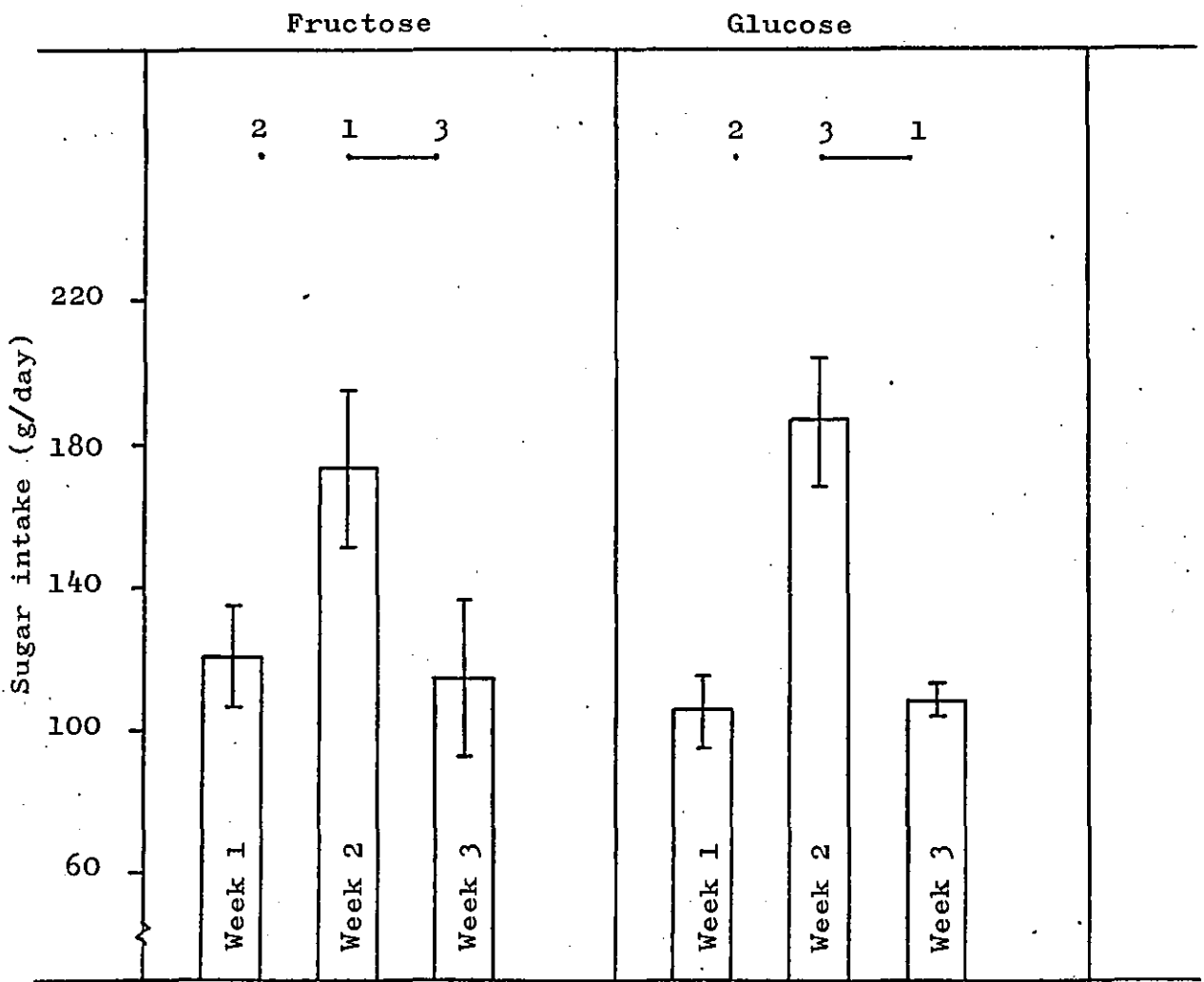


Figure 3.5.1 DIETARY SUGAR INTAKE - FEMALE DATA.

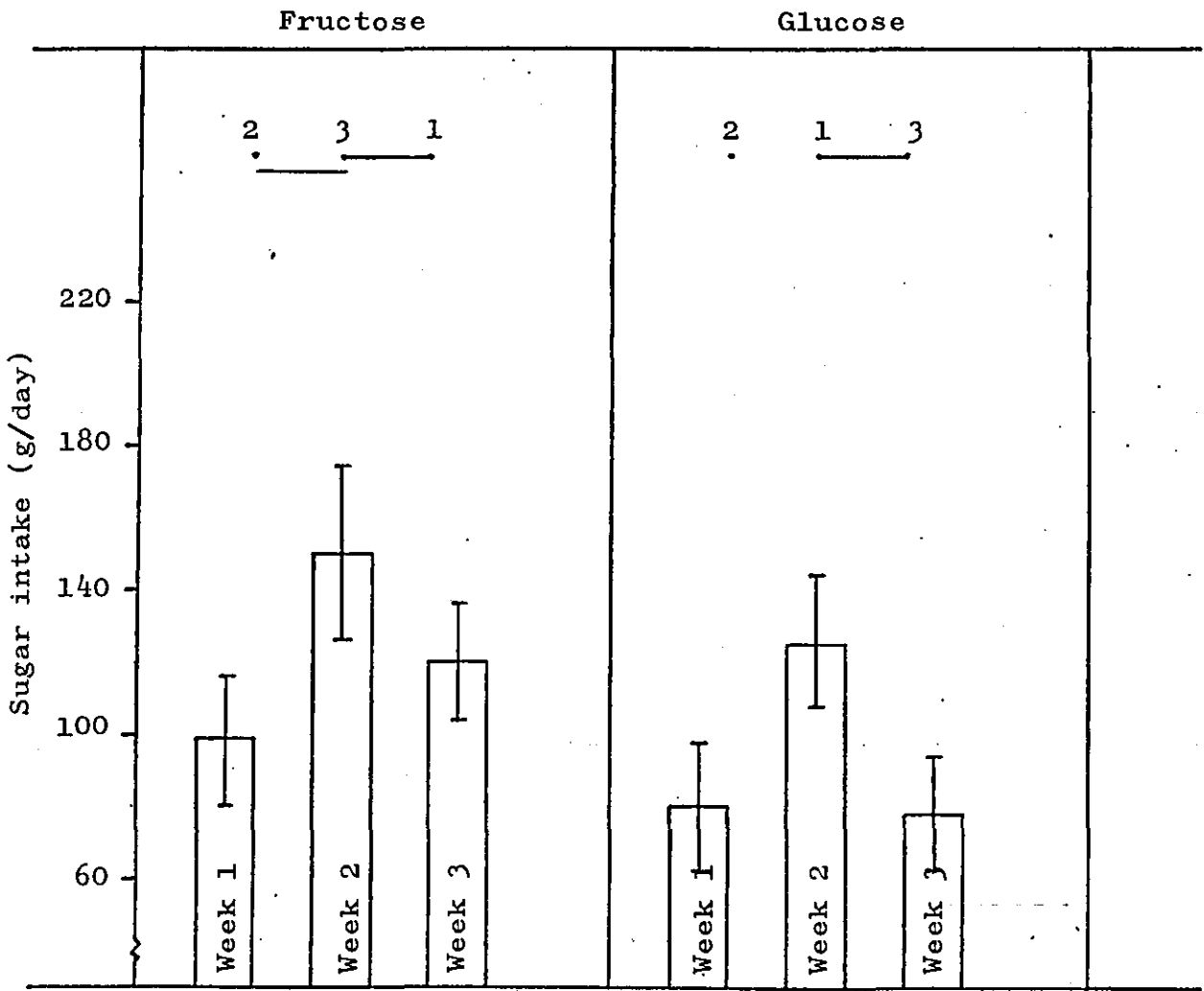


Figure 3.5.2 HORMONAL EFFECTS - MALE DATA.

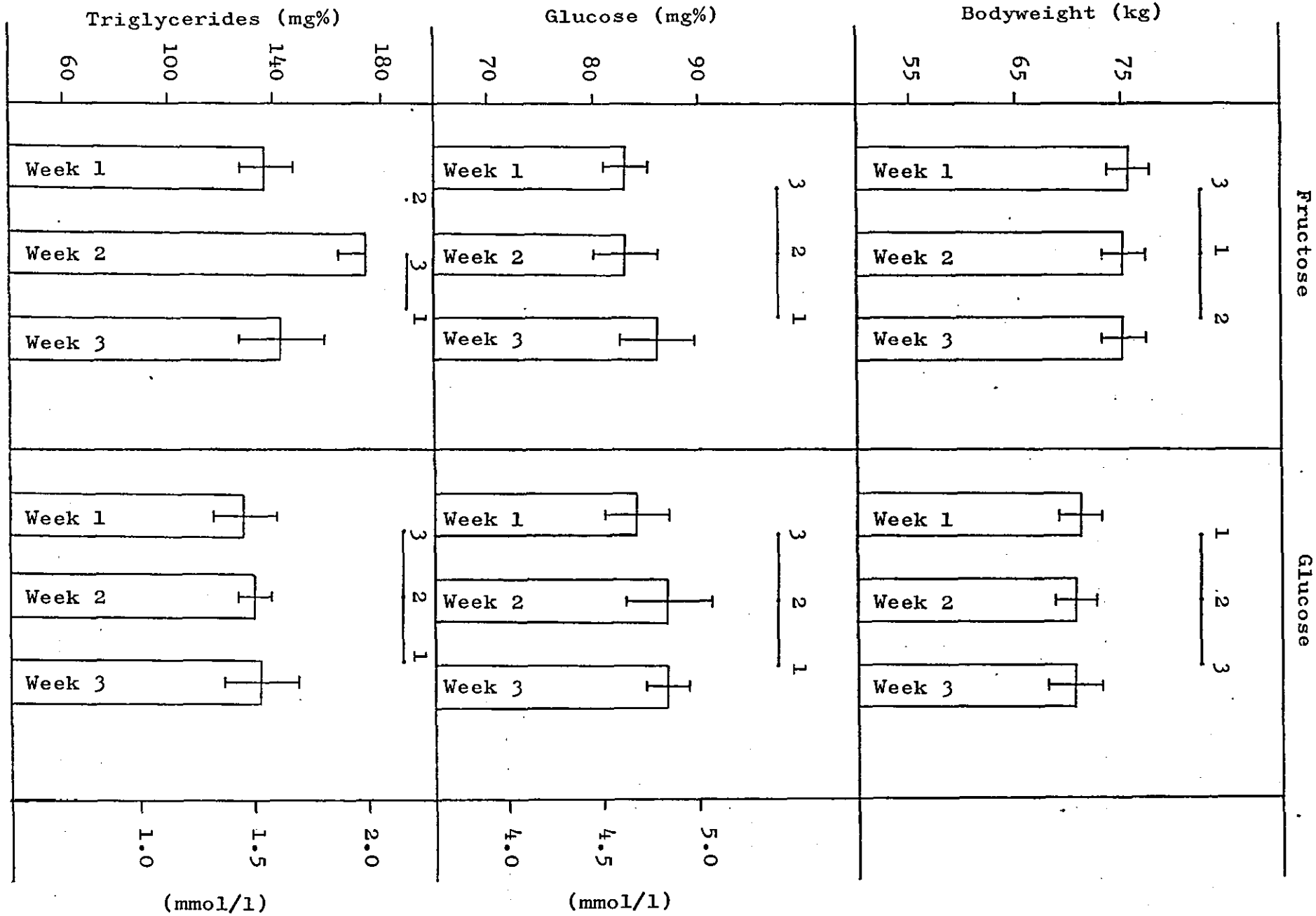


Figure 3.5.2 HORMONAL EFFECTS - FEMALE DATE.

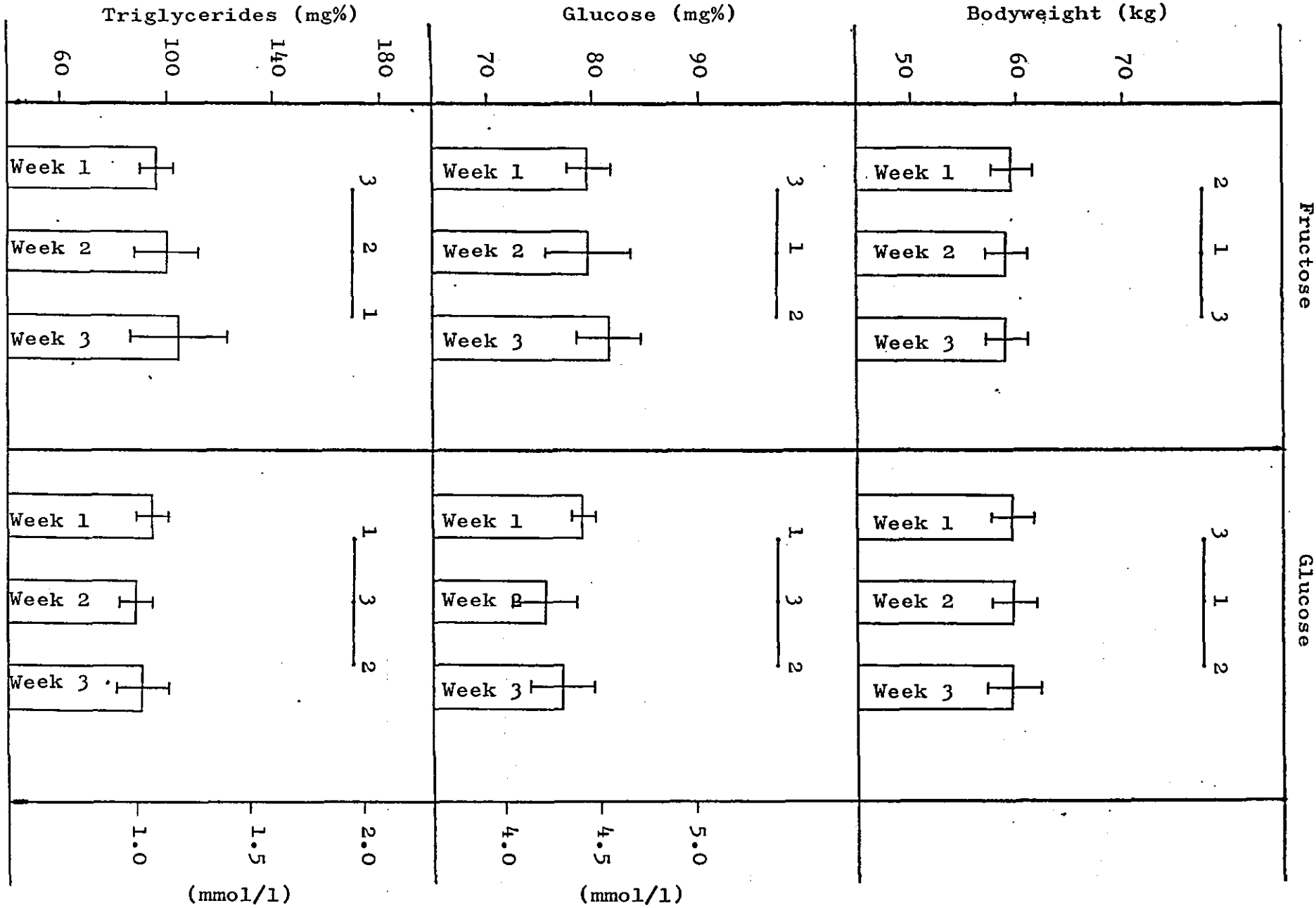


Figure 3.5.2 HORMONAL EFFECTS - MALE DATA.

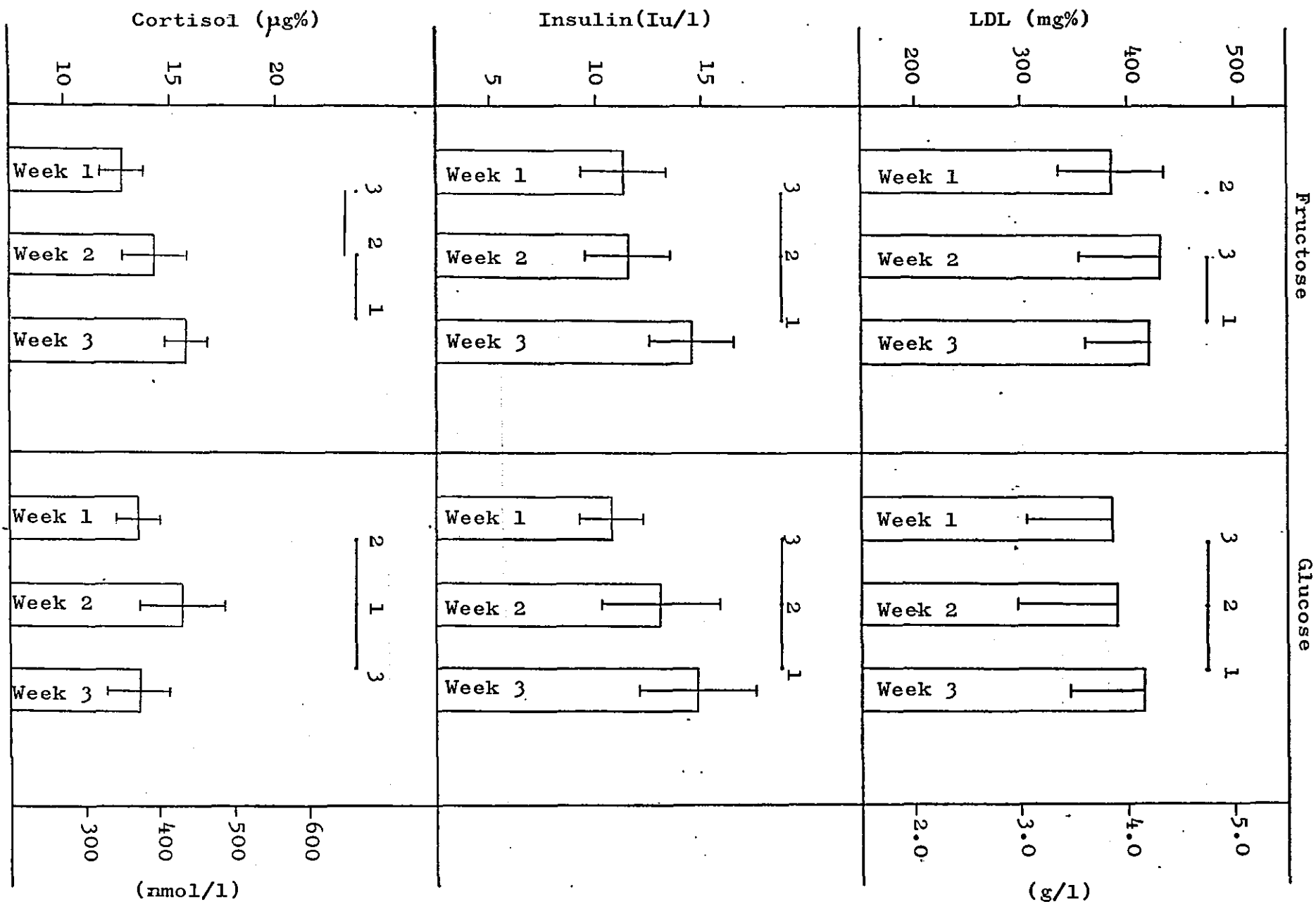


Figure 3.5.2 HORMONAL EFFECTS - FEMALE DATA.

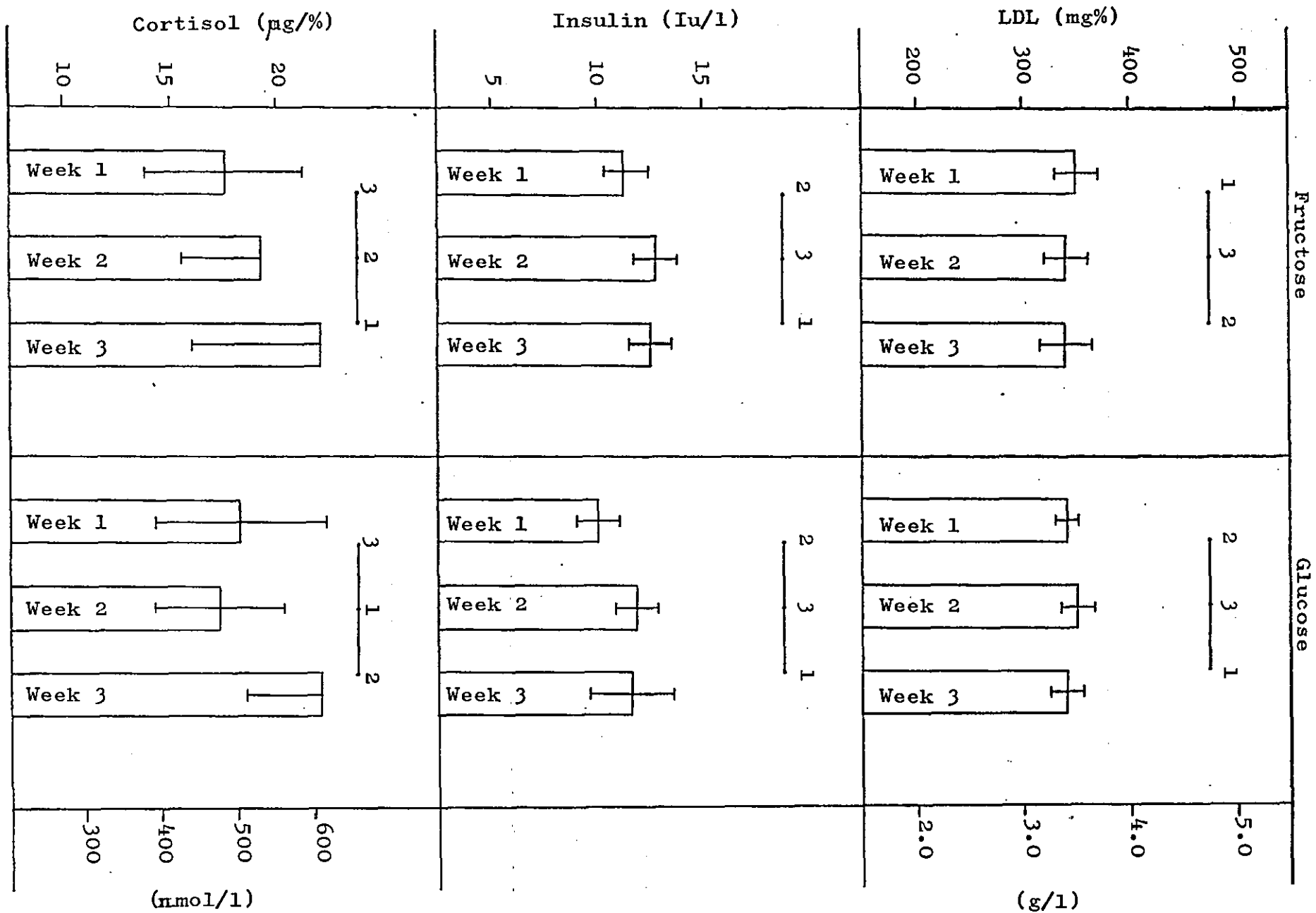


Figure 3.5.2 HORMONAL EFFECTS - MALE DATA.

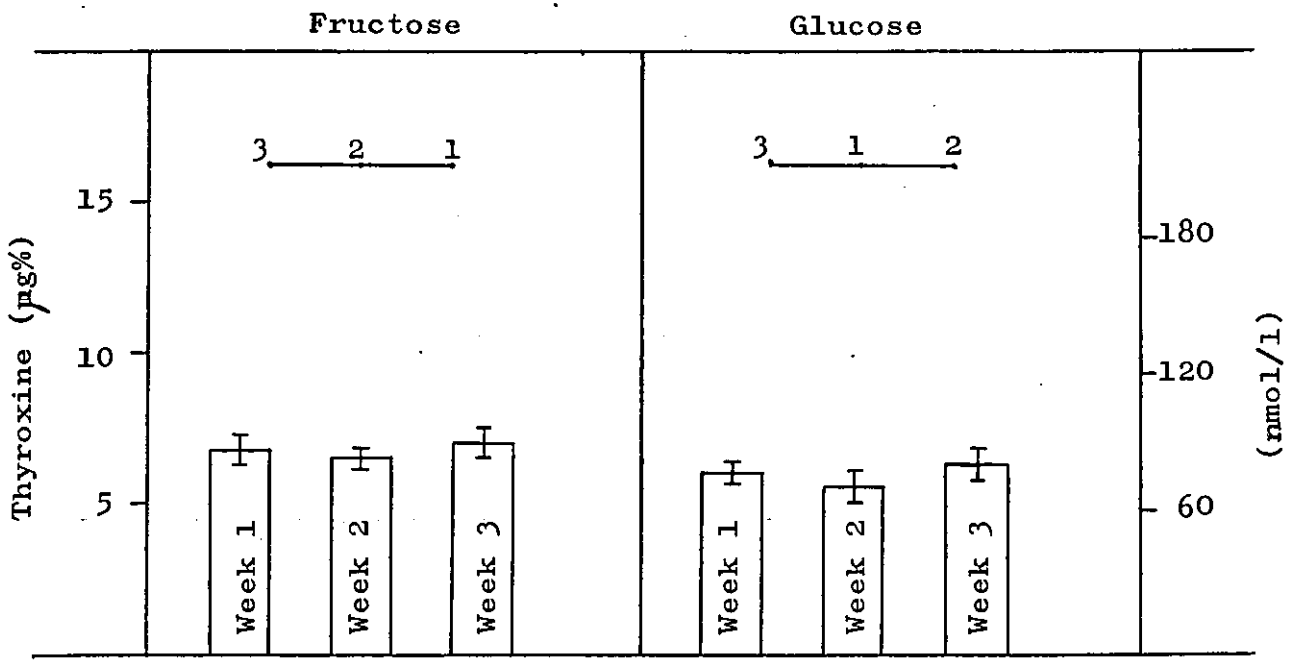
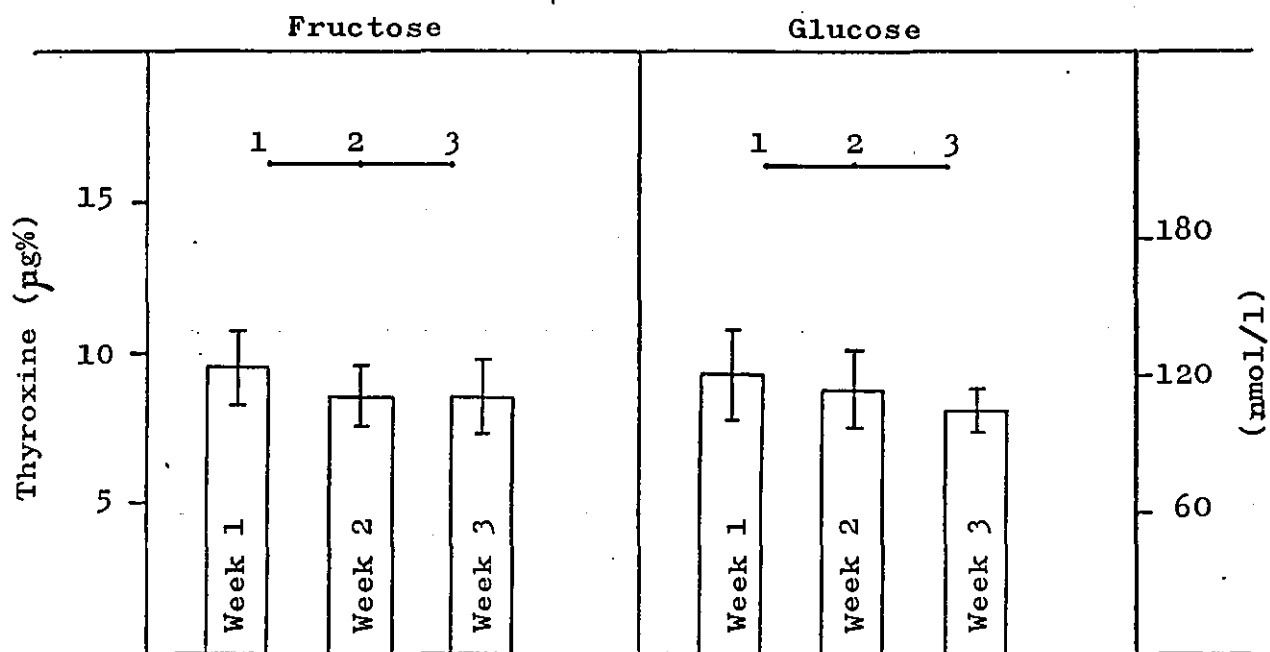


Figure 3.5.2 HORMONAL EFFECTS - FEMALE DATA.

3.5.4 DISCUSSION OF RESULTS.

The following discussion considers each variable monitored throughout the trial.

3.5.4.1 BODYWEIGHT.

For all groups no significant differences were shown between bodyweight recorded on days 8, 15, and 22. Thus, additions of glucose or fructose to the diet of young adults for a period of seven days and subsequent removal of this "extra" sugar had no effect on bodyweight. This result would probably have been achieved by compensatory mechanisms serving to regulate caloric intake, or by physiological responses acting on either an increase or decrease in caloric intake to ensure bodyweight maintenance.

It should be emphasised that this is a short-term observation and may not in any way indicate the effects that would occur over a longer time period. For example an uncompensated dietary change that might occur during the period of sugar increase, coupled with an absence of a physiological mechanism regulating bodyweight during periods of caloric excess, would obviously result in weight gain. The result of this combination of factors may not be observed in a short-term study.

3.5.4.2 BLOOD GLUCOSE.

For all groups no significant differences were shown between the fasting blood glucose concentration recorded on days 8, 15, and 22. Thus additions of glucose or fructose to the diets of young adults over a period of seven days and the subsequent removal of this "extra" sugar had no effect on blood glucose concentration. This is in agreement with the results obtained in the previous study and the same arguments apply.

3.5.4.3 BLOOD TRIGLYCERIDES.

For group 1 a significant difference was observed between the blood triglyceride concentration recorded on days 8, 15, and 22. For all other groups no significant differences were shown.

Group 1.

The blood triglyceride concentration recorded on day 15 was significantly greater than on days 8 and 22. No other differences were shown.

The triglyceride elevation, recorded on day 15, above the level determined on day 8 is in agreement with the

result in the previous study. However, although the triglyceride level on day 22 was directionally greater than on day 8, on this occasion this elevation failed to reach significance.

Fructose uptake by the liver, increased provision of triglyceride precursors, and subsequent enhanced triglyceride synthesis, and the time course of VLDL production and removal from circulation, as previously detailed, can account for the observed triglyceride elevation on day 15. That triglyceride levels had returned to near base-line levels by day 22 indicates that the seven day period of increased fructose intake had no lasting effect on hepatic triglyceride synthesis during the seven days after fructose removal. During this period (days 15 - 22) the elevated blood level of triglyceride recorded on day 15 is cleared and pre-fructose increase levels are achieved. Thus the liver responded during the period of increased fructose intake by raising the level of triglyceride synthesis, but when the "fructose-hepatic-triglyceride drive" was removed the liver reverted to previous levels of triglyceride synthesis.

For all other groups the triglyceride responses recorded in this study are in agreement with the findings of the previous study.

3.5.4.4 BLOOD LDL.

For group 1 a significant difference was observed between the blood LDL concentration recorded on days 8, 15, and 22. For all other groups no significant differences were shown.

Group 1.

No significant differences were shown between the blood concentration of LDL recorded on days 22 and 15 both of which were significantly greater than the LDL level observed on day 8. This result is in agreement with that observed in the previous study.

On day 15 blood levels of triglyceride and LDL were significantly elevated over their respective day 8 values. This observation results from a sequence of events of which the initial step is the provision of increased levels of hepatic triglyceride precursors. This leads to a raised level of hepatic triglyceride and VLDL synthesis which on degradation increases the blood LDL concentration.

The difference in the clearing rates of VLDL and LDL can account for the persistence of elevated LDL levels at day 22, VLDL being cleared in 4 - 5 hours and LDL in 2 - 5 days. Thus although hepatic triglyceride

levels return to pre-fructose increase levels by day 22 the increase in triglyceride synthesis during days 8 - 15 exerts an effect on LDL levels on day 22 as a result of the slow clearance of LDL from the blood.

For all other groups the LDL response recorded in this study are in agreement with the findings of the previous study.

3.5.4.5 BLOOD INSULIN.

For all groups no significant differences were shown between the fasting blood concentration of insulin recorded on days 8, 15, and 22. Thus additions of glucose or fructose at a level of 1g/kg BW per day for seven days to the free-choice diets of young adults, and the subsequent withdrawing of this supplement had no demonstrable effect on the level of insulin in fasting blood.

This result is not in agreement with the findings of Szanto and Yudkin (1969) who compared fasting insulin levels and insulin response to oral glucose when subjects were fed diets high in sucrose (and therefore fructose) or high in starch over a fourteen day period. Both fasting insulin and insulin response during a glucose tolerance test were found to be significantly

higher after the feeding of the diet high in sucrose in comparison to the high starch diet, the base-line values, and after the rest period before dietary crossover.

A possible explanation for this discrepancy between the two sets of data is the level at which sucrose (and therefore fructose) was fed during the test period., During this period subjects received on average 442g of sucrose per day which would provide a considerably higher level of fructose than was administered in the above study. It is particularly interesting to note that in the study conducted by Szanto and Yudkin (1969) that the significant finding, of a raised fasting insulin level and enhanced insulin response to oral glucose, was due to only six of the nineteen subjects responding in that way. These subjects also demonstrated an increase in platelet adhesiveness and a much greater weight gain over the sucrose-trial period than the remainder of the group.

An animal study conducted by Blakely, Hallfrisch, Reiser and Prather, (1981) using rats fed fructose at a level typical of the American diet (approximately 14% of the total caloric intake) has exhibited similar findings to those of Szanto and Yudkin (1969). Rats receiving a diet containing 15% fructose recorded significantly higher

fasting blood glucose and insulin levels, and a greater insulin and glucose response to oral glucose than control rats. The carbohydrate component of the control diet contained 54% cooked corn starch in comparison to the fructose group which consisted of 15% fructose and 39% cooked corn starch.

Similar findings to those of Blakely et al (1981) in studies using rats have been reported by other workers (Hallfrisch, Cohen and Reiser, 1981; Kanarck and Orthen-Gambill, 1982). In both cases either fructose or sucrose diets resulted in a decreased ability to tolerate glucose in comparison to control groups fed glucose, starch, or a standard diet of a comparable level. In studies where glucose tolerance is impaired during the feeding of diets containing fructose some form of insulin antagonism is being observed. Either this is a direct effect of fructose on the actions of insulin or it is the result of a physiological effect induced by fructose.

However, the above animal studies were all conducted over a long time period (minimum duration 7 weeks) and the results differ from short-term studies. Lin and Anderson (1977), Sugawa-Katayama and Morita (1977) and Merkens et al (1980) using rats to compare the short term (maximum 25 days) effects of diets high in fructose, glucose, or starch have not observed any impairment of

glucose tolerance. Thus, the study duration is probably another factor that can account for the discrepancy in the results, a longer time period being required for the demonstration of insulin antagonism.

3.5.4.6 BLOOD CORTISOL.

For group 1 a significant difference was observed between the fasting blood cortisol concentrations recorded on days 8, 15, and 22. For all other groups no significant differences were shown.

Group 1.

The blood cortisol concentration recorded on day 22 was significantly greater than that observed on day 8. No other differences were shown. This result indicates that the entire test procedure results in an increase in circulating cortisol only when fructose is administered during the increase period and only when male subjects are involved. In a study conducted by Yudkin (1972) where young male subjects consumed high sucrose diets a rise in blood cortisol level was observed in some subjects. It is interesting to note that the group of subjects observed by Yudkin (1972) that exhibited this effect also demonstrated a rise in blood insulin concentration.

It would appear from these results that fructose by some mechanism acts to elicit a "stress-type" response although the stimulus of fructose does not seem to exert an immediate effect. The drive acting to produce this effect must either be due to the direct influence of repeated and persistent elevations in blood fructose or as a consequence of another internal environmental disturbance initiated by fructose. The lag time between the fructose-stimulus and the adrenal cortex response suggests that either the stimulus was particularly weak and repeated action was required or that a build up of a certain metabolite was required in order to evoke a response.

It is an important finding that cortisol levels increased over the trial period due to the insulin-antagonistic actions of cortisol. It is interesting to note that changes in insulin concentration occurred in a similar step-wise pattern observed for changes in cortisol concentration.

This result illustrates a "dual" influence of fructose on the internal environment. One action of fructose in this respect is to cause a direct disturbance of lipid metabolism which is augmented by the further action of fructose on cortisol release. Both influences on the internal environment are in a direction that

increase the risk of degenerative disease development.

For all other groups the study procedure had no influence on the level of circulating cortisol.

3.5.4.7 BLOOD THYROXINE.

For all groups no significant differences were observed between the fasting blood concentrations recorded on days 8, 15, and 22. Thus additions of glucose or fructose to the diets of young adults over a period of seven days and the subsequent removal of this "extra" sugar had no effect on blood thyroxine concentration.

3.5.5. GENERAL DISCUSSION AND CONCLUSION.

With the exception of the concentration of triglycerides in fasting blood on day 22 recorded for group 1, the results from the above study are in agreement with those recorded in the previous study. Thus the addition of fructose and glucose at a level of 1g/kg BW per day for seven days to the "free-choice" diet of young female adults or the addition of glucose at the same level to young male adults has no effect on the fasting blood concentration of glucose, triglycerides, LDL or on bodyweight, either immediately after the increase period (day 15) or seven days after return to typical diets (day 22). In addition for the same groups (2, 3

and 4) the study procedure had no effect on the fasting blood concentration of insulin, cortisol or thyroxine.

In contrast to groups 2, 3, and 4, and in agreement with previous observations, the addition of fructose at a level of 1g/kg BW per day for seven days to the "free-choice" diets of young adult males had an effect on the fasting blood concentration of triglycerides and LDL, immediately after the sugar increase period (day 15) for triglycerides and LDL, and seven days after subjects returned to their typical diets (day 22) for LDL. In all cases significant elevations of triglycerides (day 15) and LDL (day 15 and 22) were observed with respect to base-line (day 8) determinations.

The return of blood triglyceride levels to near base-line values by day 22 is not in agreement with the findings of the previous study. In this study hepatic function, with respect to triglyceride synthesis, was only altered during the period of fructose increase, when elevated levels of triglyceride precursors were available and not after the increase period when hepatic function resumed pre-fructose increase levels of triglyceride synthesis. Thus the dietary change that occurred from day 8 to day 15 had no lasting effect on hepatic triglyceride synthesis indicating that the liver only responded to meet the extra demand necessitated by the abundance of triglyceride precursors that occurred during

the period of increased fructose intake.

LDL levels remained elevated seven days after group 1 subjects had returned to their typical diets. It has been argued that LDL levels remain elevated over this period when triglyceride levels have returned to base-line values as a consequence of the relatively slow removal rate of LDL from circulation. This finding is of particular importance since it demonstrates that the initiator of the metabolic sequence leading to raised LDL levels need only be applied at intervals and not continuously. In this case the initiator was increased dietary fructose.

When fructose was administered during the period of sugar increase to young adult males the study procedure had no effect on the fasting blood concentration of insulin or thyroxine. The fasting blood concentration of cortisol was influenced during this particular study procedure, levels being elevated significantly with respect to base-line values seven days after subjects had returned to their typical diets (day 22). This response may have been due to the repeated additions of fructose, and the subsequent persistent elevations of blood fructose, acting either directly on the mechanisms associated with cortisol release or indirectly as a consequence of fructose metabolism. That elevations in blood cortisol were not immediate may reflect both the

extent of this particular stimulus required and also the typical response to the nature of this stimulus. Alternatively the sudden removal of a large proportion of fructose from the diet may have produced this response although at day 15 cortisol levels were already directionally greater than base-line values. In any event the study procedure resulted in a significant elevation of cortisol when fructose was administered to young male adults.

The step-wise pattern of cortisol increases that occurred were mirrored by similar changes in the fasting blood concentration of insulin. Cortisol is an insulin antagonist and in order for blood glucose maintenance to be achieved insulin secretion would be required to increase. This is an important consequence of a raised cortisol level that might lead eventually to β -cell exhaustion and diabetes mellitus. Even without frank diabetes mellitus, for an individual demonstrating elevated cortisol levels signifies a move in the direction of the production of an internal environment associated with diabetes mellitus i.e. insulin resistance. This may represent an initial step to the production of diabetes mellitus and associated diseases.

This study illustrates that fructose, in young adult males, can act in two ways towards the production of

an internal environment that is conducive to degenerative disease development. A "pure metabolic" route has been previously described whereby an abundance of hepatic triglyceride precursors, provided by fructose, results in persistent elevated LDL levels. An alternative route has also been indicated whereby fructose may elicit a "stress-type" response resulting in elevated blood cortisol levels. The importance of this observation is that cortisol is not only an insulin antagonist but also augments the purely "metabolic" actions of fructose.

From the above study two obvious questions arise:

i) Does the concentration of blood transcortin rise over the study period so that although total cortisol increases, the level of "free" physiologically active cortisol remains constant? ii) Is the increase in blood cortisol concentration observed over the test procedure a result of the large fluctuations in dietary fructose, or a direct consequence of persistently elevated blood fructose levels acting on the mechanisms controlling cortisol release? These questions are examined in further detail in the final experimental section where the effects of high fructose diets on the output of urinary "free" cortisol are investigated over the same test procedure.

3.6 EFFECTS IN YOUNG ADULT MALES OF FRUCTOSE AND GLUCOSE
ADDITIONS ON THE COMPOSITION OF THE FREE-CHOICE DIET
AND ON THE OUTPUT OF URINARY FREE CORTISOL.

This experimental section follows a similar design to the two previous studies in that subjects are exposed to a seven day period when their sugar intake (fructose or glucose) is increased by 1g/kg BW per day. The previous studies have illustrated that a consistent effect of increasing fructose intake by this amount in male subjects is to elevate the fasting blood concentration of triglycerides and LDL immediately after the increase period. In the case of LDL this rise persists for at least seven days after subjects return to their typical diets. In addition male subjects ingesting fructose during the test procedure demonstrate a significant increase in the fasting blood concentration of cortisol.

When considering the promotion of an internal environment conducive to degenerative disease development cortisol elevation is an important physiological effect of fructose since cortisol antagonises the actions of insulin

and also augments the detrimental metabolic effects of fructose highlighted above. However, the previous study determined total cortisol and although this level increased this does not demonstrate that the concentration of "free" physiologically active cortisol also rose. A purpose of this study is to examine the effect of the test procedure adopted in sections 3.4 and 3.5, using male subjects, on the urinary output of free cortisol.

The observation that the concentration of cortisol in fasting blood increases over the study procedure (section 3.5) can be interpreted either as a result of persistently high blood fructose levels acting slowly on the mechanisms controlling cortisol release, or alternatively this effect could be due to the large fluctuation in fructose intakes that occurred. This study explores further these alternative possibilities, in this case by the examination of urinary free cortisol output over the last day of the period of sugar increase (glucose and fructose) and seven days after subjects return to their typical diet.

An additional purpose of this study is to examine the dietary changes that occur to the free-choice diet of subjects during the period of increased sugar intake. This is achieved by a full dietary analysis.

3.6.1 EXPERIMENTAL DESIGN.

Essentially the design of this study follows the same format as the previously reported studies investigating the physiological effects of fructose and glucose. However, only male subjects participated in this study and all individuals received both sugars. In addition a full dietary analysis was conducted to determine the changes, if any that occur to the diet during the periods of sugar increase.

3.6.1.1 SUBJECT DETAILS.

Eight young adult males (range 19 - 27 years) participated in this study. All subjects were apparently healthy and non-obese. Subjects were provided with full details of the study programme (see appendix) and a questionnaire, previously detailed, to eliminate subjects thought to be at any risk as a result of their participation.

3.6.1.2 SUBJECT PARTICIPATION.

Subjects received increases of dietary fructose and glucose (1g/kg BW per day for seven days) added to their typical free-choice diets. Four subjects received fructose first during the period of sugar increase and the remainder glucose. A period of four weeks separated

the crossover to the second sugar. The complete diet of all subjects was recorded over a seven day base-line period and during the seven day sugar increase.

For each sugar tested subjects adhered to the following procedure.

Day 1 - 7 Pre-sugar increase. Recording of complete dietary details. Day 6 - 7 first 24 hour urine collection.

Day 8 - 14 Sugar increase period. Recording of complete dietary details. Addition of 1g/kg BW of glucose or fructose to free-choice diets.
Day 13 - 14 second 24 hour urine collection.

Day 15 - 22 Return to typical diet.
Day 21 - 22 third 24 hour urine collection.

3.6.1.3 TYPICAL DIETS, DIETARY RECORDING, AND INCREASED SUGAR ALLOWANCE.

Subjects, as in previous sugar increase studies, were instructed to maintain their typical diets and life-style as precisely as possible throughout the study. Food forms and instructions on their correct completion were provided (see appendix). Analysis of diet for energy, starch and dextrans, sugar, protein and fat was achieved by use of standard food tables (Paul and Southgate, 1985). Sugar was provided in the appropriate quantities in

seven sealed bags. Subjects were instructed to dissolve the contents of one bag per day in hot water and to ingest before 10 am on each sugar increase day.

3.6.1.4 URINE COLLECTION AND ANALYSIS.

Twenty-four hour urine samples were collected from each subject at the appropriate times. Subjects were fully informed of the correct collection procedure to be adopted to ensure a complete 24 hour urine sample (see appendix).

During the collection period urine was collected in polythene pots and stored in chilled "picnic-style" insulated hampers. On completion of each 24 hour urine collection the volume was determined and after thorough mixing a 10ml sample was removed and stored at -20°C for later analysis.

Analysis of urine for free cortisol was conducted on duplicate samples using a radioimmunoassay technique marketed by Amersham International plc. All analyses were conducted by the author.

3.6.1.5 STATISTICAL ANALYSIS.

Data transformation has included both parts of the study in the calculation of the percentage contribution. This

combination has been conducted since the same subjects received both sugars. Standard analysis of variance techniques have been employed to analyse transformed data. A significant difference has been accepted when $p \leq 0.05$. This occurrence is indicated by an asterisk symbol as in previous sections. Detailed comparison of means have been conducted, where appropriate, using the Newman-Keuls method.

3.6.2 RESULTS.

Data summaries, analysis of variance summary tables and detailed comparison of means are listed in the appendix. Results are illustrated in figures 3.6.1 and 3.6.2. Figure 3.6.1 contains urinary output of free cortisol data and figure 3.6.2 dietary recording data. Histogram bars have been identified by week numbers, 1, 2, and 3 for figure 3.6.1 and 1 and 2 for figure 3.6.2, which correspond to urine collections over days 6-7, 13-14 and 21-22. and dietary recordings obtained over days 1-7 and 8-14 respectively. The mean value \pm SEM are illustrated on all figures. Figures contain statistical summaries, the method employed has previously been described. On the statistical summaries the various stages of the study have been identified as follows:

F1 Week 1 fructose part of study.

F2	"	2	"	"	"	"
F3	"	3	"	"	"	"
G1	"	1	glucose	"	"	"
G2	"	2	"	"	"	"
G3	"	3	"	"	"	"

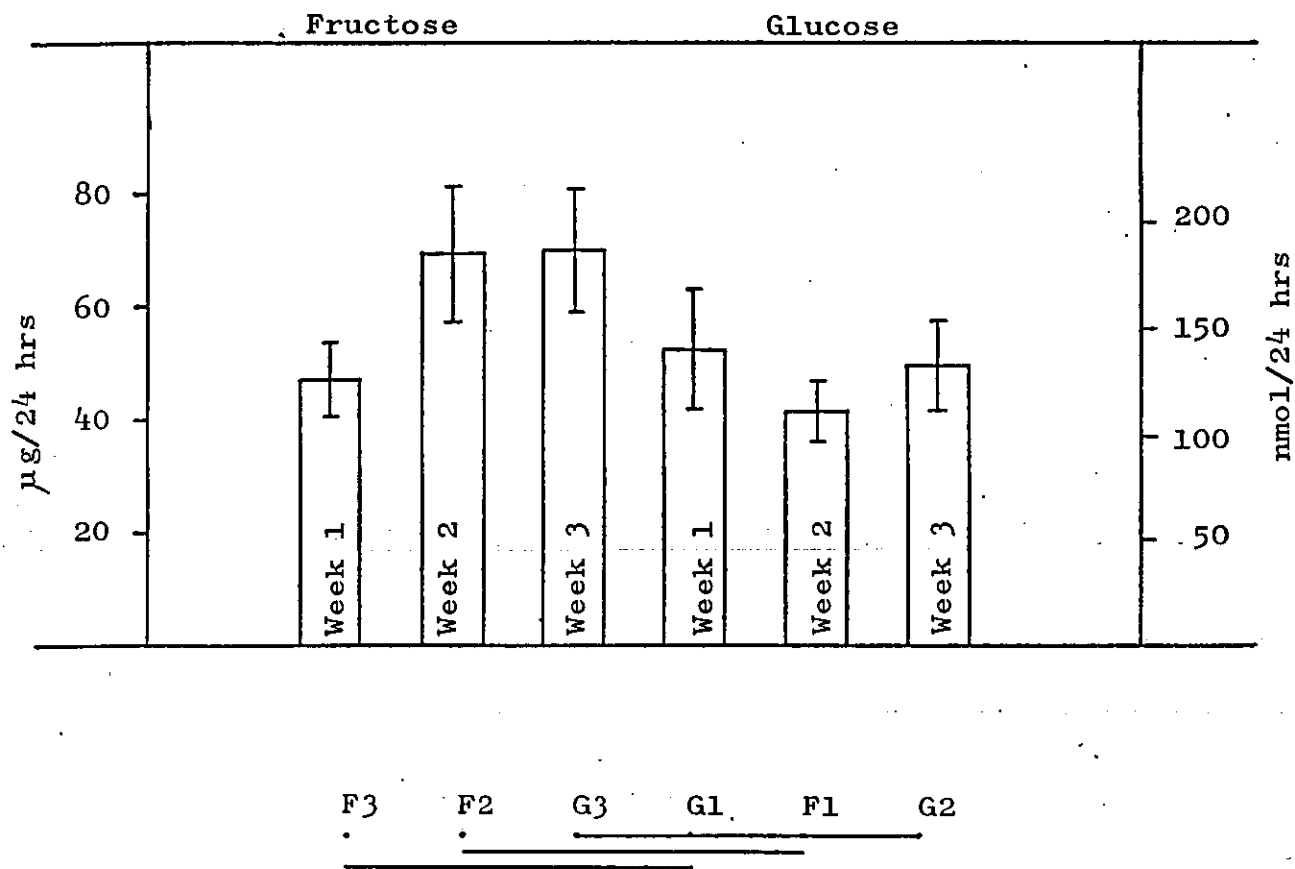
Figure 3.6.1 URINARY FREE CORTISOL OUTPUT.

Figure 3.6.2 DIETARY RECORDINGS.

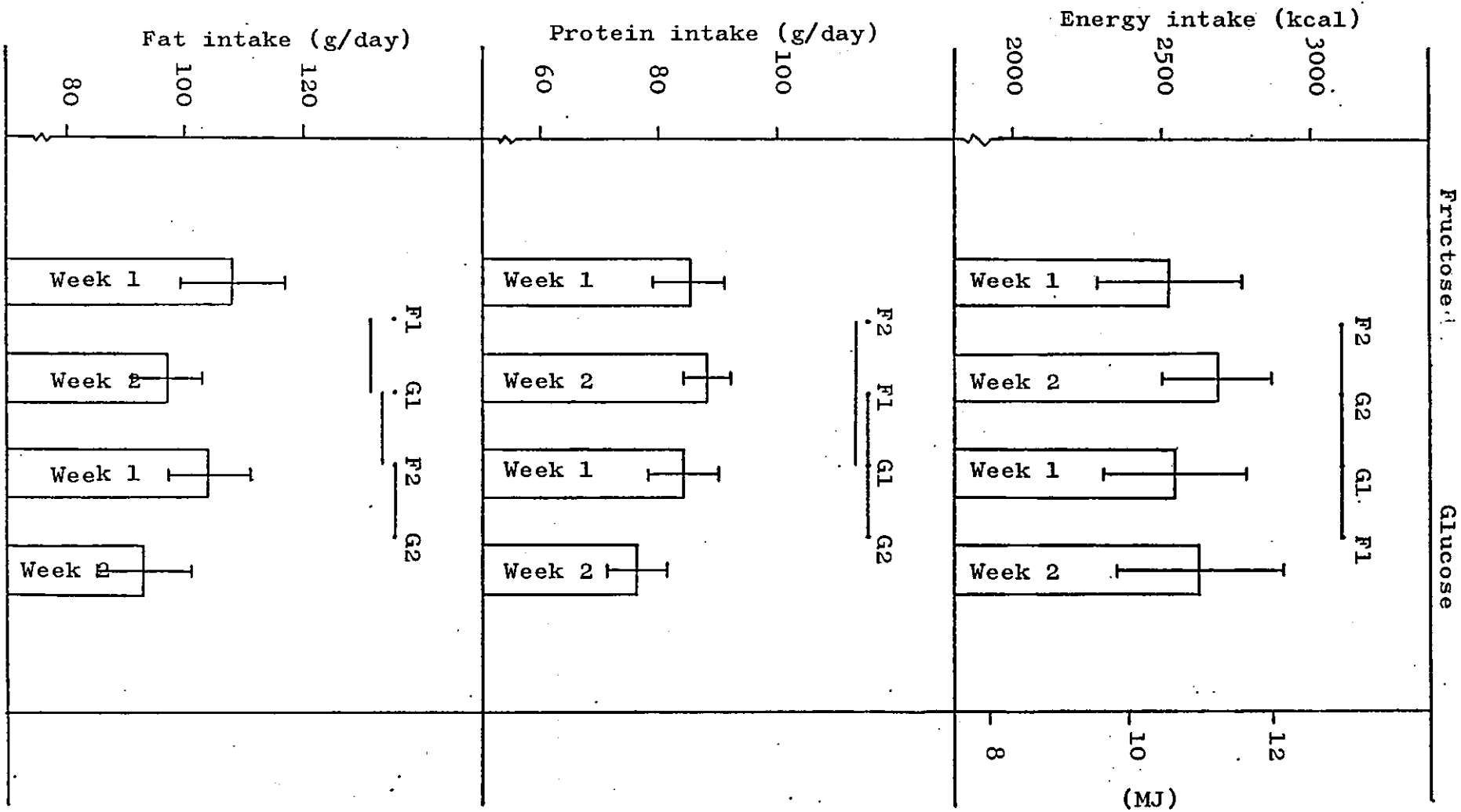
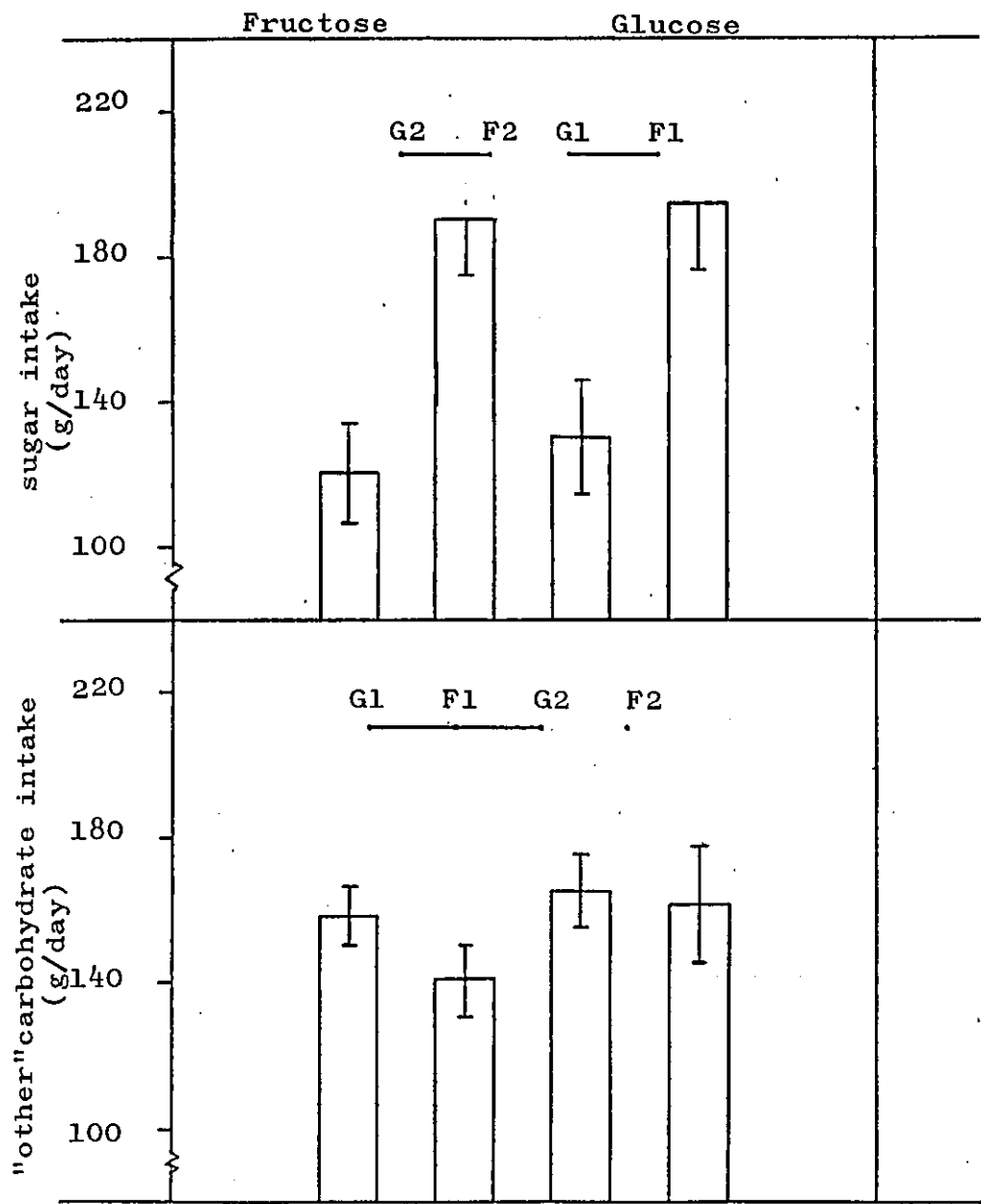


Figure 3.6.2 DIETARY RECORDINGS.



3.6.3 DISCUSSION AND CONCLUSION.

3.6.3.1 URINARY OUTPUT OF FREE CORTISOL.

A number of checks on the possible variability of the urinary output between collections were undertaken. These included urine volume, urine content of urobilinogen, blood, bilirubin, ketones, glucose and protein, pH and specific gravity. Determinations were (with the exception of volume) performed using test strips (Ames Division, Miles Laboratories) and refractometry. All values were normal and consistent for subjects between collections. Outputs of urinary free cortisol for all subjects were within typical limits for the technique employed.

The following discussion incorporates the abbreviations used on the statistical summary figures in order to identify conveniently the various stages of the study.

A significant difference between the urinary outputs of free cortisol recorded during the various parts of the study was observed. Cortisol output over days 21-22 (F3) was significantly greater than that recorded over days 6-7 (F1) and days 13-14 (G2). Cortisol output over days 13-14 (F2) was significantly greater than that observed over days 13-14 (G2). No other differences were shown.

These results support the findings of the previous study where the effects of diets high in fructose and glucose

on the fasting blood level of cortisol were examined. This indicates that the increases in total cortisol observed in fasting blood, in this case, reflected changes in unbound cortisol and thus physiologically-active cortisol.

On the last day of fructose administration cortisol output approached a significant elevation over levels recorded during days 6-7 (F1). During the same period in the glucose part of the study (days 13-14 G2) urinary cortisol output tended to be lower than that observed over day 6-7 (G1). This opposite movement produced the result of a significant elevation of cortisol output during the last day of fructose administration in comparison to cortisol output during the last day of increased dietary glucose. Thus fructose and glucose influence the functioning of the hypothalamic-pituitary-adrenal axis in different ways. Of particular relevance to the theme of this thesis is the consequence of dietary substitution of glucose by fructose which would appear to lead to an increase in circulating cortisol with the accompanying detrimental changes in metabolism.

The mechanism that produces an elevation in cortisol output during high fructose intakes, in comparison to high glucose intakes, appears to continue to function when fructose administration has ceased. Again, as highlighted in the previous study, the overall study procedure must be considered as a possible explanation for this occurrence although this only appears to have

an effect on cortisol output when fructose is the sugar added to and withdrawn from the diet. This may indicate that persistently elevated fructose levels gradually influence the functioning of the hypothalamic-pituitary-adrenal axis and that this response once initiated is slow to return to the previous levels of activity. Alternatively the system may be sensitive to widely fluctuating levels of fructose that occurred in this study, in a minor form during increase periods and in a severe form immediately afterwards. This has particularly interesting dietary-change implications, the above results indicating that the hypothalamic-pituitary-adrenal axis is sensitive to fluctuating levels of dietary fructose. Not only does the system appear to respond when fructose levels are elevated but also when they are reduced from a high level.

The above results demonstrate a type of response of the adrenal cortex that is typical during periods of emotional stress. In this case however, the stimulus responsible was dietary manipulation. This observation initially provides a useful insight into the route whereby dietary change can shift the internal environment in a direction that is characteristic of a degenerative disease. In this particular case the "route" is via the production of a stress response with an associated elevation of cortisol

which in turn leads to raised levels of insulin, glucose and cholesterol levels - characteristic of diabetes mellitus. This shift would be achieved by the physiological antagonism of insulin by cortisol. In addition a possible link between, and the order of which biochemical lesions of degenerative diseases appear is clearly indicated. The initial shift occurs towards an internal environment characteristic of diabetes mellitus which includes the presence of hypercholesterolaemia - one of the main recognised risk factors for CHD.

3.6.3.2 DIETARY RECORDING.

No significant differences were shown between base-line energy intakes and levels recorded during periods of increased sugar intake. However, during both dietary sugar increase periods energy intakes tended to be higher than the respective base-line values. For fructose this was more pronounced. A possible explanation for this occurrence might be the different energy cost of metabolism of the different sugars (by virtue of differing metabolic handling) or a poorer initial utilisation of fructose. Thus the energy is recorded as "intake" but a proportion of energy provided by fructose may have not been available and therefore the "true" energy intakes essentially remained identical, i.e. energy intake is exaggerated.

A significant difference was observed between protein intakes recorded on days 8 - 14 (F2) and days 8 - 14 (G2). No other significant differences were recorded.

Protein intakes during periods of dietary increases of fructose and glucose tended to move in opposite directions from their respective base-line values. Over days 8 - 14 (F2) protein intakes tended to increase whilst during days 8 - 14 (G2) protein intake tended to fall. The combined effect of these changes resulted in the significant elevation of protein intake during days 8 - 14 (F2) over protein intakes during days 8 - 14 (G2).

Significant differences were observed between the levels of fat intake recorded during the different stages of the study. No significant difference was observed between the intakes of fat recorded during both periods when dietary sugar was increased but during these periods both fat intakes were significantly reduced in comparison to their respective base-line values. Fat intake was also significantly reduced during days 8 - 14 (G2) in comparison to days 1 - 7 (F1) but dietary fat levels recorded over days 8 - 14 (F2) were not significantly below the value observed over days 1 - 7 (G1). No other significant differences were observed.

No significant difference was observed between sugar intakes recorded during increase periods both of which were significantly greater than either levels recorded during base-line sugar intake assessment. No other significant differences were observed.

A significant difference between the level of "other" carbohydrate intake was observed during the various recording periods. No significant differences were observed between the base-line intakes and the level during dietary glucose increase, all of which were significantly higher than during the fructose increase period.

The dietary composition recorded during all study periods can be described as being typical for a prosperous nation (Davidson et al, 1975). However, the intake of sugar during periods of dietary sugar increase should be considered to approach the maximum levels.

During the periods of sugar increase a disruption of the base-line diet was observed. When glucose was added to the diet this disruption involved sugar, protein (in comparison to fructose changes only) and fat intake whilst when fructose was added to the diet alterations in the intakes of sugar, other carbohydrates and fat were observed. Since energy

intakes were not significantly increased the periods when sugar was added to the free-choice diets an overall attempt to maintain base-line levels of energy intake was observed.

The significant increases in sugar intakes can be accounted for by the glucose and fructose additions. Control of sugar intake, to regulate overall caloric intake, appeared to be minimal, the tendency being for added sugar to be superimposed on the free-choice base-line levels.

This occurrence indicates a short-term inflexibility of individuals to modify the intake of this dietary constituent. A possible explanation is that the changes that were required to reduce sugar intakes to base-line levels would involve significant changes in dietary habits; for example, limiting the addition of sugar to tea and coffee. From this outlook the sugar appears to serve the purpose of satisfying a habitual need rather than one of nutrition.

In contrast to sugar intakes, a vast variety of foods exist to satisfy other nutritional needs, the intake of which can be modified, perhaps only in quantity, to maintain overall energy intake, nutritional well-being, and also satisfy the demands of palatability.

Energy intake was maintained during the period of increased sugar intake. However, this was achieved by different alterations in the balance of the prime foodstuffs for the two sugars. The control of food intake that determines the nature of a meal was thus influenced in different ways by fructose and glucose.

The different insulinogenic properties of fructose and glucose and their varying tissue distribution following ingestion has been discussed and could account for the different changes in diet that occurred to achieve energy intake maintenance. These differing physiological occurrences would produce a difference in the input of particular components to the overall system that is geared to the detection of the availability of nutrients and the determination of food choice. Thus glucose would act largely via an insulin and fat store mediated component of the mechanism whereby fructose would operate primarily via liver-mediated control. Although energy intake is maintained in both cases the "extra" energy provided by fructose must be considered to be initially inappropriately located.

PART 4

OVERVIEW

4. OVERVIEW.

4.1 AIMS AND FINDINGS.

The first purpose of this thesis was to determine if a need existed for the examination of the physiological effects of fructose with respect to diet and degenerative disease. This was investigated by consideration of the changing dietary patterns in affluent societies, epidemiological data centred on diet and disease, dietary studies examining the effects of sugars on the internal environment, and reports published by experts and expert committees. After establishing a "need" a series of studies were designed to investigate the possible involvement of fructose in the development of degenerative disease by careful selection of a number of physiological parameters. From these studies a number of valuable insights into the effects of fructose on the internal environment have been gained.

Sucrose intake has increased considerably during the period when affluent diseases such as CHD, diabetes mellitus and gout have increased in prevalence. During the same period total carbohydrate intake has declined slightly. The net effect of these changes is an increase in fructose in the diet (from sucrose) and a reduction in the contribution to the total carbohydrate

intake by glucose, i.e., glucose has been partially replaced by fructose. As a result fructose can be considered an important and a new component in the diet due to the levels that are now consumed and thus the physiological effects of this sugar require investigation.

Diets high in sucrose (or fructose) typically elevate the fasting blood concentration of triglycerides when compared to base-line levels or when starch is the carbohydrate source.. In some cases the level of blood cholesterol has also been observed to increase. These are important findings as a consequence of the possible relationships between the blood levels of certain blood lipid fractions and the development of degenerative disease. However, studies that have demonstrated the above effects have all involved the ingestion of large quantities of the carbohydrate under study. This would both badly disrupt a diet and produce unrealistic conditions to what would occur when "natural" dietary changes take place. Again indications are that further work should be conducted in this area to determine the role of sugars on influencing the internal environment.

Within the area of the diet/disease debate there is considerable controversy over the possible involvement

of sugar in the development of degenerative disease. Considering all the information currently available expert committees, set up to examine the present day diet and to recommend changes for the purpose of improving the quality of life, have recommended only that sugar should be reduced in the diet for the purpose of lessening the risk of obesity development. Although obesity itself is an extremely important affluent disease, and has associations with other degenerative diseases, sugar may have a role, other than a "passive" one of the provision of empty calories, that acts through other routes to impair the quality of life. However, the recommendations of the expert committies fail to demonstrate the full extent of the controversy regarding sugar and disease. This is highlighted excellently by the following extracts.

"First my research on coronary heart disease has convinced me beyond doubt that sugar plays a considerable part in this terrifying modern epidemic." Yudkin (1972).

"Firstly, although evidence is incomplete, such evidence as is available does not significantly incriminate sugar." (Walker, 1971).

This is a clear indication that further work should

be conducted in this area in order to clarify what at present is a confusing and contradictory situation.

In this thesis fructose and glucose have been compared in young adults. To compare the physiological effects of these two sugars is a logical exercise since, as pointed out above, fructose has partly replaced glucose in the diet. A second comparison has also been conducted between males and females. The reason for this comparison is that pre-menopausal females do not typically demonstrate the sucrose-induced hypertriglyceridic effect, nor do they usually develop CHD. The first study conducted involved the examination of the blood sugar profiles following oral ingestion of fructose and glucose at a level of 1g/kg BW.

Marked differences were shown between the profiles following oral fructose and glucose in both males and females. No differences were observed between males and females. Blood glucose following glucose demonstrated a typical pattern rising rapidly to a peak then falling equally rapidly to below base-line values. Blood fructose following fructose by contrast rose slowly and remained elevated for the entire duration of the test ($2\frac{1}{4}$ hours). The differences in profiles have been interpreted in a manner that is relevant to the central issue of this thesis i.e., the diet/disease debate.

The initial consequence of a partial substitution of glucose by fructose or simply an increase of fructose in the diet would be an elevation in the level of fructose in the blood for a prolonged period. Thus the effect of this dietary change would effectively be to introduce a novel energy carrying substance to the blood that persists over a long time period. Fructose and glucose are isomers but the exchange is essentially between unrelated substances due to the paramount importance of structure in biological systems. An important point to note is that the glucose profile is determined primarily by insulin the role of which ensures that this form of energy is distributed to the appropriate tissues. Fructose does not directly stimulate insulin release in man although fructose conversion to glucose and the subsequent elevation of glucose would stimulate insulin secretion. Insulin release under such circumstances results in a fall of blood glucose but appears to have no effect on blood fructose level. It would appear therefore, that fructose (as an energy form equivalent to glucose) enters a system that is primarily geared to physiologically cope with glucose. The only consequence of this difference between physiological effects that was observed in this study that is of particular relevance is that fructose persists in the blood. That fructose promotes different

physiological effects to glucose, apart from persistence in the blood, was examined in the second study.

The second study conducted involved the addition of 1g of glucose or fructose/kg BW per day for seven days to the free choice diets of young adults.

The addition of 1g of sugar/kg BW per day provided a diet high in sugar, with respect to base-line values, but in comparison to earlier reported studies, this level of addition is low and more realistic of a dietary change. In addition sugar was added to free-choice diets which is what would occur "naturally" if a new dietary component became abundant or popular. Four groups participated in this study male fructose (group 1), male glucose (group 2), female fructose (group 3) and female glucose (group 4). For each group comparisons were conducted between the physiological determinations recorded for the base-line, immediately after the seven day sugar increase (day 8) and seven days after subjects had returned to their typical diets.

The physiological effects examined were selected to provide indications about the change (if any) in the "quality" of the internal environment with respect to degenerative disease. The physiological effects

examined were; the fasting blood concentration of glucose, NEFA, cholesterol esters, LDL, VLDL, and uric acid, and blood coagulation time and body-weight maintenance. Bodyweight maintenance was of particular importance since additional calories were added to the diet and if weight change occurred changes in other physiological determinations may have been related to this event rather than the effects of the type of sugar added to the diet. The free sugar content of the diet was also examined over the study period since the addition of sugar during the increase period may have been compensated for by removal from the free-choice diet.

Significant changes, that resulted in a movement within the typical limits in a direction thought to be conducive to degenerative disease development, were confined to group 1. These changes involved the blood lipid profile and blood coagulation time.. An increased level of hepatic triglyceride precursors, provided by fructose, and the slow removal rate of LDL from circulation have been put forward to account for the changes in the lipid profile for group 1. The persistence of both triglyceride and LDL elevations after fructose removal is an important finding since this indicates that dietary levels of fructose do not have to remain elevated in order to cause a disruption

in the lipid profile that lasts for at least seven days. The mode of action of fructose resulting in a reduction in blood coagulation time cannot be deduced from this study or from the literature. However, irrespective of the precise mechanism involved a reduction in blood coagulation time must be viewed as a detrimental effect. Further work in this area is obviously required.

With respect to the lipid profile and blood coagulation time groups 2, 3, and 4 did not respond in a similar way to group 1. For group 3 a protective mechanism that involves oestrogenic activity has been put forward to account for the difference with group 1.

Groups 2 and 4 ingested glucose during the period of sugar increase. No detrimental changes were observed in blood coagulation time or in the lipid profile. The most likely explanation, that has been discussed, for this occurrence is that as a result of glucose-induced insulin secretion the increased levels of glucose were distributed mainly to adipose tissue and muscle as a direct consequence of insulin function. Therefore the excess carbohydrate in the form of glucose was accommodated by the peripheral tissues and not by the liver. Thus in the case of increased glucose in the diet the liver was not presented with

a surplus of triglyceride precursors as was the case when dietary fructose levels were elevated.

For all groups the study procedure had no effect on the fasting blood concentration of glucose, NEFA, and uric acid. For these metabolites the immediate concentration changes that are known to occur have no lasting effect on their later fasting concentration. In addition for all groups the study procedure had no effect on bodyweight or the amount of free sugar ingested in the free-choice diet. For bodyweight maintenance changes in the levels of other dietary constituents may have occurred to account for this effect. Alternatively physiological mechanisms geared to control bodyweight during times of caloric excess may have acted.

The third study was designed to examine the effects of the above study procedure on the fasting blood concentration of insulin, cortisol and thyroxine. These hormones were selected because of their pronounced effect on the internal environment in times of health and disease. In addition alternatives to a "purely metabolic - detrimental effect" of fructose were explored in this study.

Bodyweight, background sugar, and the fasting blood

concentration of glucose, triglycerides, and LDL were also determined. By inclusion of these physiological parameters it was possible to both confirm the results of the previous study and also to determine what effects were occurring concurrently.

With the exception of the level of blood triglycerides recorded in fasting blood at day 22 for group 1 (which had fallen to base-line levels) results for all parameters recorded in the previous study were confirmed. For hormonal events the only significant difference observed occurred in male fructose group. This difference involved the blood level of cortisol which was significantly elevated above base-line values at day 22. In addition the step-wise increase in cortisol was mirrored by a similar trend in blood insulin levels.

The increase in blood cortisol can be thought of as a "procedure effect" i.e., the addition and then removal of the sugar caused this effect. However, it is important to note that this occurred only in males when the sugar increased and withdrawn was fructose. An alternative explanation is that fructose, or a breakdown product of fructose, caused the elevation of cortisol and that the response observed is due to the nature of this type of stimulus.

The importance of this observation i.e., cortisol elevation, has been emphasised in that cortisol not only antagonises the peripheral role of insulin but also promotes the environment that the purely metabolic effects of fructose lead to. Both influences (of cortisol) lead to a "shift" of the internal environment in a direction thought to be more conducive to degenerative disease development. Thus, fructose would also appear to influence the internal environment by two routes by a "purely metabolic" effect and by increasing the level of cortisol in fasting blood. However, the techniques employed for determination of cortisol in fasting blood recorded total cortisol and this might not indicate the extent to which the level of "free" physiologically active cortisol was influenced. In order to investigate further the effect of fructose on cortisol levels study 4 was set up. This study examined the effects of increased dietary fructose (and glucose for control) on the urinary output of free cortisol in young adult males. In addition during this study the complete diet was recorded before and during the period of increased sugar intake.

The design of study 4 differs slightly to the previous studies in that a recording was made during the last day of increased sugar intake and not immediately

afterwards. This modification was included in order to determine if cortisol levels were increasing during the increase period and not as a consequence of "extra" sugar withdrawal. Comparisons of the urinary output of free cortisol were made between base-line, day 14 (last day of sugar increase) and day 22 (seven days after return to typical diets). All estimations of urinary free cortisol were based on 24 hour urine samples.

Cortisol output over day 14 was directionally greater than the base-line value and significantly different from the output recorded over this period when glucose was fed. After removal of "extra" fructose the cortisol output was significantly greater than the base-line value. Results indicate that the endocrine response to diets high in fructose and glucose are different and in addition demonstrate that raised cortisol levels persist after fructose removal. The consequences and detrimental effects of elevated cortisol levels have been discussed.

In the first two studies, that involved the addition of 1g of sugar/kg BW per day for seven days to free-choice diets, a significant elevation of sugar intake was observed. A possible outcome of this particular design may have been a reduction in "background" sugar to compensate for sugar addition. Since this

was not observed extra calories were consumed in the form of sugar during the sugar-increase period. These two studies indicated that either adjustments to the free-choice diet itself occurred (apart from sugar intake) or physiological mechanisms acted to achieve bodyweight maintenance during the period of caloric excess. Results from the final study indicate that the former explanation is more likely.

During periods of increased fructose or glucose intake the background free-choice diet was disrupted and energy intake was preserved. However, during the period of fructose addition "other" carbohydrate and fat were reduced whilst during the glucose increase period levels of fat and protein (in comparison to fructose) were restricted. The different dietary modifications observed could be triggered by the initial metabolic handling of fructose and glucose. Thus, although the overall mechanism achieved caloric restriction in both cases, (perhaps by the recognition of the state of energy stores) different components of this mechanism were stimulated by fructose and glucose. Differences between initial sugar location, major sites of metabolism, and hormonal responses to the two sugars are likely candidates for the stimulation of different parts of the mechanism controlling energy intake.

The displacement of certain prime foodstuffs during the periods of sugar increase, although achieving weight maintenance, could have other significant effects in the long-term. This dietary displacement indicates clearly the substitution of nutrient-rich components by molecules that provide as their only nutritional contribution - energy. Thus in the long term at least an imbalance of nutrients might be anticipated.

4.2 IMPLICATIONS AND SPECULATION.

Currently nutrition features prominently in the "headlines" and the public are becoming increasingly more aware or confused about the relationship between diet and disease. By far the most commonly considered dietary component is fat. It is interesting to note that "scientific terms" such as saturates, polyunsaturates and cholesterol are now part of the laymans vocabulary. Sugar is also frequently mentioned but rarely sucrose and seldom if ever fructose. To a certain extent this bias represents the difference in the importance of these dietary components with respect to degenerative disease that has been revealed from the various types of studies discussed previously. As a result of these findings the bias may have increased as more experimental effort is applied to examine the role of dietary fat in disease development. Studies

conducted in this thesis clearly demonstrate that fructose can influence the internal environment in a "detrimental" direction by two different mechanisms. It is therefore of great importance to appreciate the extent to which fructose exerts this influence, and to place the above findings in true perspective with respect to recent dietary change and disease.

Although it has been emphasised that the studies conducted were designed to provide low level increases in dietary sugar what resulted were diets at the extreme or just beyond the maximum of the range for normal sugar intakes in the United Kingdom. However, the "normal" sugar intake currently observed in the United Kingdom would have been totally abnormal 100 years ago. Therefore, over a relatively short period for man a new raw material has become increasingly important in the provision of energy - fructose - and what are considered normal levels should be viewed with caution. Thus at the onset before sugar addition diets should properly be considered high sugar diets.

The influence of fructose has been examined and when dietary levels are elevated in males important changes to blood cholesterol, coagulation and cortisol are observed. Again these changes occurred within the typical limits for individuals who at the onset consumed considerably more fructose than their

predecessors 100 years ago. Would the blood values at the beginning of the studies of triglycerides, LDL, cortisol and coagulation time also have been considered abnormal 100 years ago in line with the fructose intakes? Perhaps the stepwise increase in the contribution of fructose to the diet has been reflected in equal changes to what have become accepted as the normal concentration of certain blood constituents. The studies conducted simply extended the rise in fructose intakes, although rather rapidly in the progression, and important changes were observed with respect to current views on degenerative disease development.

The increased contribution of fructose to the diet must be dealt with by existing physiological mechanisms that have evolved to deal with a different energy supply. The system is certainly adaptable and copes with the dietary change in the short-term, but what are the long term consequences? Without looking in detail at the comparative metabolism of fructose and glucose one might consider that they are treated in the same manner and since no immediate physiological crisis is observed assume that increased dietary fructose is, in terms of health, of no consequence. However, evidence from the literature and from the dietary studies show clearly that this is not the case.

A biological system relies on structure for recognition and function. With this in mind it should not be at all surprising to find that the physiological handling of fructose and glucose is different. This difference includes absorption, conversion, transport, deposition of metabolites and endocrine responses. In terms of the quality of the internal environments produced by the varying physiological responses to these sugars fructose, in males, must be considered to be of poorer quality.

Fructose has been shown to act in two ways to produce an "inferior" environment. The "metabolic" route is essentially brought about by the organ location and principal site of metabolism of fructose following loading and the failure of fructose to stimulate insulin release and to benefit from insulin release in the same manner as glucose. In addition the absorption profile of fructose clearly demonstrates that a blood reservoir of fructose, destined for hepatic metabolism, persists for a considerable time following loading. The net effect is an elevated level of LDL and triglycerides.

The elevation of cortisol after periods of fructose feeding provides an additional and extremely important mechanism whereby the internal environment can be disrupted. Additionally this particular mechanism provides a valuable insight into a possible sequence of

events in disease manifestation.

That fructose elevates cortisol levels poses the interesting question - does fructose act as an internal stress to achieve this response? i.e., the observed response is produced as a result of the detection of an internal stress. This response could obviously only be achieved by the presence of elevated blood fructose levels that would have occurred as fructose increases its contribution to the diet. The idea that fructose produces a stress response might seem highly speculative but before the recent interest in diet and disease the same would have been thought of the notion that dietary fat had a role to play in CHD development.

Increased levels of cortisol produce a shift in the internal environment towards that characteristic of diabetes mellitus, including an elevated level of blood cholesterol - one of the main risk factors for CHD. This is achieved partly by insulin antagonism. Thus an endocrine response to fructose is capable of initiating an environmental change in a direction characteristic of one degenerative disease that includes, as one of its metabolic lesions, a major risk factor for another.

Peripheral insulin antagonism results in an increased

work load on pancreatic β -cells as an attempt is made to achieve blood glucose maintenance. It has been put forward that although this response prevents an immediate and severe hyperglycaemia the increased work load eventually leads to β -cell exhaustion. Through this progression in a "diabetic direction" dietary glucose (from starch or glucose) would become treated increasingly like fructose as a consequence of impaired insulin action. As a result the tissue distribution of glucose would be altered as glucose entry to adipose tissue and muscle becomes less efficient. In this way more hepatic triglyceride precursors would become available from glucose leading eventually to a raised LDL concentration. It is fascinating to note that fructose "behaves" like glucose in diabetes mellitus and as a consequence of an endocrine response to fructose slowly induces glucose to act in the same way.

The control of blood levels of cholesterol by dietary means or by drug treatment is of great importance in reducing the risk of and lessening the progression of CHD. Many factors influence cholesterol levels and obviously the more that can be controlled the better are the chances of achieving a reduction when required. Indications are that fructose, and sucrose, can elevate serum LDL and in an attempt to regulate cholesterol

levels should be included in a comprehensive dietetic control. Thus the reduction of sugar (or at least those containing fructose) in the diet should be viewed as playing a far more important role than "simply" reducing the risk of obesity development.

An important aspect of the cholesterol-elevating property of fructose is that the mechanisms by which this is achieved can be explained within the bounds of accepted physiology.

The above discussion has necessarily drawn on the experimental findings of this thesis and on the contribution of many others previously acknowledged. The major difference between the design of studies conducted here is that sugar additions were made to free-choice diets and the entire diet was not manipulated as part of the original design. The purpose of this approach was to mimic a "real-life" dietary situation when a component of the diet increases its overall contribution. This may happen when a particular component becomes more abundant or as a result of seasonal variation (e.g., Macdonald (1977) has estimated that during the summer months the consumption of soft drinks in 10-15 year olds may increase to a point where 30-35 per cent of the total energy intake is accounted for by this route).

Under the free-choice conditions of the studies conducted "detrimental" physiological changes were observed in male

subjects during periods of increased fructose intake. Analysis of the effect of sugar additions to the free-choice diet revealed that these changes occurred despite a disruption of diet which included a reduction in dietary fat intake. This perhaps indicates the "powerful" influence of fructose on the internal environment, an effect which is not easily masked when the remainder of the diet is free to vary.

The lack of control over the subjects' diet might be viewed as seriously limiting the value of the data obtained. However, this lack of control, as highlighted above, was intended and does represent as closely as possible a situation that occurs naturally. Studies conducted were of short-term duration and this again might be considered to limit the value of the observations since adaptation to the altered diets may have taken place. However, it is interesting to note that although the period of increased sugar intake lasted for only seven days, when fructose was provided an effect was observed on serum LDL and cortisol seven days after return to free-choice diets. It has been noted that this indicates that only a periodic high level of fructose need be applied to cause a prolonged LDL and cortisol elevation and not a continuously high fructose intake. In a typical diet it is highly probable that large fluctuations occur to produce days or weeks of

contrastingly high and low sugar intakes. The varied dietary sugar levels would act to inhibit a metabolic modification to an increased sugar intake since the drive to do so would be inconsistent.

Longer studies using this experimental approach (i.e., adding sugar to free-choice diets) would certainly make a valuable contribution in this area - but for how long should they be conducted? It might be the case that in order to make a further contribution by extending the dietary sugar increase period the length of study required may even be considered unethical. Perhaps longer term studies should now take the "opposite" approach and observe the physiological consequences of restricting the level of fructose in existing diets that are at present considered to contain "normal" amounts of this sugar.

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APPENDIX.

Subject Instructions
and Food forms.

IMPORTANT NOTICE TO SUBJECTS.

Please read before participating in this study.

If your answer is "yes" to any of the following questions please do not take part in this experiment.

Do you suffer from any of the following?

- 1) Blood clotting disorders.
- 2) Infectious blood disorders.
- 3) Diabetes mellitus.
- 4) Fructose intolerance.
- 5) Epilepsy.

Are you taking any drugs or medical advice in an attempt to alleviate a metabolic disorder?

Thank you for participating so far.

SUBJECT INFORMATION.

Blood sugar absorption profiles.

Two sugars, fructose and glucose, are under investigation in this study, the purpose of which is to examine their blood absorption profiles in young adults after oral ingestion. Subjects will be required to ingest both sugars, one sugar on the first occasion and the other at the second visit. A fasting and five further blood samples will be taken during each test procedure which lasts approximately $2\frac{3}{4}$ hours. The following time-table illustrates the procedure for one part of the study. (Arrival time can be adjusted for convenience).

8.55 a.m.	Arrive at test station in a fasting condition (free of food for at least 12 hours and of alcohol for 24 hours).
9.25 a.m.	Fasting blood sample withdrawn.
9.30 a.m.	Sugar load administered.
9.45 a.m.	1st post sugar load sample withdrawn.
10.00 a.m.	2nd " " " " "
10.30 a.m.	3rd " " " " "
11.00 a.m.	4th " " " " "
11.45 a.m.	5th " " " " "
11.50 a.m.	Subjects free to leave.

The above procedure will be repeated with the other test sugar approximately four weeks after the first visit.

SUBJECT INFORMATION.Metabolic/Hormonal effects of fructose and glucose.

Both studies (metabolic/hormonal) follow a similar design and last for a period of 22 days. The purpose of these studies is to examine the comparative metabolic and hormonal effects of fructose and glucose in young adults. Over the first seven days of the study base-line sugar intakes will be monitored by the completion of food forms listing foods high in sugar content. On day 8 a fasting blood sample will be drawn and analysed for a variety of blood constituents.

From day 8 to 14 subjects will be required to increase their sugar intake of either fructose or glucose. This will be achieved by the ingestion of sugar loads over this increase period. Sugar will be provided in the appropriate amounts in 7 sealed bags. These will require dissolving in warm water on each increase day. The daily amount of increased sugar allowance will be equal to 1g/kg BW. Thus a 70kg subject will be required to ingest 70g of "extra" sugar per day - approximately 12 teaspoonsfuls. Over the period of increased sugar intake the background sugar will again be monitored. A blood sample will be drawn on day 15 under identical conditions as for day 8.

From day 15 to 21 subjects return to their typical diets and record sugar intakes over this period. On day 22 the final blood sample will be taken. The following time-table summarises the test procedure.

SUBJECT TIMETABLE.

Please follow the timetable carefully.

Day 1 - 7 Recording of baseline sugar intake.

Day 8 Date.

8.55 a.m. Arrive at test station in a
fasting state as instructed.

9.00 a.m.- 9.30 a.m. During this time interval you
will be instructed to rest in a
test cubicle. After blood sampling
you will receive thorough instruct-
ions on how to ingest the sugar
loads provided. You are then
free to leave.

Day 9 Date. Ingest sugar load as instructed.

Day 10 Date. " " " " "

Day 11 Date. " " " " "

Day 12 Date. " " " " "

Day 13 Date. " " " " "

Day 14 Date.

Day 15 Date Repeat as for day 8.

Day 15 - 21 Recording of post sugar increase
dietary sugar levels.

Day 22 Repeat as for days 8 and 15.

INSTRUCTIONS TO SUBJECTS PRIOR TO BLOOD SAMPLING
ON DAYS 8, 15, AND 22.

Day prior to test.

- 1) No alcohol throughout the day.
- 2) Begin fast at 10 p.m. no caffeinated drinks or cigarettes after this time. Water only may be drunk to satisfy any sensation of thirst.

Day of test.

- 1) After 8.30 a.m. do not drink any more water.
- 2) Please wear clothing that is not tight about the upper arms.
- 3) Arrive at the test station by 8.55 a.m. Please try to avoid rushing to the station.

everages	Biscuits	Cakes	Cereals	Fruit	Honey	Jam, Ice Cream, Marmalade	Sweets	Tinned food	Treacle	Yoghur

SUBJECT INFORMATION.

Introduction.

Two sugars, fructose and glucose, are under investigation in this study, the basic aim of which is to observe the effect of high glucose or fructose diets on the urinary output of the hormone cortisol. Cortisol can be considered as a metabolic control molecule, the level of which in the blood influencing many metabolic events. Urinary cortisol provides a reliable indicator of the amount of active cortisol that has been present in the blood during a set time period.

Increased sugar intake causes alterations in the metabolic profile of fasting blood, the mechanism of change being incompletely understood. Theoretically cortisol can promote these changes but the effect of this dietary change on cortisol output is unknown. This study has been designed to determine whether or not cortisol output is influenced by increased dietary sugar.

Subject Involvement.

This study requires a high degree of reliable subject participation in order for results to be obtained that are worthy of serious consideration and analysis.

This study is divided into two main parts which are identical except for the period during increased sugar intake when the sugar given may be either fructose or glucose. If fructose is given first then glucose will be given in the second part.

The order of sugar administration will be randomised and single blind. The design for each part is as follows:

Week 1 (base-line data, diet and cortisol output).

During this period the entire diet is recorded on the forms provided (see instructions to subjects). In addition a 24 hour urine sample will be collected from the morning of day 6 to the morning of day 7, (see instructions to subjects).

Week 2 (dietary change and effect on urinary cortisol output).

Again the diet is recorded during this period. An addition of 1g/kg BW/day to the diet will be made at this stage (see instructions to subjects). On days 13-14 a 24 hour urine sample will be collected.

Week 3 (return to typical diet, urinary cortisol output).

Day 21-22 third 24 hour urine collection.

A break of at least 2 weeks will be given between the addition of one sugar to the diet and the addition of the other sugar.

The diary that follows serves to illustrate one part of the study.

DAY.

1			
2			
3			Recording of
4			pre-sugar
5			increase diet.
6 a.m.	24 hour urine		
7 a.m.	sample.		
8			
9			
10		Addition of	Recording of
11		sugar to the diet.	diet during
12			period of
13 a.m.	24 hour urine		increased
14 a.m.	sample.		sugar intake.
15			
16			
17			
18			
19			
20			
21 a.m.	24 hour urine		
22 a.m.	sample.		

INSTRUCTIONS TO SUBJECTS.

Food forms.

Enter the weight (or volume for beverages) of all foods consumed along with an accurate description of the food item. In addition specify details of cooking, e.g., fried, grilled. For base-line data (days 1-7 inclusive) and for diet data during periods of sugar increase (days 8-14 inclusive) the procedure is the same and diets should be recorded for the entire period of 7 days.

Urine collection.

Urine will be collected over a period of 24 hours on 6 occasions throughout the study. The exact procedure on each collection period will be as follows:

Day prior to 24 hour urine collection.

- 1) No excessive exercise to be carried out.
- 2) No alcoholic beverages to be consumed.
- 3) Retire at a time that is typical for yourself, record this time.

24 hour urine collection.

During this period no alcohol is to be consumed or excessive exercise to be carried out.

- 1) Rise at a time typical for yourself, record this time.
- 2) Void bladder, DO NOT collect this volume, record time.
- 3) Collect all urine volumes from this time for the next 24 hours in the containers provided. Store containers in a cool place.
- 4) On the night of a collection period retire at the same time as on the night prior to the urine collection.
- 5) Rise at the same time as on the morning the urine collection began.
- 6) Void bladder at the same time as on the day the urine collection began, COLLECT this volume.

For all future 24 hour urine collections all conditions and times met in the first collection period must be followed precisely.

Increased Sugar Intake.

Sugar will be provided in sealed packages at the beginning of each sugar increase period. The sugar should be dissolved in hot water and consumed after a meal.

SUBJECT	RECORDING PERIOD		SUGAR
INDIVIDUAL FOOD ITEMS		WEIGHT (g)	COOKING METHOD

Kit Estimations.

Instructions.

Test-Combination

β -Lipoprotein β -Lipoproteins

Colorimetric method
Cat.No. 124 921 for 45 tests

Method 557
Burstein, M., and J. Samuels, (1958), La Presse Médicale 66: 974,
Watson, D. (1960), Clin. Chim. Acta 8: 637.

Test principle
The β -lipoproteins are precipitated with CaCl_2 /heparin and their concentration is determined by complexing the cholesterol fraction (5% on average) with sulfuric acid/acetic acid/acetic anhydride.
Normal values in serum: 360-640 mg/100 ml \pm 3.6-6.4 g/l

References: Preblich, W., and E. Koettgen, (1969), Arzt. Lab. 16: 30.

Sample material
Serum, heparinized plasma.

Reagents
Contents Initial concentrations of solutions:

- 1 Standard cholesterol 200 mg/100 ml
glacial acetic acid 100 g/l
- 2 Heparin/ CaCl_2 0.0125%
heparin 25 mmol/l
 CaCl_2 2.5 mmol/l
NaCl 0.9% 0.9% 0.9%
- 3 Color reagent:
acetic acid 7.0 mol/l
acetic anhydride 6.5 mol/l

Additional reagent:
Sulfuric acid, conc., A.R.

Quality control
For control of accuracy: Precipitation

β -Lipoproteins

Test-Combination

Preparation and stability of solutions

- 1, 2 Use solutions undiluted.
Stable up to the expiry date specified when stored at +15 to 25°C.
Close bottles after use.
- 3

Caution! Strongly corrosive reagents.
Use only safety pipettes.

Sample preparation
Hemolysis interferes with the test.
Perform assay immediately.

Precipitation of β -lipoproteins:

Pipette into 10-ml centrifuge tube:		
sample		0.05 ml
solution 2		2.00 ml
Mix well. Let stand for 15 min at 20-25°C, then centrifuge for 15 min at 3000 rpm. Pour off supernatant carefully and discard. Then let tube stand inverted 30 min on a filter paper for 5 min and remove any residual supernatant with filter paper. Use the precipitate for cholesterol determination.		

Procedure

Wavelength: Hg 578 nm (560-580 nm).
Cuvette: 1 cm light path.
Incubation temperature: 20-25°C.
Measure against blank.

One blank and one standard are sufficient for each assay series.

Pipette into dry test tubes (blank and standard) or onto precipitate in centrifuge tube (sample):

	blank	standard	sample
dist. water	0.05 ml	0.05 ml	0.05 ml
solution 1	2.50 ml	2.50 ml	2.50 ml

Mix and let blank, standard, and sample stand in a water bath for 5 min at 20-25°C. Add:

sulfuric acid	0.50 ml	0.50 ml	0.50 ml
---------------	---------	---------	---------

Mix quickly and leave in water bath for a further 10 min. Pour solutions into dry cuvettes and measure the absorbances of sample (A_{sample}) and standard (A_{standard}) against the blank.

If the cholesterol fraction of β -lipoproteins exceeds 600 mg/100 ml or β -lipoprotein values exceed 1700 mg/100 ml, dilute 0.2 ml serum with 0.2 ml of 0.9% NaCl solution and repeat the assay (result $\times 2$).

Calculation
of the concentration (c) of β -lipoprotein cholesterol and of the β -lipoproteins in the sample:

Component	mg/100 ml	g/l
cholesterol	$c = 200 \times \frac{A_{\text{sample}}}{A_{\text{standard}}}$	$c = 2.0 \times \frac{A_{\text{sample}}}{A_{\text{standard}}}$
β -lipoproteins	$c = 670 \times \frac{A_{\text{sample}}}{A_{\text{standard}}}$	$c = 6.7 \times \frac{A_{\text{sample}}}{A_{\text{standard}}}$

Please note

Use only dry test tubes and glass cuvettes.

It is especially important that the precipitate which has been centrifuged out be completely dissolved upon addition of sulfuric acid. It is occasionally impossible to precipitate the β -lipoproteins from hyperlipemic sera even after dilution.

The cholesterol reaction is very sensitive to temperature, and it is therefore absolutely essential to leave the test tubes in the water bath while adding the sulfuric acid.

During storage at temperatures below 15°C, the glacial acetic acid in bottle 1 may freeze, thereby precipitating the cholesterol. If this happens, place the stoppered bottle in a water bath not above 50°C for 20 min to liquefy the glacial acetic acid and to redissolve the cholesterol completely.

Since sulfuric acid is very viscous, the solutions must be mixed thoroughly.

Solutions 1 and 3 contain acetic acid and acetic anhydride, which are caustic. If solutions come into contact with skin or mucous membranes, flush immediately with large quantities of water. If solutions come into contact with eyes, immediately flush liberally with water and consult an ophthalmologist.

Boehringer Mannheim GmbH
Diagnostics

Jan. 1979

Cholesterol C-system

CHOD-PAP method
Enzymatic colorimetric method

Cat. No. 290319 for 4 x 32 ml
Cat. No. 237574 for 4 x 100 ml
Cat. No. 236691 for 4 x 500 ml

Method
Siedel, J., et al. (1981), *J. Clin. Chem. Clin. Biochem.* 19: 838.
Stähler, F., et al. (1977), *Med. Lab.* 30: 23.
Trinder, P. (1959), *Ann. clin. Biochem.* 6: 24.

Test principle
cholesterol ester + H₂O $\xrightarrow{\text{cholesterol esterase}}$ cholesterol + RCOOH
cholesterol + O₂ $\xrightarrow{\text{cholesterol oxidase}}$ Δ^4 -cholestenone + H₂O₂
2 H₂O₂ + 4-aminophenazone + phenol $\xrightarrow{\text{POD}}$ 4-(p-benzoquinone-mono-
imine)-phenazone + 4 H₂O

Clinical interpretation
The following limits are recommended to permit recognition of
the risk factor hypercholesterolemia:
suspect if above 220 mg/100 ml = 5.7 mmol/l
elevated if above 260 mg/100 ml = 6.7 mmol/l

Reference: Schettler, G., and E. Häsel, (1978), *Arbeitsmed. Sozialmed.*
Präventivmed. 10: 25.

Sample material
Serum, heparinized plasma or EDTA plasma

Reagents
Contents Concentrations
in the test:
Tris buffer 100 mmol/l; pH 7.7
Magnesium aspartate 50 mmol/l
4-aminophenazone 1 mmol/l
phenol 8 mmol/l
3,4-dichlorophenol 8 mmol/l
hydroxyphenylthio-sulfonate 0.3%
cholesterol esterase ≥ 0.4 U/ml
cholesterol oxidase ≥ 200 U/ml
peroxidase ≥ 0.2 U/ml

Quality control
For control of accuracy: PreciNorm® U, PreciPath® U,
PreciLip® E, L
For precision control: PreciNorm® UPX

COLOMBO

Preparation and stability of solutions
Dissolve contents of one bottle of kit with
Cat. No. 290319 in ca. 32 ml redist. water
(fill up to mark on bottle neck)

Cat. No. 237574 in 100 ml redist. water

Cat. No. 236691 in 500 ml redist. water.

The reagent solution is ready to use after 10 minutes.

Stable for four weeks at +2 to +8°C.
seven days at +15 to 25°C.

Sample preparation
The sample can be stored up to six days at +4°C
six days at +20 to 25°C.

Procedure
Wavelength: Hg 546 nm (470-580 nm)
Spectrophotometer: 500 nm

Glass cuvette: 1 cm light path

Incubation temperature: 20-25°C or 37°C

Measure against reagent blank (RB).

One reagent blank is sufficient for each assay series.

Pipette into cuvette:		
	RB	Sample
sample material	-	0.02 ml
reagent solution	2.00 ml	2.00 ml
Mix and incubate RB and sample for 10 min at 20-25°C or 5 min at 37°C. Read absorbance of sample against RB within 1 hour (A_{sample}).		

If values exceed 700 mg/100 ml (= 18.1 mmol/l), dilute
0.1 ml of sample material with 0.2 ml of 0.9% NaCl solution
and repeat assay (result x 3).

Calculation
of the concentration (c) of cholesterol in the sample:

Wavelength	mg/100 ml	mmol/l
Hg 546 nm	$c = 853 \times A_{\text{sample}}$	$c = 22.1 \times A_{\text{sample}}$
500 nm	$c = 575 \times A_{\text{sample}}$	$c = 14.9 \times A_{\text{sample}}$

Please note

If difficulties encountered in quality control point to inadequate linear response on the part of the photometer, or if measurements cannot be taken at Hg 546 nm or 500 nm, a calibration curve must be constructed using PreciLip® Cholesterol (Cat. No. 125512). The cholesterol values are read off the curve.

Hemoglobin concentrations up to 200 mg/100 ml (= 0.124 mmol/l) do not affect the test.



Boehringer Mannheim GmbH
Diagnostica

January 1982

Test-Combination

Non-esterified Fatty Acids

Colorimetric method
Cat.No. 128 055 for 50-150 tests

Method

Duncombe, W. G. (1964), Clin. Chim. Acta 9:122.

Test principle

The non-esterified fatty acids are converted to chloroform-soluble copper salts; the copper in the organic layer is subsequently measured colorimetrically. The concentration of non-esterified fatty acids is proportional to the absorbance of the copper-containing chloroform.

Normal values in serum

0.09-0.6 meq/1000 ml

Reference: Horowitz, P. J. N., et al. (1968), Clin. Chim. Acta 14:89.

Sample material

Serum

Reagents

Contents	Initial concentrations of solutions:
1 Buffer/Cupric nitrate	
triethanolamine buffer	0.45 mol/l, pH 7.8
cupric nitrate	0.27 mol/l
2 Diethyldithiocarbamate	8 mmol/l

Additional reagents:

Chloroform, A.R.

Palmitic acid, A.R. (>99.5%)

or

Stearic acid, A.R. (>99.5%)

Preparation and stability of solutions

- 1 Use solutions undiluted.
- 2 Store bottles tightly closed. Stable up to the expiry date specified when stored at +15 to 25°C.

Use safety pipettes to pipette the chromogen solution. Contact with the mouth and mucous membranes must absolutely be avoided.

Fatty acid standard: 0.5 meq fatty acids/l

Prepare the standard solution by weighing out 12.82 mg of palmitic acid A.R. or 14.22 mg of stearic acid A.R. and rinsing this into a 100-ml volumetric flask with chloroform, subsequently making the solution up to the mark.

Keep flask stoppered and store in a dark place at +15 to 25°C. Stable for six months.

Sample preparation

Since the concentration of non-esterified fatty acids in serum is influenced by many factors, fasting blood should be taken under conditions as nearly standardized as possible.

→ Hemolysis interferes with the test.

Use only fresh serum.

Procedure

Wavelength: Hg 438 nm (420-450 nm)

Cuvette: 1 cm light path

Temperature: 20-25°C

Measure against blank.

One blank and one standard are sufficient for each assay series.

The extraction of the copper salts should be carried out in ground-glass-stoppered centrifuge tubes using a mechanical shaker. (Adhesive tape may be used to prevent the stoppers from working loose during the shaking procedure.)

The stoppers must never be greased.

Normal centrifuge tubes, with plastic stoppers resistant to chloroform, may be used in place of ground-glass-stoppered tubes.

Pipette into 10-ml ground-glass-stoppered centrifuge tubes:

	blank	standard	sample
chloroform	5.0 ml	5.0 ml	5.0 ml
standard	-	0.2 ml	-
serum	-	-	0.2 ml
redist. water	0.2 ml	-	-
solution 1	1.0 ml	1.0 ml	1.0 ml

Shake vigorously for 10 min using a mechanical shaker, then centrifuge for 5 min. Carefully draw off the blue-green aqueous layer (together with the protein layer) by means of a fine-tipped pipette connected to a water-jet aspirator. Care must be taken to remove the layers completely.

Pipette into test tubes:

chloroform layer	2.0 ml	2.0 ml	2.0 ml
solution 2	0.2 ml	0.2 ml	0.2 ml

Mix, and after 10 min read absorbances of sample (A_{sample}) and standard (A_{standard}) against the blank.

If the concentration of non-esterified fatty acids exceeds 2.0 meq/1000 ml serum, repeat the assay with 0.1 ml serum (result x 2).

Calculation

of the concentration (c) of non-esterified fatty acids in serum:

$$c = 0.5 \times \frac{A_{\text{sample}}}{A_{\text{standard}}} \text{ [meq/1000 ml]}$$

Please note

When removing the chloroform layer, care must be taken not to touch the wall of the centrifuge tube with the pipette tip so that traces of the aqueous phase still present do not enter the pipette.

After removal, wipe the outside of the pipette tip with filter paper, and then let the chloroform run into the test tube such that the pipette tip does not come into contact with either the wall of the tube or the liquid in the tube.

The entire contents of the tube must be thoroughly mixed during the shaking process if well-reproducible results are to be obtained.

Small amounts of copper oxide may form on the wall of bottle 1 during storage. This does not interfere with the determination. A small precipitate may form in solution 2 during storage. This does not interfere with the determination.

If the final volume is not sufficient to fill the cuvette, use 3.0 ml of the chloroform layer and add 0.3 ml of solution 2. The calculation remains the same.



Boehringer Mannheim GmbH
Diagnostica

Test-Combination

Triglycerides (Neutral Fat)

UV-method

Cat.No. 124 958 for 3 x 4 tests

Cat.No. 125 032 for 3 x 17 tests

Cat.No. 124 966 for 3 x 35 tests (3 x 100 mg/ml)

Automated Analyze Boehringer Mannheim

Method

Enzymatic triglyceride assay after saponification with ethanolic KOH

References: Eggstein, M. (1969), *Klin. Wochs.* 44: 287-294; Eggstein, M. and F. H. Kreutz, *ibid.* 44: 282; Schmidt, F. M. et al. (1968), *Z. klin. Chem.* 8: 158.

Test principle

Triglycerides $\xrightarrow{\text{KOH}}$ glycerol + fatty acids

glycerol + ATP $\xrightarrow{\text{GK}}$ glycerol-3-phosphate + ADP

ADP + PEP $\xrightarrow{\text{PK}}$ pyruvate + ATP

pyruvate + NADH + H⁺ $\xrightarrow{\text{LDH}}$ lactate + NAD⁺

Clinical interpretation

The following limits are recommended to permit recognition of the risk factor hypertriglyceridemia:

suspect if above 150 mg/100 ml = 1.71 mmol/l

elevated if above 200 mg/100 ml = 2.29 mmol/l

References: Schettler, G. and E. Müllert, (1976), *Arbeitsmed. Sozialmed.* 10: 25.

The following normal values were determined for a selected group of subjects:

74-172 mg/100 ml = 0.85-1.97 mmol/l

References: Eggstein, M. (1969), *Klin. Wochs.* 44: 287.

Sample material

Serum, heparinized plasma or EDTA plasma

Reagents

Controls

1. Buffer

triethanolamine buffer

MgSO₄

0.1 mol/l, pH 7.4

4 mmol/l

2. NADH/ATP/PEP

8 mmol/l, 33 mmol/l, 11 mmol/l

3. LDH/PK

800 U/ml, 130 U/ml

4. GK

150 U/ml

Additional reagents (absolutely glycerol-free):

Ethanol potassium hydroxide, ca. 0.5 N (Cat.No. 125 296)

Magnesium sulfate solution, ca. 0.15 mol/l (Cat.No. 125 318)

Quality control

For control of accuracy: Precinorm® U and Precilip®

For precision control: Precinorm® UPK

For control of accuracy: Precinorm® U and Precilip®

For precision control: Precinorm® UPK

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For precision control: Precinorm® UPK

Preparation and stability of solutions

1. Cat.No. 124 958: Dissolve contents in 40 ml redist. water.

Cat.No. 125 032: Dissolve contents in 150 ml redist. water.

Cat.No. 124 966: Use solution undiluted.

Stable for one year at +15 to +25°C.

2. Cat.No. 124 958: Dissolve contents of one bottle in 0.6 ml redist. water.

Cat.No. 125 032: Dissolve contents of one bottle in 2.0 ml redist. water.

Cat.No. 124 966: Dissolve contents of one bottle in 4.0 ml redist. water.

Stable for two weeks at +4°C.

Use solutions undiluted.

Stable up to the expiry date specified when stored at +2 to +8°C.

3. Sample preparation

The sample can be stored up to three days at +4°C.

Dilute strongly lipemic sera 1+9 with 0.9% NaCl solution (absorbance difference $\Delta A \times 10$).

A reagent blank (RB) must be determined once for each pack by running the assay using redist. water instead of sample. $A_{\text{RB}} = A_{\text{RB}}$

A_{RB} is subtracted from the absorbance difference obtained for each sample.

4. Saponification

Pipette into centrifuge tube:

sample 0.2 ml

ethanolic potassium hydroxide 0.5 ml

Mix, close centrifuge tube with Parafilm® and incubate in water bath for either 20 min at 70°C, 30 min at 55°C, or 60 min at 37°C, then cool to 20-25°C. Add:

magnesium sulfate solution 1.0 ml

Mix well, centrifuge, and use 0.5 ml of the clear supernatant for the determination.

Procedure

Wavelength: Hg 365 nm, 340 nm or Hg 334 nm

Cuvette: 1 cm light path

Incubation temperature: 20-25°C

Measure against air.

Single assays

Pipette into cuvette:

solution 1 2.40 ml

solution 2 0.10 ml

supernatant 0.50 ml

suspension 3 0.02 ml

Mix with plastic spatula and incubate for ca. 10 min at 20-25°C, then measure absorbance A_{A} . Add:

suspension 4 0.02 ml

Mix, and after 10 min read absorbance A_{B} . $A_{\text{B}} - A_{\text{A}} = \Delta A$

Assay series

Prepare a stock solution by mixing the following volumes for every 10 assays: solution 1: 25.0 ml; solution 2: 1.0 ml; suspension 3: 0.2 ml

For larger series, use correspondingly larger volumes. The stock solution is stable for one day at +15 to +25°C or two days at +4°C.

Assay series

Prepare a stock solution by mixing the following volumes for every 10 assays: solution 1: 25.0 ml; solution 2: 1.0 ml; suspension 3: 0.2 ml. For larger series, use correspondingly larger volumes. The stock solution is stable for one day at +15 to +25°C or two days at +4°C.

Pipette into cuvette:	
stock solution	2.50 ml
supernatant	0.50 ml
Mix with plastic spatula and incubate for ca. 10 min at 20-25°C, then measure absorbance A_{A} . Add:	
suspension 4	0.02 ml
Mix, and after 10 min read absorbance A_{B} . $A_{\text{B}} - A_{\text{A}} = \Delta A$	

If the absorbance difference ΔA exceeds 0.400 at Hg 365 nm or 0.800 at Hg 334 nm/340 nm

or the triglyceride concentration exceeds 650 mg/100 ml

the triglyceride concentration exceeds 650 mg/100 ml dilute the sample as specified for strongly lipemic sera under "Sample preparation".

Calculation

The calculation factor is the same for single assays and assay series as the deviation is less than 1%.

Calculate the triglyceride concentration (c) as follows:

Wavelength	Hg 365 nm	340 nm	Hg 334 nm
c [mg/100 ml]	1330 x ΔA	718 x ΔA	732 x ΔA
c [mmol/l]	15.20 x ΔA	8.20 x ΔA	8.37 x ΔA

Please note

Ethanol potassium hydroxide may take on a yellow-brown colour on prolonged storage. This does not affect the test.

To correct for the free glycerol in routine assays, subtract 10 mg triglyceride/100 ml (=0.11 mmol/l) from the triglyceride value calculated above.

When using control sera, please use the nominal value specified by the manufacturer. Instructions for determination of the free glycerol in research investigations are available free of charge on request.

Parafilm® is a registered trademark of Marathon Products, Neenah, Wis./USA.

Boehringer Mannheim GmbH
Diagnostics

Test-Combination

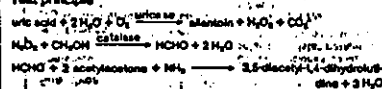
Uric-acid[®]

Uric Acid
Enzymatic colorimetric method
Cat.No. 124 753 for ca. 50 tests
Cat.No. 124 761 for ca. 180 tests

Method
Uricase/catalase/colour reaction

References: Kageyama, H. (1971), Clin. Chim. Acta 31: 421

Test principle



Normal values in serum

men: 3.4–7.0 mg/100 ml = 202–418 μmol/l
women: 2.4–5.7 mg/100 ml = 142–339 μmol/l

References: Thielig, W. et al. (1973), Dtsch. Med. Wochs. 98: 390

Sample material
Serum, heparinized plasma, urine

Reagents

Contents	Initial concentrations of solutions
1. Buffer ammonium phosphate buffer, 0.6 mol/l, pH 7.0 methanol, 1.7 mol/l catalase, 2700 U/ml	
2. Acetylacetone/Methanol 0.42 mol/l; 2.6 mol/l	
3. Standard uric acid 6 mg/100 ml (= 367 μmol/l) Uricase 5 U/ml, 80% glycerol	
5. Uric-acid reagent ammonium phosphate buffer, 0.57 mol/l, pH 7.0 methanol, 1.7 mol/l acetylacetone, 20 mmol/l catalase, 1% (= 270 U/ml)	

Quality control

In the normal range: Precinorm[®] U, Precitip[®]
In the pathologic range: Precipath[®] U
For precision control: Precinorm[®] UPX

Test-Combination

Preparation and stability of solutions

1. Cat.No. 124 753: Dilute contents of one bottle with 50 ml redist. water.
Cat.No. 124 761: Use solution undiluted.
Stable up to the expiry date specified when stored at +4°C.
Close bottle after use.
- 2, 3, and 4. Use solutions undiluted.
Stable up to the expiry date specified when stored at +2 to +8°C.
5. Prepare a stock solution of uric-acid reagent as indicated in the table below at least 30 min prior to starting the assays, and store in a brown bottle.
Stable for one month at +4°C
seven days at +15 to 25°C.
Bring to 20–25°C before use.

No. of samples	solution 1, ml	solution 2, ml	No. of samples	solution 1, ml	solution 2, ml
approx.					
5	30	1.5	80	400	20.0
10	50	2.5	100	500	25.0
20	100	5.0	120	600	30.0
30	150	7.5	160	800	40.0
50	250	12.5	180	900	45.0

Sample preparation

Serum can be stored up to five days at +4°C or +20 to 25°C.

Urine: Dilute 1 part fresh urine with 9 parts by volume of redist. water.

Procedure

Wavelength: Hg 405 nm (400–420 nm)

Spectrophotometer: 410 nm

Glass cuvette: 1 cm light path

Incubation temperature: 37°C

Measure against sample blank.

If readings cannot be taken at 405 or 410 nm, A_{405} must be measured against A_{405} . In this case, the standard (solution 3) is assayed like the sample material.

Pipette onto bottoms of test tubes:		
	sample blank	sample
sample material solution 5 (uric-acid reagent)	0.50 ml 5.00 ml	–
Mix well. Add:		
solution 4		0.02 ml
pipette out of sample-blank test tube		2.50 ml
Mix well and incubate sample and sample blank for at least 60 min at 37°C. Do not expose to direct sunlight! Measure absorbance of sample against sample blank (A_{sample}).		

* Transfer remainder from Pipette back to test tube.

Calculation

a) using a factor:

Calculate the concentration (c) of uric acid as follows:

Wavelength	Hg 405 nm	410 nm
serum or plasma	$c \text{ (mg/100 ml)} = 25 \times \frac{A_{\text{sample}}}{A_{\text{standard}}}$ $c \text{ (μmol/l)} = 1490 \times \frac{A_{\text{sample}}}{A_{\text{standard}}}$	$24.6 \times \frac{A_{\text{sample}}}{A_{\text{standard}}}$ $1460 \times \frac{A_{\text{sample}}}{A_{\text{standard}}}$
urine	$c \text{ (mg/100 ml)} = 250 \times \frac{A_{\text{sample}}}{A_{\text{standard}}}$ $c \text{ (mmol/l)} = 14.9 \times \frac{A_{\text{sample}}}{A_{\text{standard}}}$	$24.6 \times \frac{A_{\text{sample}}}{A_{\text{standard}}}$ $14.6 \times \frac{A_{\text{sample}}}{A_{\text{standard}}}$

b) sample against standard:

	mg/100 ml	μmol/l
serum or plasma	$c = 6 \times \frac{A_{\text{sample}}}{A_{\text{standard}}}$	$c = 357 \times \frac{A_{\text{sample}}}{A_{\text{standard}}}$

A table of values for measurement "sample against standard" is available on request.

	mg/100 ml	mmol/l
urine	$c = 60 \times \frac{A_{\text{sample}}}{A_{\text{standard}}}$	$c = 3.6 \times \frac{A_{\text{sample}}}{A_{\text{standard}}}$

Please note

If the mean absorbance of a quadruplicate measurement lies outside the range $A_{\text{standard}} = 0.240 \pm 0.012$ or $A_{\text{standard}} = 0.244 \pm 0.012$, the uric acid concentration cannot be calculated via the factor, so that measurements must be taken against the standard.

If difficulties point to inadequate linear response on the part of the photometer, construct a calibration curve using Preciset[®] Uric Acid (Cat. No. 125 628) and obtain the uric acid values by reading off the curve. See the pack insert for details.

When running assay series (using flow-through or suction cuvettes), it is advisable to measure all sample blanks first, followed by the samples.

Any turbidity that may appear in solution 1 can be eliminated by filtration. This does not affect the test.

Prolonging the incubation up to one hour beyond the time indicated does not affect the test.

Solutions 1 and 2 contain methanol, which is poisonous. Do not swallow or inhale vapors. Avoid contact with skin. If solution comes into contact with skin or mucous membranes, flush immediately with large quantities of water. If solution comes into contact with eyes, immediately flush liberally with water and consult an ophthalmologist.

Solution 3 contains sodium azide as stabilizer. Ingestion of large quantities can produce vasodilation and a fall in blood pressure. Symptomatic therapy is indicated.

Boehringer Mannheim GmbH
Diagnostics

Oct. 1978

Merckotest[®]
Blood glucose
(GOD-PAP method)

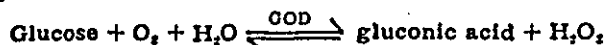
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MERCK

Reagent kit for the photometric determination of the glucose concentration in whole blood, serum and plasma

Principle¹

Glucose oxidase^{**} (GOD) catalyses the oxidation of glucose according to the following equation:



In this reaction hydrogen peroxide is formed which, in the presence of peroxidase^{***} (POD), reacts with 4-aminoantipyrine and 2,4-dichlorophenol. Oxidative coupling gives rise to antipyrilquinonimine, a red dye. The quantity formed of this dye is proportional to the glucose concentration.

Equipment

Spectrophotometer or filter photometer

Reagents

4 × ① colour reagent

1 × ② standard solution

All reagents are stable up to the expiry date stated when sealed and stored in a refrigerator.

Required in addition:

Trichloroacetic acid solution 300 mmole/l for deproteinisation, Cat. No. 9414.
The trichloroacetic acid solution is stable at room temperature.

Solutions

- (1) Colour reagent solution: 0.1 mole/l phosphate buffer and
0.1 mole/l tris buffer, pH 8
6 kU/l GOD
38 kU/l POD
0.25 mmole/l 4-aminoantipyrine
0.3 mmole/l 2,4-dichlorophenol

Dissolve the contents of bottle ① by adding 100 ml of redistilled water.
The ready-for-use colour reagent solution is stable for 4 weeks when sealed and stored in a refrigerator and for 1 week when stored at room temperature (see note 1).

- (2) Standard solution: 100 mg/100 ml = 5.5 mmole/l glucose.

The glucose standard solution is ready for use.

- (3) Trichloroacetic acid solution, Cat. No. 9414: 300 mmole/l.

The trichloroacetic acid solution is ready for use.

Procedure

One blank and 1 to 2 standards are required for each test series.

Pipette into centrifugation tubes (see note 2):			
	Sample	Standard	Blank
Trichloroacetic acid solution (3)	1.0 ml	1.0 ml	—
Whole blood or serum or plasma	0.1 ml	—	—
Standard solution (2)	—	0.1 ml	—
Mix well and centrifuge the sample. Pipette into test tubes:			
Protein-free supernatant liquor or standard mixture	0.1 ml	0.1 ml	—
Trichloroacetic acid solution (3)	—	—	0.1 ml
Colour reagent (1)	2.0 ml	2.0 ml	2.0 ml
Mix well and allow to stand for 30 minutes to 3 hours at room temperature. Avoid direct sunlight. Measure the absorbance of the sample and of the standard against the blank (see note 3).			

Absorbance maximum: 510 nm

Light path: 1 cm

Filter: between 450 and 600 nm, e. g. Hg 546 nm

¹ Based on 10 analyses per series.

^{**} β-D-Glucose: oxygen-1-oxidoreductase, EC 1.1.3.4

^{***} Donor:hydrogen peroxide oxidoreductase, EC 1.11.1.7

Insulin RIA kit

for the immunoassay of insulin in serum or plasma

Description

The kit provides a rapid, simple method for the radioimmunoassay of human Insulin in serum or plasma. The range covered by the test is 0–160 microunits/ml, and is most precise at a concentration of about 50 microunits/ml. Sufficient reagents are provided for 160 individual determinations.

Applications

Insulin, which is secreted by the β cells of the pancreas, has a variety of metabolic effects, particularly in the regulation of carbohydrate metabolism. Although the mechanisms of all its actions are not known, it has been demonstrated that Insulin exerts its main effect by governing the entry of glucose into cells by acting on the cell membrane.

The regulation of insulin levels is exerted by a feedback mechanism governed by the level of blood glucose. An increase in the blood glucose level causes a rise in insulin secretion, while a decrease in the blood glucose level causes a decrease in insulin levels. This regulation provides a constant amount of glucose for the cells of the body.

The Insulin radioimmunoassay method, however, is therefore, is valuable for experimental work on carbohydrate metabolism and for the clinical investigation of disorders of carbohydrate metabolism (1–5). It has also been used for the diagnosis of insulinoma (6).

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Protocol

- 1 Arrange assay tubes according to the scheme shown in Table 2. Each point on the standard curve should be done in triplicate and the unknowns in duplicate.

Pipetting of solutions of insulin in buffer requires special attention. Users must read notes 2 and 3 before performing an assay.

- 2 Pipette 100 μ l aliquots of buffer, insulin standard or unknown sera (see note 4) according to the scheme shown in Table 2. Deliver solutions directly to the bottom of the assay tubes in this and subsequent steps.
- 3 Pipette 100 μ l aliquots of insulin binding reagent into all tubes except 'total counts' and 'blanks'. Vortex mix the tubes and incubate at 2–4°C for 45 minutes.
- 4 Pipette 100 μ l aliquots of 125 I-labelled insulin solution to all tubes. The 'total counts' tubes may be set aside at this stage awaiting counting at step 10 of the protocol. Vortex mix the tubes and incubate at 2–4°C for not less than 2 hours 15 minutes.
- 5 Add 700 μ l buffer to all tubes (except totals), delivering the buffer to wash down the tube walls. A repeating sampler is sufficiently accurate for this step. Again vortex mix.
- 6 Place tubes in a centrifuge at room temperature and apply a force of at least 1500g for 25 minutes (± 2 min). Refrigeration or greater force will assist the separation.
- 7 At the end of the centrifugation allow the centrifuge motor to slow gradually (that is, without application of a brake). Immediately remove the tubes carefully, with the minimum of agitation and place into decanting racks. Invert the tubes gently in one continuous movement so that the precipitate will remain undisturbed at the bottom of the tubes. Avoid tapping or shaking the tubes during decantation.
- 8 Keeping the tubes inverted, place them upside down over paper tissue to drain for at least 15 minutes. Any remaining liquid in the necks of the tubes should be removed gently before returning the tubes to the upright position. Note that the precipitate may not be visible to the naked eye.
- 9 After draining, the bottoms and rims of each tube should be wiped clean using a dampened paper tissue.
- 10 Count the tubes in a γ -counter. (See note 5).

Amerlex^{*} Cortisol RIA kit

for the direct immunoassay of cortisol in human serum,
plasma or urine

Description

The Amerlex Cortisol RIA kit provides a rapid, simple and precise method for the direct measurement of cortisol in human serum, plasma and urine. The assay covers the approximate range 0–60 µg/100ml (0–1700 nmol/l). Each kit contains sufficient reagents for the evaluation of standards and unknowns up to a total of 100 determinations.

Application

Corticosteroids are synthesized from cholesterol in the adrenal cortex. The major corticosteroid produced is cortisol. Cortisol secretion is dependent on the integrity of the hypothalamic-pituitary-adrenal (HPA) axis and the steroid exerts a negative feedback influence over its own synthesis through this axis (1). Cortisol measurement is therefore an important parameter in the investigation of apparent HPA dysfunction.

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*Trade mark

Amerham

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Assay procedure (see note 2)

All the tubes in an assay should be treated identically.

It is recommended that all standards and unknowns be assayed in duplicate.

A standard curve must be included on each occasion the assay is run and whenever a new bottle of reagent is used (for the combination of reagents see note 7).

The order of the steps should be followed exactly.

- 1 Label and arrange assay tubes according to the protocol shown in Table 2.
- 2 Pipette 50 µl aliquots of the serum standards and unknowns into the assay tubes according to the protocol shown in Table 2.
- 3 Dispense 200 µl ¹²⁵I-labelled cortisol derivative solution (red) into all tubes.
- 4 Dispense 200 µl Amerlex cortisol antibody suspension (blue) into all tubes (see note 12).
- 5 Vortex mix all the tubes thoroughly. Cover the tubes, for example with plastic film, and incubate in a water bath at 37 ± 2 °C for 1 hour (see note 5).
- 6 Centrifuge all the tubes together for at least 15 minutes at 1500g at room temperature (see note 6).
- 7 Remove the tubes from the centrifuge, place them in decantation racks, and pour off and discard the supernatant liquids. The tubes should be gently inverted in one continuous movement so that the precipitate will remain undisturbed at the bottom of each tube. Avoid tapping or shaking the tubes during decantation.
- 8 Keeping the tubes inverted, place them so that they drain for 3–10 minutes on to paper tissues placed in a suitable tray. After draining, blot the rims of the inverted tubes on the tissues to remove any droplets adhering to the rim.
- 9 Count all the tubes in a suitable gamma counter (see note 10).

Amerlex T4 RIA kit

for the immunoassay of T4 (thyroxine) in human serum

Description

The Amerlex T4 RIA kit provides a simple, reliable and precise radioimmunoassay for thyroxine in human serum. The total range covered by the reagents is 0 to ~25 µg T4/100 ml.

Three pack sizes are available:

1. 50 test pack, code IM.2010, for the evaluation of standards and unknowns up to a total of 50 determinations.
2. 100 test pack, code IM.2011, for the evaluation of standards and unknowns up to a total of 100 determinations.
3. 400 test pack, code IM.2014, for the evaluation of standards and unknowns up to a total of 400 determinations.

These packs may be used on one or more occasions as required.

Application

Abnormal levels of T4 are generally an indication of thyroid malfunction^{1,2}. The measurement of T4 levels is, therefore, an important part of the investigation of the dysfunction of the hypothalamic/pituitary/thyroid axis.

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Assay procedure (see notes 1-11)

All the tubes in an assay should be treated identically. It is recommended that initially samples be determined in duplicate. Clinical and performance data quoted were obtained in this way. Experience with the technique may, however, suggest that single determinations for unknowns are adequate at the discretion of the user. Standards, however, should always be assayed in duplicate.

A standard curve must be included on each occasion the assay is run and whenever a new bottle of reagent is used (for the combination of reagents see note 6). Label and arrange the assay tubes (see note 7) according to the protocol shown in Table 1.

The order of the steps should be followed exactly.

1. Pipette 25 µl aliquots of the standards and unknown serum samples into the appropriate tubes, according to the protocol in Table 1.
2. Dispense 500 µl [¹²⁵I]thyroxine solution (red) into each tube using a precision pipette or repeating dispenser (see notes 2 and 3).
3. Dispense 500 µl Amerlex T4-antibody suspension (blue) into each tube using a precision pipette or repeating dispenser (see notes 2, 3 and 8).
4. Vortex mix all the tubes thoroughly and leave to stand at room temperature for at least 45 minutes (see note 5). (Note the colour should now be deep purple.)
5. Centrifuge all the tubes together for 15 minutes at 1500g or greater.
6. After centrifugation place the tubes carefully into suitable decantation racks, then pour off and discard the supernatant liquids. Keeping the tubes inverted, place them on a pad of absorbent tissues and allow to drain for between 5 and 10 minutes. Blot the rims of the inverted tubes on the tissue pad to remove any adhering droplets of liquid. Do not re-invert the tubes once they have been turned upright.
7. Count all the tubes in a suitable gamma counter for the time required to accumulate at least 20,000 counts in tubes 1 and 2.

DATA SUMMARIES AND
STATISTICAL ANALYSES.

FROM SECTION 3.3
ABSORPTION PROFILES.

Absorption profiles. Changes in Blood
Glucose concentrations following oral
glucose loading in male subjects.

		Glucose concentration (mg%)											
Time (mins)		0		15		30		60		90		135	
		ΔG		ΔG		ΔG		ΔG		ΔG		ΔG	
S U B J E C T S	1	85	+27	112	+51	136	+26	111	+11	96	-29	56	
	2	85	+27	112	+6	91	-15	70	-9	76	-6	79	
	3	78	+26	104	+23	101	-2	76	+2	80	-25	53	
	4	90	+23	113	+41	131	0	90	-8	82	-27	63	
	5	86	+23	109	+8	94	-10	76	-13	73	-30	56	
	6	89	+23	112	+6	95	-41	48	-24	65	-39	50	
	\bar{X}	86	+25	110	+23	108	-7	79	-7	79	-26	60	
	SE	1.6	0.8	1.2	8.2	8.2	9.0	8.6	4.9	4.1	4.5	4.5	

Absorption profiles. Changes in Blood
Glucose concentration following oral
glucose loading in female subjects.

		Glucose concentration (mg%)											
Time (mins)		0	15		30		60		90		135		
		ΔG		ΔG		ΔG		ΔG		ΔG			
S U B J E C T S	7	90	+26	116	+30	120	+25	115	-1	89	-32	58	
	8	72	+36	108	+30	102	-3	69	+1	73	-10	62	
	9	74	+29	103	+16	90	-4	70	+3	77	-13	61	
	10	84	+30	114	+30	114	-19	65	-24	60	-29	55	
	11	76	+20	96	+33	109	0	76	-16	60	-4	72	
	12	82	+19	101	+18	100	-23	59	-18	64	-5	77	
\bar{X}		80	+27	106	+26	106	-4	76	-9	71	-16	64	
SE		2.9	2.5	3.3	2.9	4.5	6.9	8.2	4.9	4.5	4.9	3.7	

Absorption profiles. Changes in blood glucose concentration following oral fructose loading in male subjects.

Glucose concentration (mg%)												
Time (mins)		0	15		30		60		90		135	
			ΔG		ΔG		ΔG		ΔG		ΔG	
S U B J E C T S	1	77	+11	88	+18	95	-18	59	-15	62	-15	62
	2	82	+15	97	+8	90	-12	70	-18	64	-24	58
	3	76	+2	78	-2	74	-2	74	+1	77	-2	74
	4	87	+3	90	-5	82	+4	91	+1	88	0	87
	5	81	+6	87	-1	80	-10	71	-5	76	-10	71
	6	84	+1	85	+4	88	-24	60	-26	58	-18	66
\bar{X}		81	+6	88	+4	85	-10	71	-10	71	-12	70
SE		1.6	2.5	2.5	3.3	3.3	4.1	4.9	4.5	4.5	3.7	4.1

Absorption profiles. Changes in blood glucose concentration following oral fructose loading in female subjects.

Glucose concentration (mg%)												
Time (mins)		0	15		30		60		90		135	
		ΔG		ΔG		ΔG		ΔG		ΔG		
S U B J E C T S	7	83	+4	87	-4	79	-5	78	-5	78	-3	80
	8	83	+9	92	+14	97	+16	99	+4	87	-3	80
	9	82	+3	85	+2	84	-21	61	-27	55	-25	57
	10	78	+8	86	+6	84	-8	70	-17	61	-18	60
	11	72	+13	85	+11	83	-6	66	-8	64	-5	67
	12	88	+7	95	-3	85	-13	75	-12	76	-9	79
\bar{X}		81	+7	88	+4	86	-6	75	-11	70	-11	71
SE		2.0	1.6	1.6	2.9	1.2	4.9	5.3	4.5	4.9	3.7	4.5

Absorption profiles. Changes in blood fructose concentration following oral fructose loading in male subjects.

Fructose concentration (mg%)												
Time (mins)		0	15		30		60		90		135	
		ΔF		ΔF		ΔF		ΔF		ΔF		
S U B J E C T S	1	0.6	+6.7	7.3	+10.3	10.9	+11.5	12.1	+9.1	9.7	+3.5	4.1
	2	0.2	+4.8	5.0	+7.9	8.1	+9.1	9.3	+10.4	10.6	+6.8	7.0
	3	0.4	+4.4	4.8	+7.8	8.2	+5.8	6.2	+6.1	6.5	+1.6	2.0
	4	0.8	+1.8	2.6	+8.7	9.5	+9.7	10.5	+7.5	8.3	+4.9	5.7
	5	0.3	+2.7	3.0	+9.7	10.0	+10.2	10.5	+8.8	9.1	+2.6	2.9
	6	0.0	+4.1	4.1	+11.0	11.0	+9.0	9.0	+5.6	5.6	+3.6	3.1
\bar{X}		0.4	+4.1	4.5	+9.2	9.6	+9.2	9.6	+7.9	8.3	+3.8	4.1
SE		0.1	0.7	0.7	0.5	0.5	1.9	0.8	0.8	0.8	0.7	0.8

Absorption profiles. Changes in blood fructose concentration following oral fructose loading in female subjects.

Fructose concentration (mg%)												
Time (mins)		0	15		30		60		90		135	
			ΔF		ΔF		ΔF		ΔF		ΔF	
S U B J E C T S	7	0.2	+2.2	2.4	+9.5	9.7	+12.7	12.9	+7.6	7.8	+4.0	4.2
	8	0.6	+2.6	3.2	+7.6	8.2	+9.1	9.7	+7.8	8.4	+3.1	3.7
	9	0.7	+2.4	3.1	+9.5	10.2	+7.6	8.3	+9.5	10.2	+0.7	1.4
	10	0.0	+1.8	1.8	+11.8	11.8	+8.5	8.5	+7.7	7.7	+6.4	6.4
	11	0.1	+6.5	6.6	+10.1	10.2	+8.8	8.9	+4.4	4.5	+1.5	1.6
	12	0.8	+5.2	6.0	+10.4	11.2	+11.7	12.5	+9.3	10.1	+1.9	2.7
\bar{X}		0.4	+3.5	3.9	+9.8	10.2	+9.7	10.1	+7.7	8.1	+2.9	3.3
SE		0.1	0.8	0.8	0.6	0.5	0.8	0.9	0.7	0.9	0.9	0.8

Absorption profiles.

Analysis of Variance Summary tables.

Comparison of male and female blood glucose following glucose.

Source of Variation	SS	df	MS	F
Treatments (time)	21312.11	11	1937.465	12.109*
Experimental Error	9600.333	60	160.006	
Total	30912.444			

Blood glucose following glucose, male and female pooled data.

Source of Variation	SS	df	MS	F
Treatments (time)	30912.444	5	4171.689	27.385*
Experimental Error	20858.444	66	152.333	
Total	10054.000			

Absorption profiles.Analysis of Variance Summary tables.

Comparison of male and female blood fructose following fructose.

Source of Variation	SS	df	MS	F
Treatments (time)	901.807	11	81.982	29.786*
Experimental Error	165.142	60	2.752	
Total	1066.949			

Blood fructose following fructose, male and female pooled data.

Source of Variation	SS	df	MS	F
Treatments (time)	897.808	5	179.562	64.149*
Experimental Error	184.743	66	2.799	
Total	1082.551			

Absorption Profiles.

Analysis of Variance Summary tables.

Comparison of male and female blood glucose following fructose.

Source of Variation	SS	df	MS	F
Treatments (time)	3697.500	11	336.136	4.019*
Experimental Error	5018.000	60	83.633	
Total	8715.500			

Blood glucose following fructose, male and female pooled data.

Source of Variation	SS	df	MS	F
Treatments (time)	3643.167	5	728.633	9.481*
Experimental Error	5072.333	66	76.854	
Total	8715.500			

Absorption profiles. Detailed comparison of means. Male and female blood glucose following oral glucose.

			Blood sample (mins after loading, M=male, F=female)											
			135M	135F	90F	60F	60M	90M	OF	OM	30F	15F	30M	15M
Means			59.500	64.167	70.500	75.667	78.500	78.667	79.667	85.500	105.833	106.333	108.000	110.833
Blood sample (mins after loading, M=male, F=female)	135M	59.000	-	4.667	11.000	16.167	19.000	19.167	20.167	26.000*	46.333*	46.833*	48.500*	50.833*
	135F	64.167		-	6.333	11.500	14.333	14.000	15.500	21.333	41.666*	42.166*	43.833*	46.166*
	90F	70.500			-	5.167	8.000	8.167	9.167	15.000	35.333*	35.833*	37.500*	39.833*
	60F	75.667				-	2.833	3.000	4.000	9.833	30.166*	30.666*	32.333*	34.666*
	60M	78.500					-	0.167	1.167	7.000	27.333*	27.833*	29.500*	31.833*
	90M	78.667						-	1.000	6.833	27.166*	27.666*	29.333*	31.666*
	OF	79.667							-	5.833	26.166*	26.666*	28.333*	30.666*
	OM	85.500								-	20.333	20.833	22.500	24.833
	30F	105.833									-	0.500	2.167	4.500
	15F	106.333										-	1.667	4.000
	30M	108.000											-	2.333
	15M	110.333												-

r=	2	3	4	5	6	7	8	9	10	11	12
q(r,60)	2.83	3.40	3.74	3.98	4.16	4.31	4.44	4.45	4.65	4.73	4.81
q(r,60)	14.614	17.558	19.314	20.553	21.483	22.257	22.928	23.497	24.013	24.426	24.839

$\sqrt{MS_{\text{error}}/n}$

Absorption profiles. Detailed comparison of means.

Male and female blood glucose following oral fructose.

		Blood sample (mins after loading M=male, F=female)											
		135M	90F	135F	90M	60M	60F	OF	OM	30M	30F	15M	15F
Means		69.667	70.167	70.500	70.833	70.833	74.833	81.000	81.167	84.833	85.333	87.500	88.333
Blood sample (mins after loading)	135M	69.667	-	0.500	0.833	1.166	1.166	5.166	11.333	11.500	15.166	15.666	17.933*18.666*
	90F	70.167	-	0.333	0.666	0.666	4.666	10.833	11.000	14.666	15.166	17.333	18.166*
	135F	70.500		-	0.333	0.333	4.333	10.500	10.667	14.333	14.833	17.000	17.833
	90M	70.833			-	-	4.000	10.167	10.334	14.000	14.500	16.667	17.500
	60M	70.833				-	4.000	10.167	10.334	14.000	14.500	16.667	17.500
	60F	74.833					-	6.167	6.334	10.000	10.500	12.667	13.500
	OF	81.000						-	0.167	3.833	4.333	6.500	7.333
	OM	81.167							-	3.666	4.166	6.333	7.166
	30M	84.833								-	0.500	8.667	3.500
	30F	85.333									-	2.167	3.000
	15M	87.500										-	0.833
	15F	88.333											-

r=	2	3	4	5	6	7	8	9	10	11	12
q(r,60)	2.83	3.40	3.74	3.98	4.16	4.31	4.44	4.55	4.65	4.73	4.81
q(r,60)	10.566	12.694	13.963	14.859	15.531	16.091	16.577	16.987	17.361	17.659	17.958

$\sqrt{\frac{MS_{error}}{n}}$

absorption profiles. Detailed comparison of

means. Male and female blood fructose following oral fructose.

			Blood sample (mins after loading M=male, F=female)											
			OM	OF	135F	15F	135M	15M	90F	90M	60M	30M	30F	60F
Means			0.383	0.400	3.333	3.850	4.133	4.467	8.117	8.300	9.600	9.617	10.117	10.133
Blood sample (mins after loading, M= male, F=female)	OM	0.383	-	0.017	2.950*	3.467*	3.750*	4.084*	7.734*	7.917*	9.217*	9.234*	9.734*	9.750*
	OF	0.400		-	2.933*	3.450*	3.733*	4.067*	7.717*	7.900*	9.200*	9.217*	9.717*	9.733*
	135F	3.333			-	0.517	0.800	1.134	4.784*	4.967*	6.267*	6.284*	6.784*	6.800*
	15F	3.850				-	0.283	0.617	4.267*	4.450*	5.750*	5.767*	6.267*	6.283*
	135M	4.133					-	0.334	3.984*	4.167*	5.467*	5.484*	5.984*	6.000*
	15M	4.467						-	3.65 *	3.833*	5.133*	5.150*	5.650*	5.666*
	90F	8.117							-	0.183	1.483	1.500	2.000	2.016
	90M	8.300								-	1.300	1.317	1.817	1.833
	60M	9.600									-	0.017	0.517	0.533
	30M	9.617										-	0.500	0.516
	30F	10.117											-	0.016
	60F	10.133												-
r=			2	3	4	5	6	7	8	9	10	11	12	
q(r,60)			2.83	3.40	3.74	3.98	4.16	4.31	4.44	4.55	4.65	4.73	4.81	
MS _{error} /n			1.917	2.303	2.533	2.695	2.817	2.919	3.007	3.081	3.149	3.202	3.258	

means. Blood glucose following oral glucose,
male and female pooled data.

		Blood sample, (mins after loading)						
		135	90	60	0	30	15	
Blood sample mins after loading	Means	61.833	74.583	77.083	82.583	106.917	108.333	
	135	61.833	-	12.750*	15.250*	20.750*	45.084*	46.500*
	90	74.583	-	2.500	8.000	32.334*	33.750*	
	60	77.083	-	5.500	29.834*	31.250*		
	0	82.583	-	24.334*	25.750*			
	30	106.917	-	1.416				
	15	108.383	-					

	r=	2	3	4	5	6
$\sqrt{\text{MS}_{\text{error}}/n}$	q(r,66)	2.827	3.396	3.735	3.974	4.154
	q(r,66)	10.072	12.100	13.308	14.159	14.800

Absorption profiles. Detailed comparison of means, Blood glucose following oral fructose, male and female pooled data.

			Blood sample, (mins after loading)					
			135	90	60	0	30	15
Blood sample (mins after loading)		Means	70.083	70.500	72.833	81.083	85.083	87.917
	135	70.083	-	0.417	2.750	11.000*	15.000*	17.834*
	90	70.500		-	2.333	10.583*	14.583*	17.417*
	60	72.833			-	8.250	12.250*	15.084*
	0	81.083				-	4.000	6.834
	30	85.083					-	2.834
	15	87.917						-

	r=	2	3	4	5	6
$q(r, 66)$		2.827	3.396	3.735	3.974	4.154
$\sqrt{\text{MS}_{\text{error}}/n}$	$q(r, 66)$	7.154	8.594	9.452	10.057	10.513

Absorption profiles. Detailed comparison of means.

Blood fructose following oral fructose, male and female pooled data.

		Blood sample (mins after loading)					
		0	135	15	90	60	30
Blood sample (mins after loading)	Means	0.392	3.733	4.158	8.208	9.867	9.875
	0	-	3.341*	3.766*	7.816*	9.475*	9.483*
	135		-	0.425	4.475*	6.134*	6.142*
	15			-	4.050*	5.709*	5.717*
	90				-	1.659	1.667
	60					-	0.008
	30						-

	r=	2	3	4	5	6
	q(r,66)	2.827	3.396	3.735	3.974	4.154
$\sqrt{\text{MS}_{\text{error}}/n}$	q(r,66)	1.365	1.640	1.804	1.919	2.006

DATA SUMMARIES
AND STATISTICAL
ANALYSES.

FROM SECTION 3,4
METABOLIC EFFECTS.

Metabolic Effects.

Group 1 (male fructose) - data summary.

Daily Sugar Intake (g)						
Days						
	1-7	(%)	8-14	(%)	15-21	(%)
S U B J E C T S	1.	91	21.36	188	44.13	147 34.51
	2.	65	20.97	169	54.52	76 24.52
	3.	123	28.41	193	44.57	117 27.02
	4.	91	31.38	120	41.38	79 27.24
	5.	121	30.95	177	45.27	93 23.79
	6.	89	29.97	140	47.14	68 22.90
	7.	95	21.59	220	50.00	125 28.41
	8.	158	27.92	237	41.87	171 30.21
	9.	88	19.73	223	50.00	135 30.27
	10.	93	27.60	153	45.40	91 27.00
\bar{X}	101	25.99	182	46.43	110	27.59
SEM	8.3	1.44	12.0	1.29	10.8	1.10

Metabolic Effects.Group 1 (male fructose) - data summary.

		Bodyweight (kg)					
		8		15		22	
Day		8		15		22	
S U B J E C T S	1.	71.2	33.19	71.3	33.24	72.0	33.57
	2.	80.5	33.40	80.2	33.28	80.3	33.32
	3.	66.2	33.28	66.3	33.33	66.4	33.38
	4.	69.8	33.43	69.4	33.24	69.6	33.33
	5.	76.4	33.39	76.0	33.22	76.4	33.39
	6.	71.2	33.15	72.1	33.57	71.5	33.29
	7.	59.1	33.68	57.9	32.99	58.5	33.33
	8.	72.9	33.30	73.4	33.53	72.6	33.17
	9.	73.8	33.32	73.4	33.14	74.3	33.54
	10.	81.1	33.31	80.9	33.22	81.5	33.47
\bar{X}		72.2	33.35	72.1	33.48	72.3	33.38
SEM		2.1	0.05	2.1	0.23	2.1	0.04

Metabolic Effects.Group 1 (male fructose) - data summary.

Blood cholesterol esters (mg%)							
		(%)		(%)		(%)	
Day	8		15		22		
S U B J E C T S	1	175	31.53	187	33.69	193	34.77
	2	215	33.33	218	33.80	212	32.87
	3	161	34.77	152	32.83	150	32.40
	4	160	29.52	176	32.47	206	38.01
	5	233	32.82	253	35.63	224	31.55
	6	144	32.80	142	32.35	153	34.85
	7	138	33.99	136	33.50	132	32.51
	8	216	33.91	200	31.40	221	34.69
	9	151	32.33	161	34.48	155	33.19
	10	193	29.65	228	35.02	230	35.33
\bar{X}	179	32.47	185	33.52	188	34.02	
SEM	11	0.56	12	0.41	12	0.60	

Metabolic Effects.

Group 1 (male fructose) - data summary.

		Blood LDL (mg%)					
		(%)		(%)		(%)	
Day		8		15		22	
S U B J E C T S	1	411	32.01	457	35.59	416	32.40
	2	443	28.29	578	36.91	545	34.80
	3	310	32.80	347	36.72	288	30.48
	4	349	31.38	395	35.52	368	33.09
	5	619	32.63	669	35.27	609	32.10
	6	351	32.23	359	32.97	379	34.80
	7	263	34.88	247	32.76	244	32.36
	8	508	32.86	471	30.47	567	36.68
	9	318	32.06	346	34.88	328	33.06
	10	436	28.59	546	35.80	543	35.61
	\bar{X}	401	31.77	442	34.69	429	33.54
	SEM	34	0.63	41	0.64	41	0.60

Metabolic Effects.

Group 1 (male fructose) - data summary.

		Blood glucose					
		(mg%)					
		8		15		22	
Day							
S U B J E C T S	1	88	33.72	89	34.10	84	32.18
	2	95	32.31	104	35.37	95	32.37
	3	91	34.60	85	32.32	87	33.08
	4	76	33.33	71	31.14	81	35.53
	5	79	32.24	82	33.47	84	34.29
	6	88	32.84	88	32.84	92	34.33
	7	83	33.60	80	32.39	84	34.01
	8	74	31.22	83	35.02	80	33.76
	9	78	31.45	90	36.29	80	32.26
	10	84	32.18	86	32.95	91	34.87
\bar{X}		84	32.85	86	33.59	86	33.67
SEM		2	0.34	3	0.50	2	0.37

Metabolic Effects.Group 1 (male fructose)- data summary.

		Blood NEFA (Meq/l)					
		(%)		(%)		(%)	
Day		8		15		22	
SUBJECTS	1	0.283	34.81	0.240	29.52	0.290	35.67
	2	0.148	13.37	0.361	32.61	0.598	54.02
	3	0.465	39.37	0.388	32.85	0.328	27.77
	4	0.335	37.14	0.275	30.49	0.292	32.37
	5	0.276	27.54	0.416	41.52	0.310	30.94
	6	0.535	43.39	0.339	27.49	0.359	29.12
	7	0.295	34.58	0.244	28.60	0.314	36.81
	8	0.371	35.67	0.327	31.44	0.342	32.88
	9	0.169	26.78	0.212	33.60	0.250	39.62
	10	0.344	29.94	0.290	25.24	0.515	44.82
	\bar{X}	0.322	32.26	0.309	31.34	0.360	36.40
	SEM	0.038	2.66	0.022	1.40	0.035	2.54

Metabolic Effects.

Group 1 (male fructose) - data summary.

		Coagulation time (mins)					
		(%)		(%)		(%)	
Day		8		15		22	
S U B J E C T S	1	7.3	32.30	7.9	34.96	7.4	32.74
	2	7.8	39.80	6.0	30.61	5.8	29.59
	3	7.1	35.82	6.4	31.84	6.6	32.84
	4	7.3	35.61	6.6	32.20	6.6	32.20
	5	8.3	36.09	7.1	30.87	7.6	33.04
	6	8.4	33.20	7.8	30.83	9.1	35.97
	7	9.2	33.82	9.0	33.09	9.0	33.09
	8	7.6	34.08	7.7	34.53	7.0	31.39
	9	8.0	34.78	7.4	32.17	7.6	33.04
	10	6.5	32.50	6.5	32.50	7.0	35.00
	\bar{X}	7.8	34.75	7.2	32.36	7.4	32.89
	SEM	0.2	0.69	0.3	0.47	0.3	0.55

Metabolic Effects.

Group 2 (male glucose) - data summary.

Daily Sugar Intake (g)							
Days							
	1-7	(%)	8-14	(%)	15-21	(%)	
SUBJECTS	11	81	26.56	150	49.18	74	24.26
	12	71	23.75	148	49.50	80	26.76
	13	147	31.01	202	42.62	125	26.37
	14	85	20.83	184	45.10	139	34.07
	15	128	33.16	161	41.71	97	25.13
	16	136	32.69	201	48.32	79	18.99
\bar{X}	108	28.00	174	46.07	99	25.93	
SEM	13.3	2.08	10.1	1.40	11.1	1.99	

Metabolic Effects.

Group 2 (male glucose) - data summary.

Bodyweight (kg)							
		(%)			(%)	(%)	
Day	8	15		22			
SUBJECTS	11	69.3	33.09	70.2	33.52	69.9	33.38
	12	73.6	33.50	73.1	33.27	73.0	33.23
	13	68.9	33.37	68.9	33.37	68.7	33.27
	14	82.1	33.54	81.2	33.17	81.5	33.29
	15	75.4	33.25	75.7	33.38	75.7	33.38
	16	69.3	33.49	68.9	33.30	68.7	33.20
\bar{X}	73.1	33.37	73.0	33.34	73.0	33.29	
SEM	2.1	0.07	2.0	0.05	2.1	0.03	

Cholesterol esters (mg%)							
	(%)		(%)		(%)		
Day	8		15		22		
S	11	161	32.07	160	31.87	181	36.06
U	12	145	32.15	154	34.15	152	33.70
B	13	215	30.98	231	33.29	248	35.73
J	14	154	31.69	156	32.10	176	36.21
E	15	187	33.45	182	32.56	190	33.99
C	16	192	33.10	191	32.93	197	33.97
T							
S							
	\bar{X}	176	32.24	179	32.82	191	34.94
	SEM	11	0.37	12	0.34	13	0.48

Metabolic Effects.

Group 2 (male glucose) - data summary.

Blood triglycerides (mg%)						
	8		15		22	
Day		(%)		(%)		(%)
11	132	32.84	125	31.09	145	36.07
S 12	77	28.84	122	45.69	68	25.47
U 13	97	27.48	125	35.41	131	37.11
B 14	69	25.09	119	43.27	87	31.64
J 15	123	35.86	101	29.45	119	34.69
E 16	170	33.14	174	33.92	169	32.94
C						
T						
S						
\bar{X}	111	30.54	128	36.47	120	32.99
SEM	16	1.66	10	2.69	15	1.71

Blood LDL (mg%)						
	8		15		22	
Day		(%)		(%)		(%)
11	282	30.65	284	30.87	354	38.48
S 12	179	30.65	217	37.16	188	32.19
U 13	553	33.49	544	32.95	554	33.56
B 14	270	31.00	295	33.87	306	35.13
J 15	493	34.77	460	32.44	465	32.79
E 16	402	35.02	362	31.53	384	33.45
C						
T						
S						
\bar{X}	363	33.06	360	32.79	375	34.15
SEM	59	0.85	50	0.91	52	0.93

Metabolic Effects.Group 2 (male glucose) - data summary.

		Blood glucose (mg%)					
		8		15		22	
		(%)		(%)		(%)	
Day		8		15		22	
SUBJECTS	11	82	31.66	89	34.36	88	33.98
	12	88	33.98	86	33.20	85	32.82
	13	77	33.19	80	34.48	75	32.33
	14	90	34.62	83	31.92	87	33.46
	15	82	34.89	71	30.21	82	34.89
	16	84	32.68	86	33.46	87	33.85
\bar{X}		84	33.50	83	32.94	84	33.56
SEM		2	0.50	3	0.66	2	0.37

		Blood NEFA (Meq/l)					
		8		15		22	
		(%)		(%)		(%)	
Day		8		15		22	
SUBJECTS	11	0.023	32.79	0.172	27.79	0.244	39.42
	12	0.782	50.85	0.368	23.93	0.388	25.23
	13	0.277	38.37	0.244	33.80	0.201	27.84
	14	0.247	29.79	0.164	19.78	0.418	50.42
	15	0.337	44.17	0.189	24.77	0.237	31.06
	16	0.333	29.34	0.411	36.21	0.391	34.45
\bar{X}		0.363	37.55	0.258	27.71	0.313	34.74
SEM		0.087	3.52	0.044	2.55	0.039	3.74

Metabolic Effects.Group 2 (male glucose) - data summary.

Blood uric acid (mg%)						
		(%)			(%)	(%)
Day		8	15		22	
SUBJECTS	11	6.1	31.12	7.2	36.73	6.3 32.14
	12	5.9	33.52	5.6	31.82	6.1 34.66
	13	5.6	33.94	5.4	32.73	5.5 33.33
	14	7.6	39.58	6.0	31.25	5.6 29.17
	15	5.7	34.13	5.4	32.34	5.6 33.53
	16	7.3	35.78	6.3	30.88	6.8 33.33
\bar{X}		6.4	34.68	6.0	32.63	6.0 32.69
SEM		0.4	1.16	0.3	0.87	0.2 0.78

Blood coagulation time (mins)							
		(%)			(%)	(%)	
Day		8	15		22		
SUBJECTS	11	9.9	37.08	8.4	31.46	8.4	31.46
	12	10.0	36.50	7.9	28.83	9.5	34.67
	13	7.4	35.92	6.3	30.58	6.9	33.50
	14	8.8	32.12	9.3	33.94	9.3	33.94
	15	6.7	32.06	6.9	33.01	7.3	34.93
	16	7.2	32.73	7.2	32.73	7.6	34.55
\bar{X}		8.3	34.40	7.7	31.76	8.2	33.85
SEM		0.6	0.96	0.5	0.76	0.5	0.52

Metabolic Effects.Group 3 (female fructose) - data summary.

Daily Sugar Intake (g)						
Days						
		1-7	(%)	8-14	(%)	15-21 (%)
S U B J E C T	17	53	23.04	122	53.04	55 23.91
	18	109	28.09	167	43.04	112 28.87
	19	125	23.11	250	46.21	166 30.68
	20	46	22.33	119	57.77	41 19.90
	21	83	35.17	110	46.61	43 18.22
	22	47	24.48	82	42.71	63 32.81
\bar{X}		77	25.75	142	47.49	80 26.76
SEM		13.9	2.01	24.3	2.44	20.1 2.44

Metabolic Effects.Group 3 (female fructose) - data summary.

Bodyweight (kg)						
Day	8		15		22	
17	65.3	33.38	65.3	33.38	65.0	33.23
S 18	59.8	33.37	59.4	33.15	60.0	33.48
U 19	64.3	33.56	63.7	33.25	63.6	33.19
B 20	52.8	33.46	52.3	33.14	52.7	33.40
J 21	59.1	33.37	58.8	33.20	59.2	33.43
E 22	55.2	33.25	55.4	33.37	55.4	33.37
C						
T						
S						
\bar{X}	59.4	33.40	59.2	33.25	59.3	33.37
SEM	2.0	0.04	2.0	0.04	2.0	0.05

Blood cholesterol esters						
Day	8		15		22	
17	158	33.47	150	31.78	164	34.75
S 18	192	36.16	155	29.19	184	34.65
U 19	167	34.58	159	32.92	157	32.51
B 20	207	34.85	197	33.16	190	31.99
J 21	182	34.27	175	32.96	174	32.77
E 22	172	35.03	159	32.38	160	32.59
C						
T						
S						
\bar{X}	180	34.73	166	32.07	172	33.21
SEM	7	0.36	7	0.61	5	0.48

Metabolic Effects.Group 3 (female fructose) - data summary.

Blood triglycerides (mg%)						
		(%)			(%)	(%)
Day	8	15		22		
SUBJECTS	17	69	24.04	110	38.33	108 37.63
	18	93	33.94	104	37.96	77 28.10
	19	98	39.68	74	29.96	75 30.36
	20	107	30.84	114	32.85	126 36.37
	21	64	31.84	75	37.31	62 30.85
	22	89	36.48	72	29.51	83 34.02
\bar{X}	87	32.80	92	34.32	89	32.89
SEM	7	2.19	8	1.66	10	1.52

Blood LDL (mg%)							
	(%)		(%)		(%)		
Day	8		15		22		
S U B J E C T S	17	272	31.74	274	31.97	311	36.29
	18	292	36.18	223	27.63	292	36.18
	19	311	34.90	278	31.20	302	33.89
	20	373	33.10	386	34.25	368	32.65
	21	362	33.58	378	35.06	338	31.35
	22	331	33.43	322	32.53	337	34.04
\bar{X}	324	33.82	310	32.11	325	34.07	
SEM	16	0.63	26	1.07	11	0.79	

Metabolic Effects.Group 3 (female fructose) - data summary.

Blood glucose (mg%)						
	8		15		22	
Day		(%)		(%)		(%)
S 17	77	33.33	80	34.63	74	32.03
U 18	77	32.08	80	33.33	83	34.58
B 19	82	33.33	81	32.93	83	33.74
J 20	79	31.60	83	33.20	88	35.20
E 21	83	35.32	76	32.34	76	32.34
C 22	82	33.20	79	31.98	86	34.82
T						
S						
\bar{X}	80	33.14	80	33.07	82	33.79
SEM	1	0.53	1	0.38	3	0.54

Blood NEFA (MEq/l)						
	8		15		22	
Day		(%)		(%)		(%)
S 17	0.261	28.52	0.302	33.01	0.352	38.47
U 18	0.238	27.90	0.307	35.99	0.308	36.11
B 19	0.422	33.90	0.418	33.57	0.405	32.53
J 20	0.330	30.53	0.306	28.31	0.445	41.17
E 21	0.837	40.18	0.810	38.89	0.436	20.93
C 22	0.329	29.27	0.333	29.63	0.462	41.10
T						
S						
\bar{X}	0.403	31.72	0.413	32.23	0.401	35.05
SEM	0.091	1.90	0.082	1.60	0.025	3.12

Metabolic Effects.Group 4 (female glucose)-data summary.

Daily Sugar Intake (g)						
Days						
	1-7	(%)	8-14	(%)	15-21	(%)
23	125	31.17	166	41.40	110	27.43
24	157	28.49	231	41.92	163	29.58
25	81	19.38	232	55.50	105	25.12
26	73	22.39	91	27.91	162	49.69
27	38	23.90	79	49.69	42	26.42
28	50	25.51	99	50.51	47	23.98
\bar{X}	87	25.14	150	44.49	105	30.37
SEM	18.6	1.73	28.7	3.98	21.6	3.94

Metabolic Effects.Group 4 (female glucose) - data summary.

		Bodyweight (kg)					
		(%)		(%)		(%)	
Day		8		15		22	
SUBJECTS	23	50.5	33.36	50.2	33.16	50.7	33.49
	24	51.8	33.44	51.7	33.38	51.4	33.18
	25	60.2	33.35	60.2	33.35	60.1	33.30
	26	66.1	33.25	66.3	33.35	66.4	33.40
	27	58.3	33.35	58.3	33.35	58.2	33.30
	28	65.0	33.20	65.4	33.40	65.4	33.40
\bar{X}		58.7	33.33	58.7	33.33	58.7	33.35
SEM		2.7	0.04	2.8	0.04	2.7	0.04

Blood cholesterol esters							
(mg%)							
		(%)		(%)		(%)	
Day		8		15		22	
S U B J E C T S	23	192	34.04	187	33.16	185	32.80
	24	145	33.88	135	31.54	148	34.58
	25	176	32.47	180	33.21	186	34.32
	26	201	34.18	187	31.80	200	34.01
	27	216	34.89	202	32.63	201	32.47
	28	180	32.97	183	33.52	183	33.52
\bar{X}		185	33.74	179	32.64	184	33.62
SEM		10	0.36	9	0.33	8	0.35

Metabolic Effects.Group 4 (female glucose)-Data summary.

Blood triglycerides (mg%)						
Day	8	15	22			
23	96	33.10	101	34.83	93	32.07
24	73	30.80	77	32.49	87	36.71
25	61	30.65	67	33.67	71	35.68
26	94	33.69	88	31.54	97	34.77
27	103	34.33	102	34.00	95	31.67
28	86	34.82	87	35.22	74	29.96
\bar{X}	86	32.90	87	33.63	86	33.48
SEM	7	0.73	6	0.57	4	1.07

Blood 'LDL' (mg%)						
Day	8	15	22			
23	328	34.38	308	32.29	318	33.33
24	268	32.25	273	32.85	290	34.90
25	284	35.06	270	33.33	256	31.60
26	313	32.81	335	35.12	306	32.08
27	371	32.95	385	34.19	370	32.86
28	334	31.69	361	34.25	359	34.06
\bar{X}	316	33.19	322	33.67	317	33.14
SEM	15	0.52	19	0.42	18	0.50

Metabolic Effects.Group 4 (female glucose) - data summary.

		Blood glucose (mg%)					
		8		15		22	
		(%)		(%)		(%)	
Day		8		15		22	
S U B J E C T S	23	95	33.93	94	33.57	91	32.50
	24	92	34.46	88	32.96	87	32.58
	25	84	32.43	86	33.20	89	34.36
	26	77	34.38	71	31.70	76	33.93
	27	76	32.90	77	33.83	78	33.77
	28	77	34.68	72	32.43	73	32.88
\bar{X}		84	33.80	81	32.87	82	33.34
SEM		3	0.37	4	0.28	3	0.32

		Blood NEFA (Meq/l)					
		8		15		22	
		(%)		(%)		(%)	
Day		8		15		22	
S U B J E C T S	23	0.251	34.72	0.237	32.78	0.235	32.50
	24	0.257	30.78	0.295	35.33	0.283	33.89
	25	0.199	31.49	0.222	35.13	0.211	33.39
	26	0.621	34.52	0.592	32.91	0.586	32.57
	27	0.617	34.20	0.587	32.54	0.600	33.26
	28	0.537	34.34	0.505	32.29	0.522	33.38
\bar{X}		0.414	33.34	0.406	33.50	0.406	33.17
SEM		0.081	0.71	0.071	0.40	0.074	0.22

Metabolic Effects.Analysis of variance summary tables.Between Group analysis - base line values.Dietary Sugar.

Source of Variation	SS	df	MS	F
Treatments	2311.064	3	770.355	0.659
Experimental error	28051.900	24	1168.829	
Total	30362.964	27		

Bodyweight.

Source of Variation	SS	df	MS	F
Treatments	1257.469	3	419.156	11.846
Experimental error	849.179	24	35.382	
Total	2106.648	27		

Blood cholesterol esters.

Source of Variation	SS	df	MS	F
Treatments	277.791	3	92.597	0.121
Experimental error	18299.066	24	762.461	
Total	18576.857	27		

Metabolic Effects.

Analysis of variance summary tables.

Between Group analysis - base line values.(cont.)

Blood triglycerides.

Source of Variation	SS	df	MS	F
Treatments	4778.363	3	1592.787	1.815
Experimental error	21060.065	24	877.544	
Total	25839.428	27		

Blood LDL

Source of Variation	SS	df	MS	F
Treatments	36036.607	3	12012.202	1.312
Experimental error	219685.25	24	9153.552	
Total	255721.85	27		

Blood glucose.

Source of Variation	SS	df	MS	F
Treatments	62.695	3	20.898	0.547
Experimental error	916.733	24	38.197	
Total	979.428	27		

Metabolic Effects.Analysis of variance summary tables.Between Group analysis - base line values.(cont.)Blood NEFA

Source of Variation	SS	df	MS	F
Treatments	0.041	3	0.013	0.413
Experimental error	0.794	24	0.033	
Total	0.835	27		

Blood Uric Acid

Source of Variation	SS	df	MS	F
Treatments	24.431	3	8.143	13.806
Experimental error	14.156	24	0.589	
Total	38.588	27		

Coagulation time

Source of Variation	SS	df	MS	F
Treatments	4.487	3	1.495	1.215
Experimental error	29.539	24	1.230	
Total	34.027	27		

Metabolic Effects.Analysis of variance summary tables.Within Group 1 (male fructose) analysis.Dietary Sugar Intake.

Source of Variation	SS	df	MS	F
Treatments	2584.446	2	1292.223	78.403*
Experimental error	445.009	27	16.482	
Total	3029.454	29		

Bodyweight.

Source of Variation	SS	df	MS	F
Treatments	0.055	2	0.028	1.264
Experimental error	0.588	27	0.022	
Total	0.643	29		

Blood cholesterol esters.

Source of Variation	SS	df	MS	F
Treatments	59.139	2	29.569	3.785*
Experimental error	210.94	27	7.813	
Total	270.079	29		

Metabolic Effects.Analysis of variance summary tables.Within Group 1 (male fructose) analysis (cont.)Blood Triglycerides

Source of Variation	SS	df	MS	F
Treatments	280.200	2	140.100	8.441*
Experimental error	448.121	27	16.597	
Total	728.321	29		

Blood LDL

Source of Variation	SS	df	MS	F
Treatments	43.148	2	21.574	5.606*
Experimental error	103.906	27	3.848	
Total	147.053	29		

Blood Glucose

Source of Variation	SS	df	MS	F
Treatments	5.200	2	2.600	1.538
Experimental error	45.659	27	1.691	
Total	50.859	29		

Metabolic Effects.Analysis of variance summary tables.Within Group 1 (male fructose) analysis (cont.)Blood NEFA

Source of Variation	SS	df	MS	F
Treatments	145.563	2	72.781	1.413
Experimental error	1390.791	27	51.511	
Total	1536.354	29		

Blood Uric Acid

Source of Variation	SS	df	MS	F
Treatments	8.069	2	4.035	0.858
Experimental error	126.890	27	4.700	
Total	134.960	29		

Blood Coagulation time.

Source of Variation	SS	df	MS	F
Treatments	31.544	2	15.772	4.690*
Experimental error	90.792	27	3.363	
Total	122.336	29		

Metabolic Effects.

Analysis of variance summary tables.

Within Group 2 (male glucose) analysis.

Dietary Sugar Intake.

Source of Variation	SS	df	MS	F
Treatments	1445.225	2	722.612	36.174*
Experimental error	299.644	15	19.976	
Total	1744.869	17		

Bodyweight

Source of Variation	SS	df	MS	F
Treatments	0.020	2	0.010	0.598
Experimental error	0.251	15	0.017	
Total	0.271	17		

Blood cholesterol esters.

Source of Variation	SS	df	MS	F
Treatments	24.327	2	12.163	12.588*
Experimental error	14.493	15	0.966	
Total	38.820	17		

Metabolic Effects.Analysis of variance summary tables.Within Group 2 (male glucose) analysis (cont).Blood triglycerides.

Source of Variation	SS	df	MS	F
Treatments	106.667	2	53.334	2.067
Experimental error	387.031	15	25.802	
Total	493.698	17		

Blood LDL.

Source of Variation	SS	df	MS	F
Treatments	8.730	2	4.365	0.901
Experimental error	72.676	15	4.845	
Total	81.405	17		

Blood glucose.

Source of Variation	SS	df	MS	F
Treatments	1.396	2	0.698	0.421
Experimental error	24.895	15	1.660	
Total	26.292	17		

Metabolic Effects.Analysis of variance summary tables.Within Group 2 (male glucose) analysis (cont.)Blood NEFA.

Source of Variation	SS	df	MS	F
Treatments	308.257	2	154.129	2.345
Experimental error	986.018	15	65.735	
Total	1294.276	17		

Blood Uric Acid.

Source of Variation	SS	df	MS	F
Treatments	16.308	2	8.154	1.514
Experimental error	80.797	15	5.386	
Total	97.105	17		

Blood Coagulation time.

Source of Variation	SS	df	MS	F
Treatments	23.282	2	11.641	3.303
Experimental error	52.860	15	3.524	
Total	76.142	17		

Metabolic Effects.Analysis of variance summary tables.Within Group 3 (female fructose) analysis.Dietary Sugar Intake.

Source of Variation	SS	df	MS	F
Treatments	1997.624	2	998.812	31.357*
Experimental error	477.791	15	31.853	
Total	2475.415	17		

Bodyweight.

Source of Variation	SS	df	MS	F
Treatments	0.070	2	0.035	2.998
Experimental error	0.176	15	0.012	
Total	0.246	17		

Blood cholesterol esters.

Source of Variation	SS	df	MS	F
Treatments	21.392	2	10.696	7.243*
Experimental error	22.150	15	1.477	
Total	43.541	17		

Metabolic Effects.Analysis of variance summary tables.Within Group 3 (female fructose) analysis (cont.)Blood Triglycerides.

Source of Variation	SS	df	MS	F
Treatments	8.769	2	4.384	0.223
Experimental error	295.200	15	19.680	
Total	303.969	17		

Blood LDL.

Source of Variation	SS	df	MS	F
Treatments	13.686	2	6.843	1.579
Experimental error	64.996	15	4.333	
Total	78.681	17		

Blood Glucose.

Source of Variation	SS	df	MS	F
Treatments	1.784	2	0.892	0.638
Experimental error	20.970	15	1.398	
Total	22.754	17		

Metabolic Effects.Analysis of variance summary tables.Within Group 3 (female fructose) analysis (cont.)Blood NEFA.

Source of Variation	SS	df	MS	F
Treatments	33.458	2	16.729	0.525
Experimental error	478.160	15	31.877	
Total	511.618	17		

Blood Uric Acid.

Source of Variation	SS	df	MS	F
Treatments	23.970	2	11.985	2.227
Experimental error	80.743	15	5.383	
Total	104.714	17		

Coagulation time

Source of Variation	SS	df	MS	F
Treatments	4.309	2	2.155	0.322
Experimental error	100.323	15	6.688	
Total	104.632	17		

Metabolic Effects.Analysis of variance summary tables.Within Group 4 (female glucose) analysis.Dietary Sugar Intake.

Source of Variation	SS	df	MS	F
Treatments	1202.076	2	601.038	8.735*
Experimental error	1032.075	15	68.805	
Total	2234.152	17		

Bodyweight.

Source of Variation	SS	df	MS	F
Treatments	0.108	2	0.054	0.734
Experimental error	1.103	15	0.074	
Total	1.211	17		

Blood cholesterol esters.

Source of Variation	SS	df	MS	F
Treatments	4.309	2	2.154	3.029
Experimental error	10.669	15	0.711	
Total	14.977	17		

Metabolic Effects.

Analysis of variance summary tables.

Within Group 4 (female glucose) analysis (cont.)

Blood Triglycerides.

Source of Variation	SS	df	MS	F
Treatments	1.769	2	0.885	0.220
Experimental error	60.234	15	4.016	
Total	62.003	17		

Blood LDL.

Source of Variation	SS	df	MS	F
Treatments	1.038	2	0.519	0.367
Experimental error	21.204	15	1.414	
Total	22.242	17		

Blood Glucose.

Source of Variation	SS	df	MS	F
Treatments	2.604	2	1.302	2.014
Experimental error	9.699	15	0.647	
Total	12.303	17		

Metabolic Effects.Analysis of variance summary tables.Within Group 4 (female glucose) analysis (cont.)Blood NEFA.

Source of Variation	SS	df	MS	F
Treatments	0.330	2	0.165	0.096
Experimental error	25.698	15	1.713	
Total	26.029	17		

Blood Uric Acid.

Source of Variation	SS	df	MS	F
Treatments	47.498	2	23.749	1.307
Experimental error	272.613	15	18.174	
Total	320.111	17		

Coagulation time.

Source of Variation	SS	df	MS	F
Treatments	6.301	2	3.150	0.793
Experimental error	59.557	15	3.970	
Total	65.858	17		

Metabolic Effects.
Detailed comparison of means.
Between group base-line values.

Bodyweight.

		Group			
		4	3	1	2
Group	Means	58.7	59.4	72.2	73.1
	4	58.7	-	0.7	13.5
	3	59.4	-	12.8	13.7
	1	72.2	-	-	0.9
	2	73.1	-	-	-
r		=	2	3	4
q(r,24)			2.92	3.53	3.90
$\sqrt{\text{MSerror}/\bar{n}}$ q(r,24)			6.342	7.667	8.47

Blood Uric Acid.

		Group			
		3	4	1	2
Group	Means	3.9	4.2	6.3	6.4
	3	3.9	-	0.3	2.4
	4	4.2	-	2.1	2.2
	1	6.3	-	-	0.1
	2	6.4	-	-	-
r		=	2	3	4
q(r,24)			2.92	3.53	3.90
$\sqrt{\text{MSerror}/\bar{n}}$ q(r,24)			0.819	0.990	1.094

Metabolic Effects.

Detailed comparison of means.

Within Group 1 (male fructose).

Sugar Intake.

		Day		
		8	22	15
Day	Means	25.988	27.587	46.428
	8	25.988	-	1.599
	22	27.587	-	18.841
	15	46.428	-	-

r=	2	3
q(r,27)	2.875	3.510
$\sqrt{\text{MSerror}/n}$ q(r,27)	3.691	4.506

Blood Cholesterol esters.

		Day		
		8	15	22
Day	Means	31.848	32.934	35.217
	8	31.848	-	1.086
	15	32.934	-	2.283
	22	35.217	-	-

r=	2	3
q(r,27)	2.77	3.51
$\sqrt{\text{MSerror}/n}$ q (r,27)	2.448	3.103

Metabolic Effects.

Detailed comparison of means.

Within Group 1 (male fructose)(cont.)

Blood Triglycerides.

		Day		
		8	22	15
Day	Means	29.514	33.491	36.995
	8	29.514	-	3.977
	22	33.491	-	7.481
	15	36.995	-	3.50

r=	2	3
q(r,27)	2.77	3.51
$\sqrt{\text{MSerror}/n}$ q(r,27)	3.569	4.522

Blood LDL.

		Day		
		8	22	15
Day	Means	31.773	33.539	34.689
	8	31.773	-	1.766
	22	33.539	-	3.916
	15	34.689	-	1.15

r=	2	3
q(r,27)	2.77	3.51
$\sqrt{\text{MSerror}/n}$ q(r,27)	1.718	2.177

Metabolic Effects.

Detailed comparison of means.

Within Group 1 (male fructose)(cont.)

Coagulation time.

		Day		
		15	22	8
Day	Means	32.360	32.886	34.750
	15	32.360	-	0.526
	22	32.886	-	2.390
	8	34.750	-	1.864

r=	2	3
q(r,27)	2.77	3.51
$\sqrt{\text{MSerror}/n}$ q(r,27)	1.606	2.035

Metabolic Effects.Detailed comparison of means.Within Group 2 (male glucose)Sugar Intake.

		Day		
		22	8	15
Day	Means	25.930	28.000	45.888
	22 25.930	-	2.07	19.958
	8 28.000		-	17.888
	15 45.888			-
r =			2	3

q(r,15)

3.015

3.675

 $\sqrt{\text{MSerror}/n}$ q(r,15)

5.501

6.706

Blood Cholesterol Esters.

		Day		
		8	15	22
Day	Means	32.240	32.817	34.943
	8 32.240	-	0.577	2.703
	15 32.817		-	2.126
	22 34.943			-
r =			2	3

q(r,15)

3.015

3.675

 $\sqrt{\text{MSerror}/n}$ q(r,15)

1.210

1.475

Metabolic Effects.

Detailed comparison of means.

Within Group 3 (female fructose).

Sugar Intake.

		Day		
		8	22	15
Day	Means	25.750	26.760	47.490
	8	25.750	-	1.01
	22	26.760	-	21.740
	15	47.490	-	20.730

r=	2	3
q(r,15)	3.015	3.675
$\sqrt{\text{MSerror}/n}$ q(r,15)	6.947	8.468

Blood Cholesterol Esters.

		Day		
		15	22	8
Day	Means	32.065	33.210	34.727
	15	32.065	-	1.145
	22	33.210	-	2.662
	8	34.727	-	1.517

r=	2	3
q(r,15)	3.015	3.675
$\sqrt{\text{MSerror}/n}$ q(r,15)	1.496	1.823

Metabolic Effects.

Detailed comparisons of means.

Within Group 4 (female glucose).

Sugar Intake.

		Day		
		8	22	15
Day	Means	25.140	30.370	44.488
	8	25.140	-	5.230
	22	30.370	-	19.348
	15	44.488	-	14.118

r=	2	3
q(r,15)	3.015	3.675
$\sqrt{\text{MSerror}/n}$ q(r,15)	10.210	12.445

DATA SUMMARIES
AND STATISTICAL
ANALYSES.

FROM SECTION 3.5
HORMONAL EFFECTS.

Hormonal Effects.

Group 1 (male fructose) data summary.

Daily Sugar Intake (g)							
Day	1-7	(%)	8-14	(%)	15-21	(%)	
SUBJECT	1	115	24.84	197	42.55	151	32.61
	2	160	27.35	246	42.05	179	30.60
	3	136	34.26	163	41.06	98	24.69
	4	71	24.48	145	50.00	74	25.52
	5	122	39.61	115	37.34	71	23.05
\bar{X}	121	30.12	173	42.60	114	27.29	
SEM	14.6	2.95	22.5	2.06	21.6	1.83	

Bodyweight (kg)							
Day	8	(%)	15	(%)	22	(%)	
SUBJECT	1	79.8	33.36	79.7	33.32	79.7	33.32
	2	80.8	33.43	79.9	33.06	81.0	33.51
	3	73.4	33.30	73.4	33.30	73.6	33.39
	4	72.0	33.46	71.0	32.99	72.2	33.55
	5	71.3	33.26	72.0	33.58	71.1	33.16
\bar{X}	75.5	33.36	75.2	33.25	75.5	33.39	
SEM	2.0	0.04	1.92	0.01	2.0	0.07	

Hormonal Effects.

Group 1 (male fructose) data summary (cont.)

Blood Glucose (mg%)							
Day	8	(%)	15	(%)	22	(%)	
SUBJECT	1	83	31.80	89	34.10	89	34.10
	2	79	32.11	82	33.33	85	34.55
	3	81	33.06	76	31.02	88	35.92
	4	81	34.76	79	33.91	73	31.33
	5	91	33.09	90	32.73	94	34.18
\bar{X}	83	32.96	83	33.02	86	34.02	
SEM	2	0.52	3	0.55	4	0.75	

Blood triglyceride (mg%)							
Day	8	(%)	15	(%)	22	(%)	
SUBJECT	1	101	28.45	137	38.59	117	32.96
	2	138	28.69	188	39.09	155	32.23
	3	145	30.53	187	39.37	143	30.11
	4	127	32.56	164	42.05	99	25.39
	5	166	29.54	196	34.88	200	35.39
\bar{X}	135	29.95	174	38.80	143	31.26	
SEM	11	0.75	11	1.15	17	1.71	

Hormonal Effects.Group 1 (male fructose) data summary (cont.)

Blood LDL (mg%)							
Day	8	(%)	15	(%)	22	(%)	
SUBJECT	1	246	33.65	241	32.97	244	33.38
	2	349	29.04	411	34.19	442	36.77
	3	418	32.03	445	34.10	442	33.87
	4	332	31.65	355	33.84	362	34.51
	5	582	30.89	689	36.57	613	32.54
\bar{X}	385	31.45	428	34.33	421	34.21	
SEM	56	0.75	74	0.60	60	0.72	

Blood Insulin (IU/l)							
Day	8	(%)	15	(%)	22	(%)	
SUBJECT	1	9.3	20.53	19.8	43.71	16.2	35.76
	2	10.0	28.25	12.4	35.03	13.0	36.72
	3	18.5	38.70	8.0	16.74	21.3	44.56
	4	10.5	36.21	8.5	29.31	10.0	34.48
	5	8.8	29.53	9.5	31.88	11.5	38.59
\bar{X}	11.4	30.64	11.6	31.33	14.4	38.02	
SEM	1.8	3.20	2.2	4.38	2.0	1.77	

Hormonal Effects.

Group 1 (male fructose) data summary (cont.)

Blood Cortisol($\mu\text{g}\%$)							
Day	8	(%)	15	(%)	22	(%)	
SUBJECT	1	14.5	32.66	14.9	33.56	15.0	33.78
	2	13.0	28.32	18.4	40.09	14.5	31.59
	3	8.8	26.43	9.7	29.13	14.8	44.44
	4	11.9	30.75	11.9	30.75	14.9	38.50
	5	16.0	30.48	16.5	31.43	20.0	38.10
\bar{X}	12.8	29.73	14.3	32.99	15.8	37.28	
SEM	1.2	1.07	1.6	1.91	1.0	2.21	

Blood Thyroxine(μg%)							
Day	8	(%)	15	(%)	22	(%)	
SUBJECT	1	5.4	31.04	6.0	34.48	6.0	34.48
	2	7.2	31.44	7.2	31.44	8.5	37.12
	3	7.2	31.44	7.4	32.31	8.3	36.25
	4	6.6	33.50	6.7	34.01	6.4	32.49
	5	7.2	37.11	6.4	32.99	5.8	29.90
\bar{X}	6.7	32.91	6.7	33.05	7.0	34.05	
SEM	0.4	1.14	0.3	0.55	0.6	1.31	

Hormonal Effects.Group 2 (male glucose) data summary.

Daily Sugar Intake (g)							
Days	1-7	(%)	8-14	(%)	15-21	(%)	
SUBJECT	6	139	30.68	209	46.14	105	23.18
	7	71	23.13	129	42.04	107	34.85
	8	100	25.25	201	50.76	95	23.99
	9	120	25.53	224	47.66	126	26.81
	10	96	25.95	166	44.86	108	29.19
\bar{X}	105	26.11	186	46.29	108	27.60	
SEM	11.5	1.24	17.1	1.45	5.3	2.10	

Bodyweight (kg)							
Day	8	(%)	15	(%)	22	(%)	
SUBJECT	6	68.2	33.29	68.0	33.19	68.7	33.53
	7	70.0	33.38	70.3	33.52	69.4	33.10
	8	68.0	33.50	67.8	33.40	67.2	33.10
	9	69.3	33.45	68.8	33.21	69.1	33.35
	10	77.9	33.33	77.9	33.33	77.9	33.33
\bar{X}	70.7	33.39	70.6	33.33	70.5	33.28	
SEM	1.8	0.04	1.9	0.06	1.9	0.08	

Hormonal Effects.Group 2 (male glucose) data summary (cont.)

Blood Glucose (mg%)							
Day	8	(%)	15	(%)	22	(%)	
SUBJECT	6	85	31.25	94	34.56	93	34.19
	7	84	33.33	79	31.35	89	35.32
	8	92	33.33	97	35.15	87	31.52
	9	81	32.14	87	34.52	84	33.33
	10	76	32.34	78	33.19	81	34.47
\bar{X}	84	32.48	87	33.75	87	33.77	
SEM	3	0.39	4	0.68	2	0.65	

Blood Triglyceride (mg%)							
Day	8	(%)	15	(%)	22	(%)	
SUBJECT	6	84	27.91	117	38.87	100	33.22
	7	135	32.14	128	30.48	157	37.38
	8	152	32.69	140	30.12	173	37.20
	9	113	32.95	126	36.74	104	30.32
	10	150	33.11	153	33.78	150	33.11
\bar{X}	127	31.76	133	34.00	137	34.25	
SEM	13	0.90	6	1.71	15	1.35	

Hormonal Effects.Group 2 (male glucose) data summary (cont.)

Blood LDL (mg%)							
Day	8	(%)	15	(%)	22	(%)	
SUBJECT	6	435	30.57	487	34.22	501	35.21
	7	190	31.61	163	27.12	248	41.27
	8	211	34.82	161	26.57	234	38.61
	9	560	33.04	560	33.04	575	33.92
	10	539	32.79	586	35.65	519	31.57
\bar{X}	387	32.57	391	31.32	415	36.12	
SEM	79	0.72	95	1.88	72	1.72	

Blood Insulin (IU/1)							
Day	8	(%)	15	(%)	22	(%)	
SUBJECT	6	10.9	31.41	16.4	47.26	7.4	21.33
	7	8.0	26.58	9.2	30.56	12.9	42.86
	8	9.3	31.10	6.1	20.40	14.5	48.49
	9	9.1	19.83	21.8	47.49	15.0	32.68
	10	16.4	31.30	11.9	22.71	24.1	45.99
\bar{X}	10.7	28.04	13.1	33.68	14.8	38.27	
SEM	1.5	2.25	2.8	5.84	2.7	5.02	

Hormonal Effects.Group 2 (male glucose) data summary (cont.)

Blood Cortisol (mg%)							
Day	8	(%)	15	(%)	22	(%)	
SUBJECT	6	13.3	28.00	18.7	39.37	15.5	32.63
	7	12.9	39.09	9.5	28.79	10.6	32.12
	8	12.5	34.92	13.8	38.55	9.5	26.54
	9	17.9	33.21	17.9	33.21	18.1	33.58
	10	11.3	26.78	17.0	40.28	13.9	32.94
\bar{X}	13.6	32.40	15.4	36.04	13.5	31.56	
SEM	1.1	2.27	1.7	2.19	1.6	1.28	

Blood Thyroxine (mg%)							
Day	8	(%)	15	(%)	22	(%)	
SUBJECT	6	5.4	31.95	5.4	31.95	6.1	36.09
	7	5.1	33.12	4.1	26.62	6.2	40.26
	8	6.5	33.51	5.3	27.32	7.6	39.18
	9	6.8	32.08	7.1	33.49	7.3	34.43
	10	6.4	35.75	6.0	33.52	5.5	30.73
\bar{X}	6.0	33.28	5.6	30.58	6.5	36.14	
SEM	0.3	0.69	0.5	1.50	0.4	1.71	

Hormonal Effects.Group 3 (female fructose) data summary.

Daily Sugar Intake (g)							
Days	1-7	(%)	8-14	(%)	15-21	(%)	
SUBJECT	11	149	28.33	243	46.20	134	25.48
	12	47	21.86	109	50.70	59	27.44
	13	113	29.20	128	33.08	146	37.73
	14	73	22.19	123	37.39	133	40.43
	15	107	28.16	147	38.68	126	33.16
\bar{X}	98	25.95	150	41.21	120	32.85	
SEM	17.5	1.61	24.0	3.18	15.5	2.87	

Bodyweight (kg)							
Day	8	(%)	15	(%)	22	(%)	
SUBJECT	11	58.8	33.39	58.7	33.33	58.6	33.28
	12	55.3	33.35	55.3	33.35	55.2	33.29
	13	56.9	33.47	56.7	33.35	56.4	33.18
	14	64.4	33.18	64.9	33.44	64.8	33.39
	15	62.7	33.26	62.9	33.37	62.9	33.37
\bar{X}	59.6	33.33	59.7	33.37	59.6	33.30	
SEM	1.7	0.05	1.8	0.02	1.9	0.04	

Hormonal Effects.Group 3 (female fructose) data summary (cont.)

Blood Glucose (mg%)							
Day		8	(%)	15	(%)	22	(%)
SUBJECT	11	83	35.02	76	32.07	78	32.91
	12	79	33.62	74	31.49	82	34.89
	13	85	31.60	93	34.57	91	33.83
	14	77	32.08	82	34.17	81	33.75
	15	76	33.63	74	32.63	76	33.63
	\bar{X}	80	33.19	80	32.99	82	33.80
	SEM	2	0.61	4	0.60	3	0.32

Blood Triglycerides (mg%)							
Day	8	(%)	15	(%)	22	(%)	
SUBJECT	11	98	29.70	105	31.82	127	38.49
	12	100	30.96	88	27.25	135	41.80
	13	114	30.16	121	32.01	143	37.83
	14	86	39.82	61	28.24	69	31.94
	15	89	32.84	128	47.23	54	19.94
\bar{X}	97	32.70	101	33.31	106	34.00	
SEM	5	1.86	12	3.61	18	3.86	

Hormonal Effects.Group 3 (female fructose) data summary (cont.)

Blood LDL (mg%)						
Day	8	(%)	15	(%)	22	(%)
S 11	359	34.75	339	32.82	335	32.43
U 12	293	34.11	305	35.51	261	30.38
B 13	361	34.38	321	30.57	368	35.05
J 14	344	34.09	326	32.31	339	33.60
E 15	381	31.65	416	34.55	407	33.80
C						
T						
\bar{X}	348	33.80	341	33.15	342	33.05
SEM	15	0.55	19	0.87	24	0.79

Blood Insulin (IU/l)						
Day	8	(%)	15	(%)	22	(%)
S 11	13.2	36.67	11.9	33.06	10.9	30.28
U 12	7.8	24.68	10.9	34.49	12.9	40.82
B 13	13.3	33.00	12.5	31.02	14.5	35.98
J 14	13.9	29.89	17.9	38.50	14.7	31.61
E 15	9.9	32.35	11.9	38.89	8.8	28.76
C						
T						
\bar{X}	11.6	31.32	13.0	35.19	12.4	33.49
SEM	1.2	1.98	1.3	1.53	1.1	2.19

Hormonal Effects.Group 3 (female fructose) data summary (cont.)

Blood Cortisol ($\mu\text{g}\%$)						
Day	8	(%)	15	(%)	22	(%)
S 11	22.8	26.21	28.8	33.10	35.4	40.69
U 12	30.0	31.58	27.0	28.42	38.0	40.00
B 13	9.2	23.47	15.4	39.29	14.6	37.25
E 14	20.0	39.37	15.4	30.32	15.4	30.32
C 15	7.0	27.13	10.8	41.86	8.0	31.01
T						
\bar{X}	17.8	29.55	19.5	34.60	22.3	35.85
SEM	4.5	2.78	3.6	2.58	6.0	2.20

Blood Thyroxine ($\mu\text{g}\%$)						
Day	8	(%)	15	(%)	22	(%)
S 11	10.0	37.59	8.3	31.20	8.3	31.20
U 12	9.9	31.13	10.5	33.02	11.4	35.85
B 13	7.2	33.65	7.6	35.51	6.6	30.84
E 14	13.4	37.43	11.0	30.73	11.4	31.84
C 15	5.5	35.71	5.0	32.47	4.9	31.82
T						
\bar{X}	9.2	35.10	8.5	32.59	8.5	32.31
SEM	1.4	1.22	1.1	0.84	1.3	0.91

Hormonal Effects.

Group 4 (female glucose) data summary.

Daily Sugar Intake (g)							
Day	1-7	(%)	8-14	(%)	15-21	(%)	
SUBJECT	16	39	19.40	99	49.25	63	31.34
	17	53	28.96	80	43.72	50	27.32
	18	71	29.22	109	44.86	63	25.93
	19	133	30.37	165	37.67	140	31.96
	20	111	31.18	170	47.75	75	21.07
\bar{X}	81	27.83	125	44.65	78	27.52	
SEM	17.7	2.14	18.1	2.01	16.0	1.98	

Bodyweight (kg)							
Day	8	(%)	15	(%)	22	(%)	
SUBJECT	16	60.0	33.37	60.0	33.37	59.8	33.26
	17	53.7	33.35	53.1	32.98	54.2	33.67
	18	67.2	33.40	67.3	33.45	66.7	33.15
	19	56.5	33.18	57.1	33.53	56.7	33.29
	20	57.1	33.24	57.1	33.24	57.6	33.53
\bar{X}	58.9	33.31	58.9	33.31	59.0	33.38	
SEM	2.3	0.04	2.4	0.10	2.1	0.10	

Hormonal Effects.Group 4 (female glucose) data summary (cont.)

Blood Glucose (mg%)							
Day	8	(%)	15	(%)	22	(%)	
SUBJECT	16	82	32.93	82	32.93	85	34.14
	17	79	32.65	81	33.47	82	33.88
	18	81	34.62	73	31.20	80	34.19
	19	78	34.36	79	34.80	70	30.84
	20	74	35.75	64	30.92	69	33.33
\bar{X}	79	33.66	76	32.66	77	33.28	
SEM	1	0.65	3	0.72	3	0.63	

Blood Triglyceride (mg%)							
Day	8	(%)	15	(%)	22	(%)	
SUBJECT	16	76	34.08	79	35.43	68	30.49
	17	110	32.26	108	31.67	123	36.07
	18	82	33.75	79	32.51	82	33.75
	19	101	36.73	91	33.09	83	30.18
	20	104	33.77	99	32.14	105	34.09
\bar{X}	95	34.12	91	32.97	92	32.92	
SEM	7	0.73	6	0.66	10	1.13	

Hormonal Effects.Group 4 (female glucose) data summary (cont.)

Blood LDL (mg%)						
Day	8	(%)	15	(%)	22	(%)
S 16	293	33.33	307	34.93	279	31.74
U 17	322	32.79	335	34.11	325	33.10
B 18	342	32.08	357	33.49	367	34.43
E 19	376	34.78	362	33.49	343	31.73
C 20	356	31.70	385	34.28	382	34.02
T						
\bar{X}	338	32.94	349	34.06	339	33.0
SEM	14	0.54	13	0.27	18	0.56

Blood Insulin (IU/l)						
Day	8	(%)	15	(%)	22	(%)
S 16	11.7	29.85	10.0	25.51	17.5	44.64
U 17	6.8	23.94	13.8	48.59	7.8	27.46
B 18	10.0	31.35	12.7	39.81	9.2	28.84
E 19	12.1	32.10	12.6	33.42	13.0	34.48
C 20	9.5	33.33	9.3	32.63	9.7	34.04
T						
\bar{X}	10.0	30.11	11.7	35.99	11.4	33.89
SEM	0.9	1.64	0.9	3.88	1.7	3.02

Hormonal Effects.Group 4 (female glucose) data summary (cont.)

Blood Cortisol ($\mu\text{g}\%$)							
Day	8	(%)	15	(%)	22	(%)	
SUBJECT	16	11.4	34.13	8.8	26.35	13.2	39.52
	17	33.4	35.84	26.8	28.76	33.0	35.41
	18	17.8	28.12	19.5	30.81	26.0	41.07
	19	12.4	27.37	9.80	21.63	23.1	50.99
	20	15.5	28.44	23.0	42.20	16.0	29.36
\bar{X}	18.1	30.78	17.68	29.95	22.3	39.27	
SEM	4.0	1.75	3.2	3.42	3.5	3.56	

Blood Thyroxine ($\mu\text{g}\%$)							
Day	8	(%)	15	(%)	22	(%)	
SUBJECT	16	4.2	25.46	5.9	35.76	6.4	38.79
	17	10.7	38.63	9.9	35.74	7.1	25.63
	18	7.3	34.27	5.9	27.70	8.1	38.03
	19	10.7	38.91	9.3	33.82	7.5	22.27
	20	13.2	35.77	12.6	34.15	11.1	30.08
\bar{X}	9.2	34.61	8.7	33.43	8.0	31.96	
SEM	1.6	2.45	1.3	1.49	0.8	2.7	

Hormonal Effects.Analysis of variance summary tables.Between Group analysis - base line data.Sugar Intake.

Source of Variation	SS	df	MS	F
Treatments	5347.600	3	1782.533	1.381
Experimental error	20653.600	16	1290.850	
Total	26001.200	19		

Bodyweight.

Source of Variation	SS	df	MS	F
Treatments	1011.998	3	337.333	17.194*
Experimental error	313.908	16	19.619	
Total	1325.906	19		

Blood Glucose.

Source of Variation	SS	df	MS	F
Treatments	80.550	3	26.850	1.326
Experimental error	324.000	16	20.250	
Total	404.550	19		

Hormonal Effects.Analysis of variance summary tables.Between Group analysis - base line data (cont.)Blood Triglycerides.

Source of Variation	SS	df	MS	F
Treatments	6364.550	3	2121.517	4.915*
Experimental error	6906.400	16	431.650	
Total	13270.950	19		

Blood LDL.

Source of Variation	SS	df	MS	F
Treatments	9707.750	3	3235.917	0.263
Experimental error	196929.200	16	12308.075	
Total	206636.950	19		

Blood Insulin.

Source of Variation	SS	df	MS	F
Treatments	7.894	3	2.631	0.272
Experimental error	154.536	16	9.659	
Total	162.430	19		

Hormonal Effects.Analysis of variance summary tables.Between Group analysis - base line data (cont.)Blood Cortisol.

Source of Variation	SS	df	MS	F
Treatments	113.932	3	37.977	0.818
Experimental error	743.140	16	46.446	
Total	857.072	19		

Blood Thyroxine.

Source of Variation	SS	df	MS	F
Treatments	41.202	3	13.734	2.435
Experimental error	90.228	16	5.639	
Total	131.430	19		

Hormonal Effects.Analysis of variance summary tables.Within Group 1 (male fructose) analysis.Dietary Sugar Intake.

Source of Variation	SS	df	MS	F
Treatments	663.608	2	331.804	12.188*
Experimental error	326.683	12	27.224	
Total	990.291	14		

Bodyweight.

Source of Variation	SS	df	MS	F
Treatments	0.079	2	0.040	1.254
Experimental error	0.380	12	0.032	
Total	0.459	14		

Blood Glucose.

Source of Variation	SS	df	MS	F
Treatments	3.509	2	1.755	0.930
Experimental error	22.633	12	1.886	
Total	26.142	14		

Hormonal Effects.Analysis of variance summary tables.Within Group 1 (male fructose) analysis (cont.)Blood triglycerides.

Source of Variation	SS	df	MS	F
Treatments	227.880	2	113.940	14.258*
Experimental error	95.895	12	7.991	
Total	323.775	14		

Blood LDL.

Source of Variation	SS	df	MS	F
Treatments	26.582	2	13.291	5.547*
Experimental error	28.755	12	2.396	
Total	55.336	14		

Blood Insulin.

Source of Variation	SS	df	MS	F
Treatments	166.067	2	83.034	1.529
Experimental error	651.776	12	54.315	
Total	817.843	14		

Hormonal Effects.Analysis of variance summary tables.Within Group 1 (male fructose) analysis (cont.)Blood Cortisol.

Source of Variation	SS	df	MS	F
Treatments	143.535	2	71.767	4.435*
Experimental error	194.204	12	16.184	
Total	337.739	14		

Blood Thyroxine.

Source of Variation	SS	df	MS	F
Treatments	3.880	2	1.940	0.353
Experimental error	66.022	12	5.502	
Total	69.902	14		

Hormonal Effects.

Analysis of variance summary tables.

Within Group 2 (male glucose) analysis.

Dietary Sugar Intake.

Source of Variation	SS	df	MS	F
Treatments	1248.773	2	624.386	47.959*
Experimental error	156.230	12	13.019	
Total	1405.003	14		

Bodyweight.

Source of Variation	SS	df	MS	F
Treatments	0.029	2	0.015	0.735
Experimental error	0.239	12	0.020	
Total	0.268	14		

Blood Glucose.

Source of Variation	SS	df	MS	F
Treatments	5.479	2	2.739	1.588
Experimental error	20.701	12	1.725	
Total	26.179	14		

Hormonal Effects.Analysis of variance summary tables.Within Group 2 (male glucose) analysis (cont).Blood Triglycerides.

Source of Variation	SS	df	MS	F
Treatments	18.751	2	9.375	0.986
Experimental error	114.093	12	9.508	
Total	132.843	14		

Blood LDL.

Source of Variation	SS	df	MS	F
Treatments	61.928	2	30.964	2.660
Experimental error	139.666	12	11.639	
Total	201.594	14		

Blood Insulin

Source of Variation	SS	df	MS	F
Treatments	262.353	2	131.177	1.224
Experimental error	1285.776	12	107.148	
Total	1548.130	14		

Hormonal Effects.Analysis of variance summary tables.Within Group 2 (male glucose) analysis (cont.)Blood Cortisol.

Source of Variation	SS	df	MS	F
Treatments	56.674	2	28.337	1.470
Experimental error	231.289	12	19.274	
Total	287.963	14		

Blood Thyroxine.

Source of Variation	SS	df	MS	F
Treatments	35.515	2	17.757	1.666
Experimental error	127.897	12	10.658	
Total	163.412	14		

Hormonal Effects.

Analysis of variance summary tables.

Within Group 3 (female fructose) analysis.

Dietary Sugar Intake.

Source of Variation	SS	df	MS	F
Treatments	584.103	2	292.051	8.365*
Experimental error	418.979	12	34.915	
Total	1003.082	14		

Bodyweight.

Source of Variation	SS	df	MS	F
Treatments	0.011	2	0.005	0.764
Experimental error	0.086	12	0.007	
Total	0.097	14		

Blood Glucose.

Source of Variation.	SS	df	MS	F
Treatments	1.803	2	0.902	0.651
Experimental error	16.615	12	1.385	
Total	18.418	14		

Hormonal Effects.Analysis of variance summary tables.Within Group 3 (female fructose) analysis (cont.)Blood Triglycerides.

Source of Variation	SS	df	MS	F
Treatments	4.256	2	2.128	0.041
Experimental error	626.894	12	52.241	
Total	631.150	14		

Blood LDL.

Source of Variation	SS	df	MS	F
Treatments	1.630	2	0.815	0.293
Experimental error	33.420	12	2.785	
Total	35.051	14		

Blood Insulin

Source of Variation	SS	df	MS	F
Treatments	37.704	2	18.852	1.020
Experimental error	221.843	12	18.487	
Total	259.546	14		

Hormonal Effects.

Analysis of variance summary tables.

Within Group 3 (female fructose) analysis (cont.)

Blood Cortisol.

Source of Variation	SS	df	MS	F
Treatments	111.258	2	55.629	1.736
Experimental error	384.611	12	32.051	
Total	495.869	14		

Blood Thyroxine.

Source of Variation	SS	df	MS	F
Treatments	23.669	2	11.835	2.353
Experimental error	60.367	12	5.031	
Total	84.037	14		

Hormonal Effects.

Analysis of variance summary tables.

Within Group 4 (female glucose) analysis.

Dietary Sugar Intake.

Source of Variation	SS	df	MS	F
Treatments	960.730	2	480.365	22.982
Experimental error	250.824	12	20.902	
Total	1211.554	14		

Bodyweight.

Source of Variation	SS	df	MS	F
Treatments	0.016	2	0.008	0.238
Experimental error	0.402	12	0.034	
Total	0.418	14		

Blood Glucose.

Source of Variation	SS	df	MS	F
Treatments	4.911	2	2.456	1.185
Experimental error	24.876	12	2.073	
Total	29.787	14		

Hormonal Effects.Analysis of variance summary tables.Within Group 4 (female glucose) analysis (cont.)Blood Triglycerides.

Source of Variation	SS	df	MS	F
Treatments	4.648	2	2.324	0.624
Experimental error	44.687	12	3.724	
Total	49.335	14		

Blood LDL.

Source of Variation	SS	df	MS	F
Treatments	3.972	2	1.986	1.754
Experimental error	13.591	12	1.133	
Total	17.563	14		

Blood Insulin.

Source of Variation	SS	df	MS	F
Treatments	88.724	2	44.362	0.990
Experimental error	537.860	12	44.822	
Total	626.584	14		

Hormonal Effects.Analysis of variance summary tables.Within Group 4 (female glucose) analysis (cont.)Blood Cortisol.

Source of Variation	SS	df	MS	F
Treatments	266.052	2	133.026	2.907
Experimental error	549.175	12	45.765	
Total	815.227	14		

Blood Thyroxine.

Source of Variation	SS	df	MS	F
Treatments	17.605	2	8.802	0.337
Experimental error	330.800	12	26.100	
Total	313.196	14		

Hormonal Effects.

Detailed comparison of means.

Within Group 1 (male fructose)

Sugar Intake

		Day		
		22	8	15
Day	Means	27.294	30.112	42.600
	22	27.294	-	2.818
	8	30.112	-	15.306
	15	42.600	-	12.488
		r	2	3
		q(r,12)	3.08	3.77
$\sqrt{\text{MSerror}/n}$		q(r,12)	7.187	8.797

Blood Triglycerides

		Day		
		8	22	15
Day	Means	29.954	31.256	38.796
	8	29.954	-	1.302
	22	31.256	-	9.256
	15	38.796	-	7.540
		r	2	3
		q(r,12)	3.08	3.77
$\sqrt{\text{MSerror}/n}$		q(r,12)	3.894	4.766

Hormonal Effects.Detailed comparison of means.Within Group 1 (male fructose) (cont.)Blood LDL

		Day		
		8	22	15
Day	Means	31.452	34.214	34.334
	8	31.452	-	2.762
	22	34.214	-	0.120
	15	34.334		-

r= 2 3

q(r,12) 3.08 3.77

$\sqrt{\text{MSerror}/n}$ q(r,12) 2.132 2.610

Blood Cortisol

		Day		
		8	15	22
Day	Means	29.728	32.992	37.282
	8	29.728	-	3.264
	15	32.992	-	4.290
	22	37.282		-

r= 2 3

q(r,12) 3.08 3.77

$\sqrt{\text{MSerror}/n}$ q(r,12) 5.541 6.783

Hormonal Effects.

Detailed comparison of means.

Within Group 2 (male glucose)

Sugar Intake

		Day		
		8	22	15
Day	Means	26.108	27.604	46.168
	8	26.108	-	1.496
	22	27.604	-	20.060
	15	46.168	-	18.564
		-	-	-
		r	2	3
		q(r,12)	3.08	3.77
$\sqrt{\text{MSerror}/n}$	q(r,12)	4.970	6.083	

Hormonal Effects.

Detailed comparison of means.

Within Group 3 (female fructose)

Sugar Intake

		Day		
		8	22	15
Day	Means	25.948	32.848	41.210
	8	25.948	-	6.900
	22	32.848	-	15.262
	15	41.210	-	8.362
		r	2	3
		q(r,12)	3.08	3.77
$\sqrt{\text{MSerror}/n}$	q(r,12)		8.139	9.962

Hormonal Effects.

Detailed comparison of means.

Within Group 4 (female glucose)

Sugar Intake

		Day		
		22	8	15
Day	Means	27.524	27.826	44.650
	22	27.524	-	0.302
	8	27.826	-	17.126
	15	44.650	-	16.824
		-	-	-
		r	2	3
		q(r,12)	3.08	3.77
$\sqrt{MS_{error}/n}$		q(r,12)	6.297	7.708

DATA SUMMARIES
AND STATISTICAL
ANALYSES.

FROM SECTION 3.6
EFFECTS ON URINARY FREE CORTISOL.
DIETARY RECORDING.

Urinary free cortisol output - data summary.

FRUCTOSE							GLUCOSE					
	($\mu\text{g}/24\text{hrs}$)	(%)	($\mu\text{g}/24\text{hrs}$)	(%)	($\mu\text{g}/24\text{hrs}$)	(%)	($\mu\text{g}/24\text{hrs}$)	(%)	($\mu\text{g}/24\text{hrs}$)	(%)	($\mu\text{g}/24\text{hrs}$)	(%)
	Week 1		Week 2		Week 3		Week 1		Week 2		Week 3	
1	58.1	18.14	62.8	19.61	69.8	21.79	49.5	15.45	36.4	11.36	43.7	13.64
2	35.8	12.26	53.2	18.22	52.7	18.05	30.6	10.48	58.8	20.14	60.9	20.86
3	72.3	15.08	87.8	18.31	125.8	26.24	89.2	18.60	33.6	7.01	70.8	14.77
4	70.3	13.82	143.2	28.15	70.5	13.86	96.1	18.89	43.7	8.59	85.0	16.71
5	31.8	14.29	40.1	18.01	55.9	25.11	31.2	14.02	36.8	16.53	26.8	12.04
6	38.2	12.24	66.3	21.24	53.8	17.24	67.1	21.50	59.2	18.97	27.5	8.81
7	34.4	11.62	70.2	23.71	93.6	31.61	26.2	8.85	33.7	11.38	38.0	12.83
8	28.8	17.31	27.0	16.23	30.5	18.33	21.8	13.10	22.3	13.40	36.0	21.64
\bar{X}	46.2	14.35	68.8	20.44	69.1	21.53	51.5	15.11	40.6	13.42	48.6	15.16
SEM	6.3	0.85	12.5	1.37	10.3	2.05	10.4	1.54	4.5	1.68	7.5	1.55

Urinary free cortisol output.

Analysis of variance summary table.

Source of Variation	SS	df	MS	F
Treatments	467.494	5	93.499	7.646*
Experimental error	807.590	42	19.288	
Total	1275.084			

Detailed comparison of means.

		Collections.						
		G2	F1	G1	G3	F2	F3	
Collections	Means	13.42	14.35	15.11	15.16	20.44	21.53	
	G2	13.42	-	0.93	1.69	1.74	7.02	8.11
	F1	14.35		-	0.76	0.81	6.09	7.18
	G1	15.11			-	0.05	5.33	6.42
	G3	15.16				-	5.28	6.37
	F2	20.44					-	1.09
	F3	21.53						-
r=		2	3	4	5	6		
q(r,42)		2.85	3.44	3.79	4.04	4.23		
$\sqrt{\text{MSerror}/n}$ q(r,42)		4.42	5.33	5.88	6.26	6.56		

Dietary recording - data summaries.Daily energy intake.

		Fructose				Glucose			
		Week 1		Week 2		Week 1		Week 2	
		kcal	(%)	kcal	(%)	kcal	(%)	kcal	(%)
S U B J E C T	1	2493	24.39	2698	26.40	2414	23.62	2615	25.59
	2	2641	21.65	2984	24.46	3042	24.94	3521	28.95
	3	3182	25.26	3141	24.93	3070	24.37	3206	25.45
	4	2336	26.08	2432	27.10	2222	24.76	1986	22.13
	5	1912	23.94	2049	25.65	1939	24.28	2087	26.13
	6	2107	24.80	2383	28.05	1962	23.10	2043	24.05
	7	2152	23.58	2375	26.03	2346	25.71	2253	24.69
	8	3383	25.75	3164	24.08	3305	25.16	3286	25.01
\bar{X}		2526	24.43	2687	25.84	2538	24.49	2626	25.25
SEM		229	0.05	175	0.48	232	0.30	276	0.68

Daily fat intake.

		Fructose				Glucose			
		Week 1		Week 2		Week 1		Week 2	
		g	(%)	g	(%)	g	(%)	g	(%)
S U B J E C T	1	115	26.56	110	25.40	102	23.56	106	24.48
	2	109	24.77	99	22.50	115	26.14	117	26.59
	3	133	26.92	119	24.09	131	26.52	111	22.47
	4	90	28.57	72	22.86	89	28.25	64	20.32
	5	92	25.41	88	24.31	95	26.24	87	24.03
	6	84	27.54	81	26.56	79	25.90	61	20.00
	7	88	24.93	84	23.80	93	26.35	88	24.93
	8	152	30.16	119	23.61	127	25.20	106	21.03
\bar{X}		108	26.85	97	24.14	104	26.02	93	22.98
SEM		8.6	0.66	6.4	0.47	6.6	0.47	7.5	0.85

Daily protein intake.

		Fructose				Glucose			
		Week 1		Week 2		Week 1		Week 2	
		g	(%)	g	(%)	g	(%)	g	(%)
	1	90	25.71	89	25.43	87	24.86	84	24.00
	2	72	22.93	85	27.07	80	25.48	77	24.52
S	3	111	26.06	112	26.29	102	23.94	101	23.71
U									
B	4	81	26.21	92	29.77	73	23.63	63	20.39
J									
E	5	69	24.30	77	27.11	71	25.00	67	23.59
C									
T	6	80	26.58	93	30.90	66	21.93	62	20.60
	7	70	24.06	79	27.15	75	25.77	67	23.02
	8	105	27.49	75	19.63	114	29.84	88	23.04
	\bar{X}	85	25.42	88	26.67	84	25.06	76	22.86
	SEM	5.7	0.54	4.2	1.19	5.9	0.81	4.9	0.54

Daily "other" carbohydrate intake.

		Fructose				Glucose			
		Week 1		Week 2		Week 1		Week 2	
		g	(%)	g	(%)	g	(%)	g	(%)
	1	156	22.71	165	24.02	183	26.64	183	26.64
	2	197	23.68	177	21.27	216	25.96	242	29.09
S	3	161	25.28	141	22.14	180	28.26	155	24.33
U									
B	4	148	28.08	108	20.49	148	28.08	123	23.34
J									
E	5	128	26.07	110	22.40	132	26.88	121	24.64
C									
T	6	150	26.69	138	24.56	141	25.09	133	23.67
	7	148	28.03	118	22.35	137	25.95	125	23.67
	8	175	23.88	174	23.74	181	24.69	203	27.69
	\bar{X}	158	25.55	141	22.62	165	26.44	161	25.38
	SEM	7.3	0.71	10.0	0.49	10.5	0.46	15.8	0.76

Dietary recording - data summaries (cont.)

Daily sugar intake.

		Fructose				Glucose			
		Week 1		Week 2		Week 1		Week 2	
		g	(%)	g	(%)	g	(%)	g	(%)
	1	115	19.83	177	30.52	118	20.35	170	29.31
	2	130	15.46	215	25.57	204	24.26	292	34.72
S	3	109	18.48	167	28.31	113	19.15	201	34.07
U									
B	4	116	18.83	206	33.44	114	18.51	180	29.22
J									
E	5	74	18.23	126	31.04	68	16.75	138	33.99
C									
T	6	103	18.43	180	32.20	104	18.61	172	30.77
	7	109	19.46	170	30.36	116	20.71	165	29.46
	8	210	22.68	281	30.35	202	21.81	233	25.16
	\bar{X}	121	18.93	190	30.22	130	20.02	194	30.84
	SEM	13.9	0.71	16.1	0.85	16.9	0.82	17.1	1.15

Dietary recordings.Analysis of variance summary tables.Daily energy intake.

Source of variation	SS	df	MS	F
Treatments	10.811	3	3.604	1.750
Experimental error	57.640	28	2.059	
Total	68.451	31		

Daily fat intake.

Source of variation	SS	df	MS	F
Treatments	74.428	3	24.809	7.797*
Experimental error	89.104	28	3.182	
Total	163.532	31		

Daily protein intake.

Source of variation	SS	df	MS	F
Treatments	60.377	3	20.126	3.792*
Experimental error	148.629	28	5.308	
Total	209.006	31		

Daily "other" carbohydrate intake.

Source of variation	SS	df	MS	F
Treatments	65.562	3	21.854	7.100*
Experimental error	86.174	28	3.078	
Total	151.736	31		

Dietary recordings.

Analysis of variance summary tables (cont.)

Daily sugar intake.

Source of variation	SS	df	MS	F
Treatments	984.659	3	328.220	50.903*
Experimental error	180.537	28	6.448	
Total	1165.196	31		

Dietary recordings.
Detailed comparison of means.

Daily fat intake.

		Recording period.			
		G2	F2	G1	F1
Recording period	Means	22.98	24.14	26.02	26.85
	G2	-	1.16	3.04	3.87
	F2		-	1.88	2.71
	G1			-	0.83
	F1				-
		r=	2	3	4
		q(r,28)	2.90	3.50	3.86
$\sqrt{\text{MSerror}/n}$		q(r,28)	1.83	2.21	2.43

Daily protein intake.

		Recording period.			
		G2	G1	F1	F2
Recording period	Means	22.86	25.06	25.42	26.67
	G2	-	2.20	2.56	3.81
	G1		-	0.36	1.61
	F1			-	1.25
	F2				-
		r=	2	3	4
		q(r,28)	2.90	3.50	3.86
$\sqrt{\text{MSerror}/n}$		q(r,28)	2.36	2.85	3.14

Dietary recordings.

Detailed comparison of means (cont.)

Daily "other" carbohydrate intake.

		Recording period			
		F2	G2	F1	G1
Recording period	Means	22.62	25.38	25.55	26.44
	F2	22.62	-	2.76	2.93
	G2	25.38	-	0.17	1.06
	F1	25.55		-	0.89
	G1	26.44			-
		r=	2	3	4
		q(r,28)	2.90	3.50	3.86
		$\sqrt{\text{MSerror}/n}$ q(r,28)	1.80	2.17	2.39

Daily sugar intake.

		Recording period			
		F1	G1	F2	G2
Recording period	Means	18.93	20.02	30.22	30.84
	F1	18.93	-	1.09	11.29
	G1	20.02	-	10.20	11.91
	F2	30.22		-	0.62
	G2	30.84			-
		r=	2	3	4
		q(r,28)	2.90	3.50	3.86
		$\sqrt{\text{MSerror}/n}$ q(r,28)	2.60	3.14	3.47

