THE ROLE OF INSULIN IN RELATION TO STRESS AND NUTRITIONAL STATE :

GLUCOSE HOMEOSTASIS AFTER SURGERY, IN OBESITY AND OLD AGE

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ABSTRACT

Since the discovery of the hormone (Banting and Best, 1922), extensive research on insulin has been going on. Insulin is important not only in relation to carbohydrate metabolism, and thus in relation to diabetes mellitus, but also as one of the important hormones in protein and fat metabolism.

There is much evidence showing an impairment of insulin action in stress, whether the stress is physical or psychological. The way in which insulin resistance develops and its effects on metabolism may vary in different stress situations. This work investigates three forms of physical stress and attempts to show the role of insulin in each of these situations. The metabolic and clinical implications of this problem are discussed.

First, the effects of surgery were studied in eleven patients. Their mean age was 52 years, mean weight was 68 kg, and mean % ideal body weight was 94%. A nitrogen blance study showed that the negative balance after surgery coincided with elevated levels of plasma glucagon, non-esterified fatty acids, branched-chain amino acids, urinary free cortisol, urinary 17-OH-corticosteroids and with a decrease of total plasma amino acids. A temporary insulin resistance occurred in post-surgical patients, shown by hyperglycaemia and hyperinsulinaemia during a two hour glucose infusion. The clinical significance of hyperglycaemia is discussed.

The second study was of twenty nine obese patients (fasting blood glucose <6.11 m mol/l). Their mean age was 40 years, mean weight was 109 kg, and mean % ideal body weight was 166% (from 115% to 233%). These patients showed on average an impaired response to the oral glucose tolerance test (oral GTT), intravenous glucose tolerance test (iv GTT) and intravenous glucose insulin tolerance test (iv GITT)

for insulin sensitivity. This impairment was related to hyperinsulinaemia which followed glucose administration (oral or iv). These obese patients seemed to fall into two groups: those with % ideal body weight $< 160\%$ showed impaired tolerance to glucose but relatively normal plasma insulin responses; those $> 160\%$ showed marked hyperinsulinaemia. It is suggested that these responses represent those of 'active' and 'passive' obesity, but that the former may include a pre-clinical stage when insulin sensitivity is very high.

Thirdly, twenty three geriatric patients (fasting blood glucose < 6.11 m mol/l) were studied. Their mean age was 79 years and mean weight was 56 kg. These patients also showed an impairment in the oral GTT, iv GTT and iv GITT. The impairment was greater than that found in obese patients. Insulin response to glucose administration (oral or iv) was sluggish, but the actual levels of insulin were not significantly lower than those found in young normal subjects (except for the peak value during iv GTT). The major cause of impaired glucose tolerance was diminished insulin sensitivity, either in the peripheral tissue, or, more probably in the liver, resulting in relative inability to switch off glucose output. An intravenous alanine tolerance test was carried out in eight elderly subjects (mean age was 78 years, mean weight was 52 kg), to assess gluconeogenic capacity of the liver, and again indicated the relative inability of endogenous insulin to suppress glucose production.

PART I

INSULIN AND GLUCOSE HOMEOSTASIS

CHAPTER 1

INTRODUCTION

A. History:

Insulin is a hormone which is traditionally associated with diabetes mellitus. Although this hormone was discovered not more than fifty years ago (Banting and Best, 1922), the disease itself had long been recognized. It was first described by a Roman physician, Aretaeus of Cappadocia, as "a moist and cold wasting of flesh and limbs into the urine" (A.D. 30 to A.D. 90). 'Diabetes' is a Greek word meaning 'siphon', and is descriptive of the body siphoning away through the urine. It was a dreaded disease and remained so for many years to come. Avicenna (Ibn Sina) a famous Arab physician (930-1033) (Fig. 1), gave a very complete description of the disorder, including some of the complications, such as diabetic gangrene, furunculosis, phthisis, and also the presence of a honey-like substance in the patient's urine. But many centuries before Aretaeus and Avicenna, old Chinese, Japanese and Hindu writings indicate that the disease had long been known to these peoples also.

The observations of Thomas Willis (1682) of glycosuria in urine however, marked the beginning of a new era when glycosuria was becoming an accepted diagnostic test for diabetes mellitus. Brunner (1683) observed in animal experiments that polyuria and polydipsia occurred after removal of the pancreas, but it was Cawley (1788) who probably was the first to associate diabetes with the pancreas when he found multiple calculi and destruction of pancreatic tissue at an autopsy of a patient who had died from diabetes.

Figure 1. An old woodcut of Avicenna (Ibn Sina) (950 - 1033).
(Courtesy of the Royal Society of Medicine, London).
(Garland, 1908)


AVICENNA
ex Codice antiquo Galeni.

Insulin is produced in the islets of Langerhans, named after Paul Langerhans (1869) who first discovered them and described their structure embedded in the tissue of the pancreas. Their function and significance were then however still unknown.

The metabolic relationship between diabetes mellitus and the pancreas was clearly shown by Von Mering and Minkowski (1890). They found that pancreatectomy on dogs produced hyperglycaemia and glycosuria and the dogs finally died in ketosis and coma. Their conclusion was that the pancreas elaborates a substance that keeps the blood sugar low and restores the metabolism to normal.

The whole world owes its thanks to Frederick Banting and Charles Best (1922), who discovered "the blood sugar lowering substance" now known as insulin. Their discovery led to the therapeutic use of insulin, thus saving thousands of lives, and has opened the doors to the possibility of its ultimate synthetic production.

It was not long before Abel (1926) achieved the crystallization of the hormone, but over twenty more years passed before Sanger and his co-workers successfully pioneered the study of the sequence of amino acids in the insulin molecule (Sanger, 1949; Ryle, Sanger, Smith & Kitai, 1955).

Since its discovery and especially at present, extensive research on insulin has been going on in relation not only to diabetes, but also to wider aspects of body metabolism. Insulin is, no doubt, the principal hormone in carbohydrate metabolism, but it is also an equally important hormone in protein (Manchester, 1970)

and in fat metabolism (Avruch, Carter and Martin, 1972). This gives a significance much wider than the specialised limits of diabetes. In particular, since insulin has a central role in the disposal of energy and in synthesis of protein, its relationship to short and long term nutritional status is receiving increasing attention.

B. Insulin and its Structure:

Insulin is produced and stored in the pancreatic islets of Langerhans (Mcleod, 1922). There is no evidence that it is produced normally elsewhere in the body (Best, Jephcott and Scott, 1932). Although in some very rare cases it is produced in non-pancreatic tumours (Shames, Dhurandar and Blackard, 1968; Omenn, 1970).

Insulin is a polypeptide, and consists of two parallel chains of amino acids. They are the A (acid) and B (basic) chains, and are joined to each other by two disulphide bridges. The third disulphide bridge is connecting two cystein-molecules within the A chain (Steiner, Kemmler, Clark, Oyer and Rubenstein, 1972). Although the detailed amino acids composition of insulin differs somewhat from one species to another, this two-chain structure and the relative positions of the three pairs of disulphide bridges are constant structural features (Fig. 2). Several kinds of experimental evidence indicates that these bridges are essential to the normal structural integrity and biological activity of the hormone (Humbel, Bosshard and Zahn, 1972).

Insulin is derived from a larger single polypeptide precursor, 'proinsulin' or 'big insulin'. In proinsulin, the A and B chain of insulin are joined in series by a further sequence of amino acids (the C-peptide), thus forming a continuous chain, with the same three pairs of disulphide bridges as in insulin (Fig. 3). (Oyer, Cho,

Figure 2. Primary structure of human insulin (from Steiner, et.al., 1972).

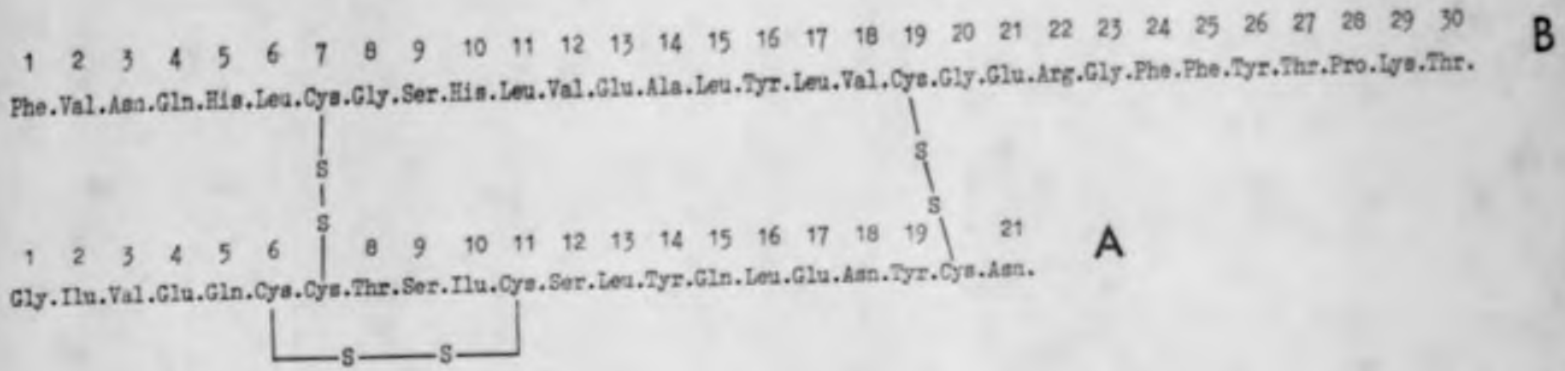
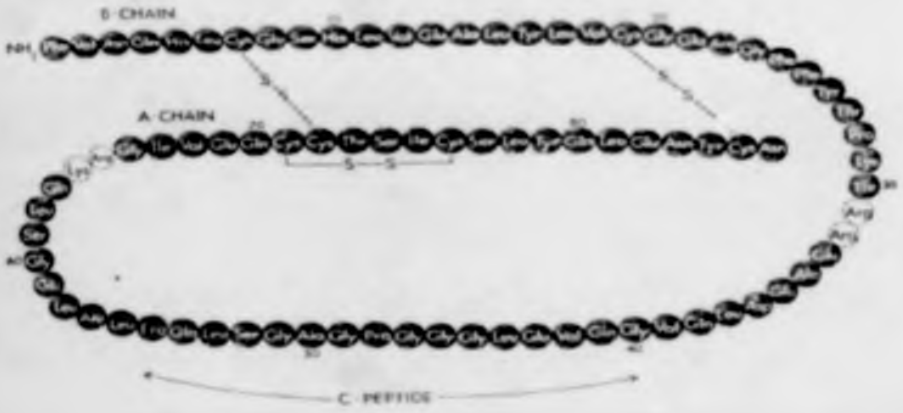

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Phe.Val.Asn.Gln.His.Leu.Cys.Gly.Ser.His.Leu.Val.Glu.Ala.Leu.Tyr.Leu.Val.Cys.Gly.Glu.Arg.Gly.Phe.Phe.Tyr.Thr.Pro.Lys.Thr.
B
S
S
S
S
1 2 3 4 5 6 8 9 10 11 12 13 14 15 16 17 18 19 21
Gly.Ile.Val.Glu.Gln.Cys.Cys.Thr.Ser.Ile.Cys.Ser.Leu.Tyr.Gln.Leu.Glu.Asn.Tyr.Cys.Asn.
A
S
S

Figure 3. Primary structure of human proinsulin (from Oyer, et.al., 1971).


B CHAIN
A CHAIN
C PEPTIDE

Peterson and Steiner, 1971). Proinsulin is synthesized in the β-cell and is then cleaved within the β-cell by proteolytic action into two-fragment insulin and C-peptide (Clark, Cho, Rubenstein and Steiner, 1969).

Some proinsulin is released with insulin into the circulation and although cross reacting with insulin antibodies, it does not appear to be a serious source of error in insulin immunoassays. However, proinsulin is also biologically active, although its effectiveness is only between 2 to 20 per cent of the biological activity of insulin (Rubenstein and Steiner, 1971).

CHAPTER II

GLUCOSE HOMEOSTATIS

A. Energy Balance:

Energy balance in man is a complex and highly integrated system of supply and utilization of energy, derived mainly from carbohydrate, fat and protein. The metabolizable energy of these nutrients is linked with requirements through energy couplers such as high energy phosphate compounds, e.g. adenosine triphosphate (ATP) and reduced forms of coenzymes, particularly reduced nicotinamide adenine dinucleotide phosphate ($NADP + H^+$). In muscle, creatine phosphate has a specialized short term role in energy storage. This system of intermittent supplies and continuous but variable demands involved the deposition of nutrient stores during periods of excess fuel intake, and conversely, their mobilization in periods of dietary nutrient deprivation. Hormonal control has an important function in this regulation of fuel supplies. Under normal circumstances, insulin action predominates during exogenous fuel excess (just after ingestion of food), whereas contra-regulatory hormones become operative during the fasting phase, when energy has to be mobilized from endogenous stores.

Carbohydrate, fat and protein from exogenous sources are hydrolysed and absorbed within the gastro-intestinal system. The major endogenous fuel stores are in the forms of gycogen in the muscle and liver, triglyceride in adipose tissue, and if all fails, protein in the peripheral muscle. The main function of protein however, is to form the structural, contractile and enzymatic component of cells.

B. Glucose as an Energy Source:

Most tissues in the body use non-esterified fatty acids (NEFA) as their main source of energy to generate ATP. But there are several tissues which are dependent on glucose. They are divided into two groups.

1. Tissues which oxidize glucose completely (rich in mitochondria):

   a. The brain and nervous tissues: The reason why these tissues do not use NEFA as their energy source is not yet clearly known. In these tissues, glucose is oxidized to pyruvate and some ATP is generated, then pyruvate is oxidized further to CO_2 in the mitochondria with much greater ATP generation.

   b. Red muscle: Although NEFA comprise the main fuel for red muscle, this tissue is able to use glucose also, and oxidises it completely to CO_2, thus generating the maximum yield of ATP.

2. Tissues dependent on glycolysis (mitochondria absent or deficient):

   a. Red blood cell: This tissue does not have mitochondria, and glucose can, therefore, be oxidized only to pyruvate. This oxidation is coupled with the reduction of pyruvate to lactate. Lactate is then transferred to the liver where it is converted back to glucose. Only small amounts of ATP are generated (2 moles per one mole of glucose) in oxidizing glucose to pyruvate, but recycling of pyruvate through lactate and the Cori cycle increases the energy yield of glycolysis considerably (see Chapter III C. on Cori cycle).

b. White muscle during exercise: This tissue does not have enough mitochondria for the complete oxidation of glucose. During exercise, therefore, lactate is formed in the red blood cells, and this lactate is then also transferred to the liver to be converted back to glucose (see Chapter III C. on Cori cycle).

C. Fate of Exogenous Glucose added to the Blood Circulation:

That "carbohydrate given by mouth can be converted into fat by the metabolic processes of the body is now an accepted fact" (Macdonald, 1967). Whether the tissues are glucose or NEFA dependent or whether mobilization of endogenous fuel stores involves glycogen or triglyceride, all may therefore, indirectly or directly be derived from dietary carbohydrate. However, the immediate fate of dietary glucose or of the glucose load during oral or intravenous glucose tolerance tests is much less certain.

In man there is a little comprehensive evidence on this question. Some suggested that up to 50 per cent of the ingested carbohydrate (oral) is taken up by the liver (Ensinck and Williams, 1972). This figure, however, is very likely to be too high, since after a glucose load, gluconeogenesis is suppressed in the liver (Madison, 1969) and studies on the rate of disappearance of glucose after oral or intravenous glucose load without using radioactive glucose tracer could be misleading. Thus experiments on rats, in which the orally administered glucose included a tracer dose of ^{14}C-glucose, showed that after 180 minutes only 15 to 18 per cent of the ingested glucose had been taken up by the liver (Curtis-Prior, Trethewey, Stewart & Hanley, 1969; Jeffcoate and Moody, 1969). However, even 15 per

cent uptake by the liver means that this organ is disproportionately active (relating to weight). It is instructive to look at some of the other organs in the same way and to compare intravenous and oral administration of glucose (Table 1). The data are from an experiment of Curtis-Prior et. al. (1969). In this experiment an intravenous dose (750 mg/kg) and an intragastric dose (1500 mg/kg) of glucose were given to rats, in each case together with a tracer dose of (U - ^{14}C) D-glucose. The results are summarized in Table 1.

The data in Table 1 show the amount of radio-active counts in each organ, therefore, they may not necessarily be that of glucose. It could be in the form of glucose products (e.g. glycogen, pyruvate, lactate). A correction figure for recycling is not included. Nevertheless, they provide us with a fair picture of the fate of exogenous glucose once it enters the circulation.

The body composition of a normal man may not necessarily be the same as that of a rat. Table 2 shows the normal body composition of a normal male, weighing 70 kg.

Table 1

Distribution of radioactivity in organs of 200 g rats following an intravenous (750 mg/kg) or intragastric (1500 mg/kg) load of glucose, together with a tracer dose of (U - ^{14}C) D-glucose (derived from Curtis-Prior et. al., 1969)

Tissue	% of body weight	% (U - ^{14}C) present				
		Intravenous load		Intragastric load		
		5 min.	40 min.	15 min.	90 min.	180 min.
1. Skeletal muscle	38	30.3	30.5	3.5	16.4	17.8
2. Skin	20	28.1	11.1	7.5	7.1	5.4
3. Blood	7	13.1	5.0	1.5	2.9	2.5
4. Liver	3	8.9	9.4	2.9	10.7	15.0
5. Adipose tissue	16	10.7	4.6	2.0	3.8	3.5
6. Alimentary tract (+ content)	5.7	5.3	3.4	60.5	14.8	8.4
7. Brain	0.7	0.7	8.0	0.3	1.1	0.8
8. Expired CO_2		0.1	1.0	0.4	11.8	31.3

Table 2

Normal body composition of a 70 kg male

Tissue	% of body weight
1. Skeletal muscle	45.0[a]
2. Blood	7.6[b]
3. Adipose tissue	19.6[b]
4. Liver	1.9[a]
5. Alimentary tract	5.8[a]
6. Brain	2.0[c]

a. Derived from Munro (1969)

b. " " Olesen (1965)

c. " " Johnston and Whillis (1954)

D. Maintenance of Blood Glucose Level:

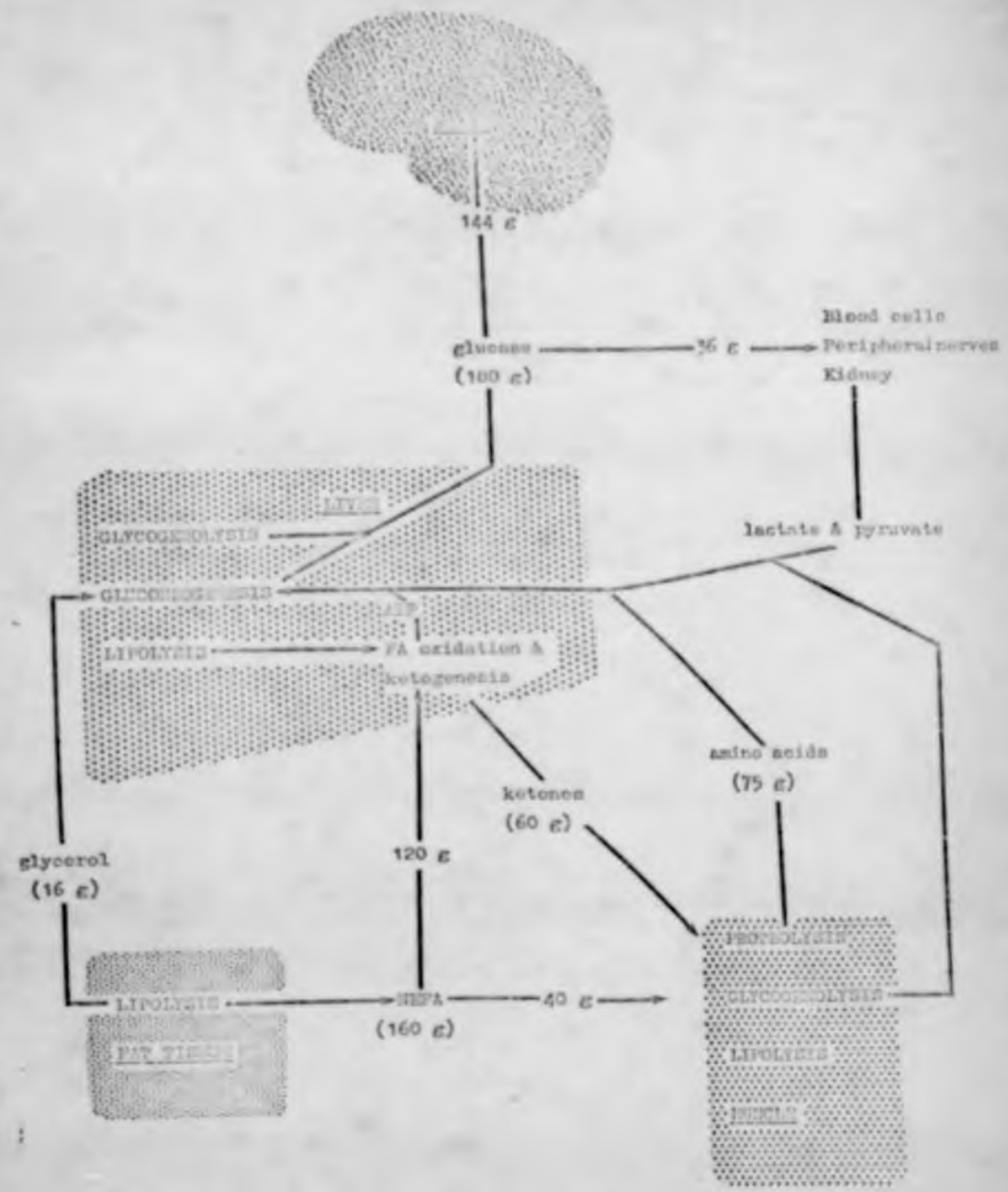
1. Basal glucose levels

During fasting, exogenous glucose is not available, and since there is always a certain demand for glucose (see Chapter II B.), the body provide it by mobilization from stores (glycogen) and by gluconeogenesis. Cahill, (1970), summarised the substrate and hormone changes during a 24 hour fast in a normal man, 70 kg in body weight, with a daily energy expenditure of 7.5 M Joules/day (1800 calorie/day) in Fig. 4.

During this period of fasting, about 180 g of glucose is produced by the liver and enters the circulation. Most of this is 'new' glucose which is derived from amino acids (75 g) from the muscle. While recycled rather than 'new' glucose is derived from pyruvate and lactate (36 g) from muscle, nerve and blood cells, and kidney medulla. Glucose is also formed from glycerol (16 g) derived from fat

NEFA from the hydrolysis of triglyceride are oxidized in the liver, and also in the muscle. Some of the energy generated in this process is used in maintaining gluconeogenesis (see Chapter III on gluconeogenesis) 80 per cent of the glucose produced by the liver will be diverted to the brain and the remainder to the cellular elements of blood, peripheral nerves and kidney medulla. Where through the process of glycolysis, it is converted back to pyruvate and lactate, which is then transported to the liver for reconversion to glucose (Fig. 4).

Figure 4. Scheme of fuel disposition in normal man, fasting for 24 hours (70 Kg. daily energy expenditure 7.5 MJ/day). Glucose directed mainly for cerebral consumption is released from hepatic glycogen, and new glucose is generated in liver from precursors derived from fat, muscle, blood cells, nerve, and renal medulla. NEFA from triglyceride hydrolysis are oxidized in muscle and liver as alternate energy sources. (from Cahill, 1970).


144 g
glucose
(100 g)
36 g
Blood cells
Peripheral nerves
Kidney
LIVER
GLYCOGENOLYSIS
lactate & pyruvate
LIPOLYSIS
FA oxidation &
ketogenesis
amino acids
(75 g)
ketones
(60 g)
120 g
glycerol
(16 g)
LIPOLYSIS
HEPA
40 g
(160 g)
PROTEOLYSIS
GLYCOGENOLYSIS
LIPOLYSIS

Cahill (1970) further suggested that in prolonged starvation there is a decline in the amount of new glucose generated in the liver with a concomittant decrease in protein catabolism. In this situation, the levels of anti insulin hormones increase in the circulation and they counteract the insulin action. (See Part II, Chapter I B. of this thesis). During this period of prolonged starvation, hepatic gluconeogenesis is reduced to the levels necessary to recycle pyruvate formed in glycolytic tissues.

2. After ingestion of food:

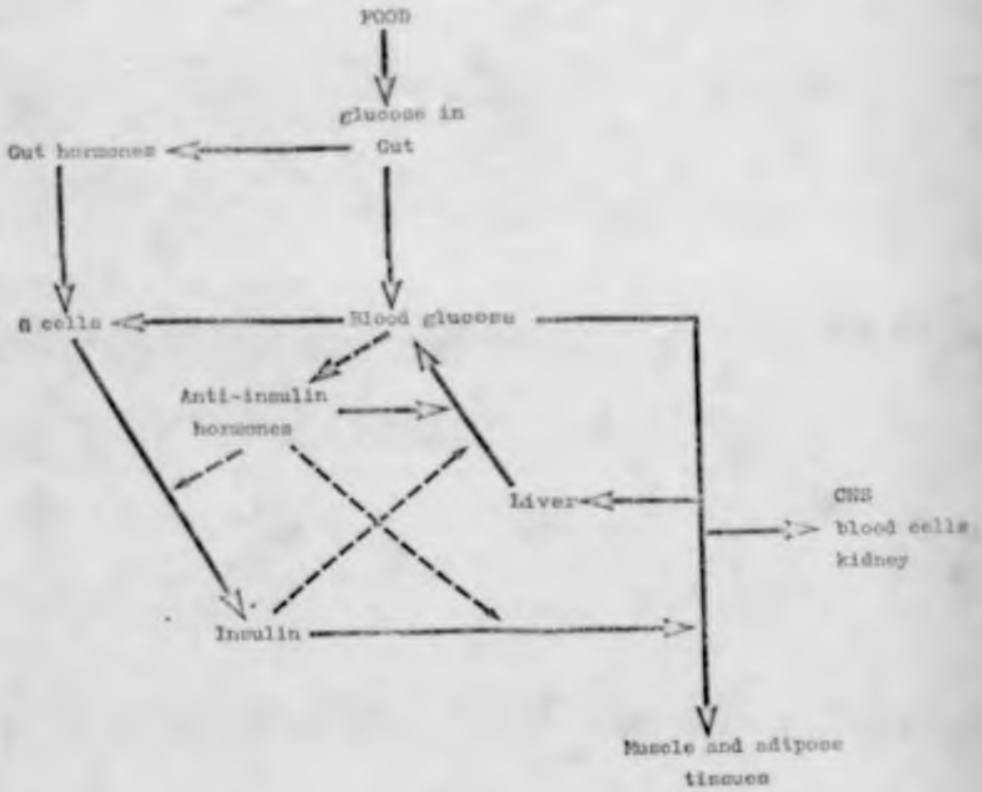
After ingestion of food, the levels of glucose, amino acids, some gut hormones (e.g. gastrin, pancreozymin-cholecystokinine, secretin and entero glucagon) are increased in the blood. These substrates and hormones stimulate increased release of insulin (Porte & Bagdade, 1970). It is known that glucose alone is a potent stimulator of insulin release, but the combination of glucose and amino acids with the hormones (entero-insular axis) is responsible for the greater release of insulin during oral glucose tolerance test (oral GTT) compared to that found during intravenous glucose tolerance test (iv GTT), where glucose loading is given directly into the blood circulation. These changes of substrates and hormonal levels during the period following the ingestion of food are summarized in Fig. 5.

When carbohydrate is fed, blood glucose rises, but seldom exceeds 8.88 m mol/l (160 mg/100 ml) and usually subsides to preprandial levels by two hours. The insulin released during nutrient absorbtion promotes storage of the metabolic fuels in appropriate compartments, (e.g. as glycogen in muscle and liver, and as triglyceride in adipose tissue). (Ensinck and Williams, 1972). In muscle and fat, insulin enhances

Figure 5. A scheme of glucose disposition after ingestion of food (carbohydrate) in Man.

Glucose is absorped in the gut and enters the circulation. Gut hormones which are increased by the presence of food in the gut, stimulate β cells. On the other hand, blood glucose alone could also stimulate β cells. β cells produce insulin which promotes glucose uptake by muscle and adipose tissues. Blood glucose inhibits the secretions of anti-insulin hormones. These hormones opose insulin actions in liver (inhibition of gluconeogenesis) and in muscle and adipose tissues (glucose uptake , inhibition of lipolysis and inhibition of protein breakdown).

Not all anti-insulin hormones exhibit every action shown in this diagram.



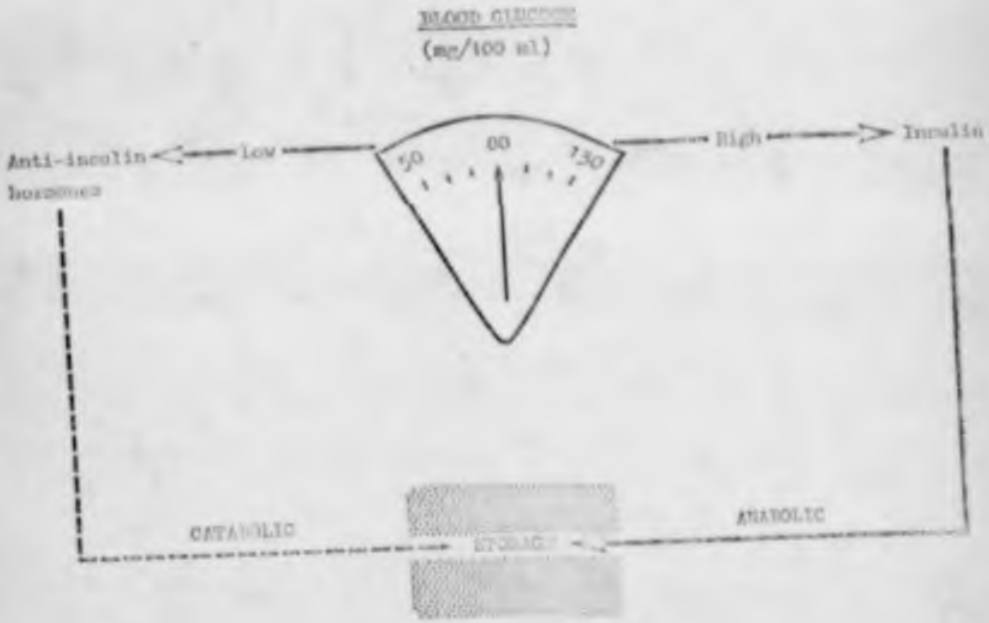
⟵ stimulate or promote.

⟵ - - - inhibit.

glucose oxidation. It also enhances glycogen synthesis, potassium and amino acid influx and protein synthesis in muscle (Reiser, 1967). Conversely, insulin inhibits the release of amino acids from muscle (Cahill, 1970). Insulin not only facilitates glucose entering adipose cells and thereby lipid synthesis, it also inhibits lipolysis. This is reflected in the decline in the circulating NEFA concentration which coincides with hyperglycaemia and elevation of insulin concentrations in blood, (Reiser, 1967; Porte and Bagdade, 1970). After ingestion of carbohydrate, glucose is taken up by the liver, and most of the carbohydrate assimilated in the liver is deposited as glycogen, under the action of glycogen synthetase, an enzyme regulated by insulin (Reiser, 1967), and concomitantly, hepatic glycogenolysis and gluconeogenesis are abruptly reduced (Madison, 1969).

Fig. 6 summarised and simplifies the control of blood glucose level by insulin and anti-insulin hormones; e.g. growth hormone, epinephrine and glucagon.

Figure 6. The control of blood glucose concentration by insulin and anti-insulin hormones.



⟵ stimulate or promote.

⟶ breakdown.

CHAPTER III
GLUCONEOGENESIS

A. Definition:

Gluconeogenesis is strictly the "synthesis of new glucose from non-carbohydrate precursors", e.g. amino acid residues. But this process overlaps considerably with that whereby glucose is resynthesized from lactate and pyruvate. The term 'gluconeogenesis' is therefore used here to include all these processes. The liver is the main organ where gluconeogenesis takes place, although in prolonged starvation, the kidney becomes an important gluconeogenic organ as well. In this situation the kidney takes up amino acids to produce 'new' glucose as well as to produce NH_3 to counteract the ketosis which is developed during starvation, (Owen, Felig, Morgan, Wahren and Cahill, 1969).

Gluconeogenesis is important during starvation and other situations when carbohydrate intake from the alimentary canal is limited and the body glycogen stores are depleted. Pyruvate, lactate, glycerol and glucogenic amino acids are converted to glucose and glycogen. These amino acids, either from alimentary canal absorption or from protein breakdown in muscle, through gluconeogenesis, become an important source of energy.

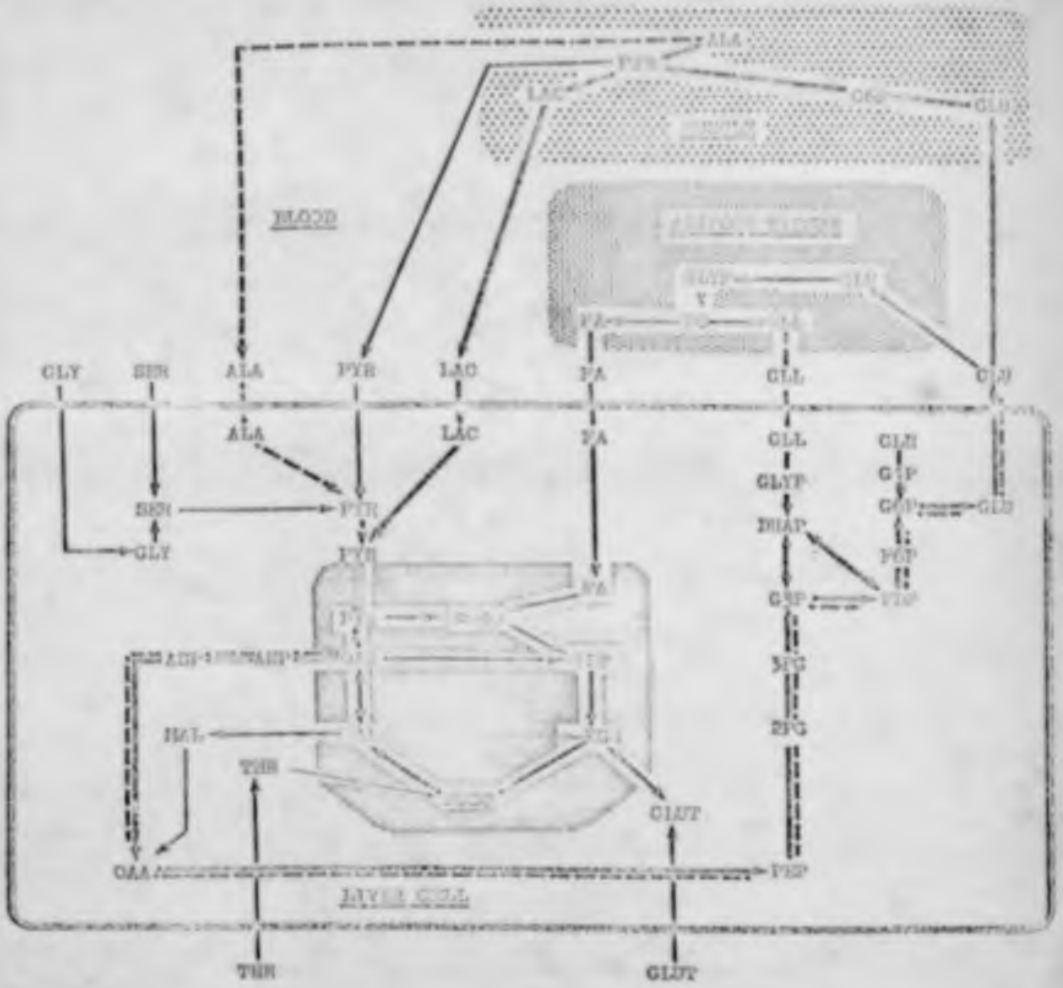
B. Liver and Gluconeogenesis:

Amino acids enter the liver cell by a membrane transport system. Lactate, alanine, serine and glycine are converted into pyruvate in the cytosol (Fig. 7). Pyruvate enters the mitochondria, is converted into oxaloacetate by pyruvate carbo-

Scheme 1. PATHWAY OF GLUCONEOGENESIS.

Abbreviations are: LAC, lactate; PYR, pyruvate; ALA, alanine; SER, serine; GLY, glycine; FA, fatty acid; AcCoA, acetyl-CoA; CIT, citrate; α-KG, α-ketoglutarate; SUCC, succinate; FUM, fumarate; MAL, malate; OAA, oxaloacetate; ASP, aspartate; GLUT, glutamate; THR, threonine; PEP, P-enol pyruvate; 2PG, 2-P-glycerate; 3PG, 3-P-glycerate; GAP, glyceraldehyde-3-P; DHAP, dihydroxyacetone-P; GLYP, glycerol-1-P; GLL, glycerol; FDP, fructose-1, 6-di-P; F6P, fructose-6-P; G6P, glucose-6-P; GLU, glucose; G1P, glucose-1-P; GLY, glycogen.

(modified from Exton, 1972).



Cori Cycle.

Gluconeogenesis; e.g. from glycerol and alanine.

xylase or to acetyl coenzyme A by pyruvate dehydrogenase. Oxaloacetate is converted into malate and aspartate which leave the mitochondria or to citrate which is mainly metabolized in the Krebs cycle. Malate and aspartate are converted back to oxaloacetate, and oxaloacetate is then converted by phospho-enolpyruvate carboxykinase into phospho-enolpyruvate (PEP) (Exton, 1972). Two moles of high energy phosphate (ATP or GTP) are needed to convert one molecule of PEP. PEP is converted into fructose - 1,6 diphosphate by a reversal of glycolysis. A further mole of ATP and a mole of (NADH + H^+) are needed for each mole of PEP utilized. Fructose - 1,6 diphosphate is hydrolysed to fructose - 6 phosphate by fructose - 1,6 diphosphatase. This enzyme is specific to tissues carrying out gluconeogenesis. Fructose - 6 phosphate is converted to glucose - 6 phosphate and glucose - 6 phosphate is converted to glucose by another enzyme specific to gluconeogenesis, glucose - 6 phosphatase. The overall conversion of two moles of lactate to one mole of glucose, therefore, requires six moles of ATP (or equivalent as GTP) and 2 moles of (NADH + H^+). The former is provided by the oxidation of NEFA and the latter by the reduction of NAD^+ to (NADH + H^+) in the conversion of lactate to pyruvate. It has been suggested that pyruvate entry into liver mitochondria is a control point for gluconeogenesis which is influenced by epinephrine, cortisol and glucagon (Adam and Haynes, 1969).

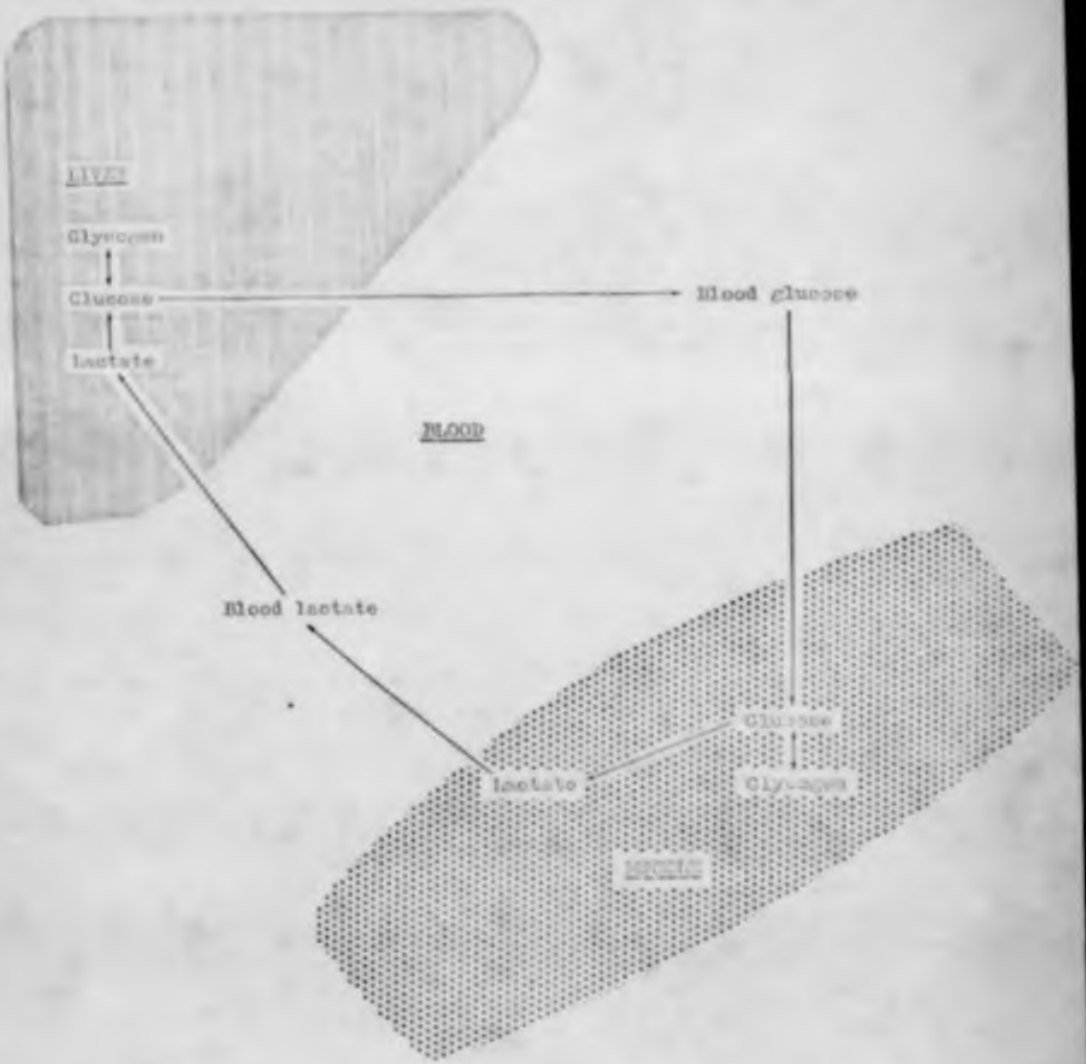
Glycerol has a small but significant contribution in gluconeogenesis. It enters gluconeogenic pathway at the level of triose-phosphate by reacting with glycerokinase to form glycerol - 1 phosphate which is then oxidised to dihydroxyacetone-phosphate by α-glycera-phosphate dehydrogenase.

C. Cori Cycle:

In muscle the complete oxidation of glucose involves production of pyruvate by a reaction in the cytosol (anaerobic) and the combustion of pyruvate by reactions in mitochondria (aerobic). Red muscle, (e.g. skeletal muscle), which is designed to work over long periods, depends upon the complete oxidation of glucose and fatty acids. Such muscle contains more mitochondria to sustain a constantly high rate of oxidation of acetyl coenzyme A and has a higher content of myoglobin to deliver the required oxygen for oxidation in the mitochondria. Whereas in white muscle (e.g. breast muscle of a chicken) which is designed for short bursts of heavy activity, utilizes ATP during such exercise at a much greater rate than could be sustained by mitochondrial oxidation. It therefore depends more upon a readily available high-energy phosphate store (viz. creatinine phosphate) and upon the rapid generation of ATP from the conversion of glucose to lactate in the cytosol. This kind of muscle has fewer mitochondria and less myoglobin. In reality, though, there is no clear cut division between red and white muscles. In exercising white muscle, glucose is converted into 2 molecules of lactate with the generation of 2 ATP in the cytosol. Lactate diffuses into the blood circulation and is taken up by the liver where it is converted back into glucose. (Fig. 8). This cyclic process of glycolysis (e.g. in muscle) and gluconeogenesis (in liver), using glucose and lactate as transport material is known as the Cori cycle (Fig. 7 and Fig. 8).

It is important to note that operation of Cori cycle does not result in a net increase in glucose formation for the body since lactate itself is derived from glucose.

Figure 8. CORI CYLE.


LIVER
Glycogen
Glucose
Lactate
Blood glucose
BLOOD
Blood lactate
Glucose
Lactate
Glycogen
MUSCLE

However, there is a transfer of energy, since NEFA from adipose tissue are oxidised in the liver to provide ATP in gluconeogenesis in the liver. Glucose recycling from lactate formed by glycolysis in blood cells, brain and other tissues may, in normal man, correspond to 10 to 33 per cent of total glucose turnover (Cahill, Herrera, Morgan, Soeldner, Steinke, Levy, Reichard and Kipnis, 1966).

CHAPTER IV
STRESS AND GLUCOSE METABOLISM

A. Definition:

The term 'stress' has a wide range of meanings depending upon the context or the situation in which this word is being used. The Concise Oxford Dictionary (Fifth Edition, 1963), gives a definition of 'stress' as: a) a constraining or impelling force, b) an effort or demand upon energy, c) an emphasis or accentuation, d) (in mechanics) a force exerted between continuous bodies or parts of a body. However, in medicine in general, 'stress' is considered as "any stimulus of such magnitude as to tend to disrupt the homeostasis of the organism" (Dosil, 1966).

B. Classification of Stress:

Stress may be classified into two types; first is physical and the other is psychological stress.

Table 3 summarized some of the different kind of stress falling into these two broad groupings.

It is very rare that one type of stress operates in isolation. It is more usual for the primary stress to lead to a secondary stress of some other type, e.g.:

1. An operation (mechanical) is followed by a decrease of food intake (nutritional).
2. An infection (physical) is followed by a decrease in food intake (nutritional).
3. An old person is feeling sad and lonely (psychological), eats less food (nutritional, physical) and is likely to succumb more easily to diseases and infections (physical).

Table 3

Classification of Stress

I Psychological

1. Environmental (e.g. unfriendly neighbours)
2. Endogenous (e.g. feeling sad)

II Physical

1. Nutritional (e.g. fasting, starvation)
2. Mechanical (e.g. fracture, operation)
3. Climatic (e.g. temperature, humidity)
4. Infection and disease
5. Physiological (e.g. pregnancy)
6. Exercise

It is obviously impossible to study all these interactions in a limited period of time. This thesis, therefore, attempts only to study three different kinds of stress, and concentrates only on measurable physical factors.

As indicated in the examples above, nutritional stress is often a secondary consequence of other forms of stress. This secondary nutritional stress usually has a component protein energy malnutrition (PEM), whatever other deficiencies may occur. The following chapter (Chapter V), therefore, deals with PEM and its effect on glucose homeostasis and insulin production and effectiveness, as a background to observations after surgery (Part II) , in obesity (Part III) and in old age (Part IV).

CHAPTER V
PROTEIN ENERGY MALNUTRITION (PEM)

A. Aetiology:

PEM is a major problem in the developing world today. Dietary deficiency of protein and energy can occur acutely from a sudden failure of food supply e.g. after a natural disaster such as flood or drought, or as a result of anorexia in illness or other acute stress, or even as a result of voluntary starvation in support of some political protest. Acute and prolonged starvation have both been studied by Cahill and his co-workers (Cahill et. al., 1966; Owen et. al. 1969; Cahill, 1970). In reality, however, in most cases nutritional deficiency is obviously not one of total starvation but various degrees of chronic or seasonal shortage of food. Some of the earlier, simple concepts of protein and calorie (energy) deficiency in the aetiology of kwashiorkor (Williams, 1933; Platt, 1958) and marasmus (Platt, 1958; Jelliffe, 1966), respectively, have become difficult to sustain (Garrow, 1966; Gopalan, 1968). Therefore, many workers prefer to use the general term of protein calorie malnutrition or protein energy malnutrition (PEM) (Waterlow and Alleyne, 1971).

However, protein, energy supplies and metabolism cannot be separated into isolated compartments. Thus PEM is related not only to derangements of protein metabolism (Waterlow and Alleyne, 1971), but also to a decrease in the ability of the body to regulate blood glucose levels owing to associated endocrine changes (Heard, Platt and Stewart, 1958; Stewart and Heard, 1959; Heard and Stewart, 1971).

B. PEM and Blood Glucose Levels:

Malnutrition is usually associated with hypoglycaemia. Baig and Edozien (1965) reported hypoglycaemia in kwashiorkow and Hadden (1967) also found hypoglycaemia in both kwashiorkor and marasmus. The actual levels of blood glucose reported vary from one investigator to another, probably owing to different degrees of severity of the disease. The levels of fasting blood glucose in marasmic children in Hungary (when some of the children later died was between 0 to 1.4 m mol/$_l$ (0 to 25 mg/100 ml), (Kerpel-Fronius and Kaiser, 1967), while reports from Uganda on children suffering from kwashiorkor showed fasting blood glucose levels less than 2.2 m mol/$_l$ (40 mg/100 ml) (Whitehead and Harland, 1966). However, James and Coore (1970) found that in series of 26 malnourished children the mean fasting blood glucose was 3.1 m mol/$_l$ (55 mg/100 ml) initially and became 3.9 m mol/$_l$ (70 mg/100 ml) after recovery. Although the degree of hypoglycaemia was significant it was perhaps not as great as might have been expected from earlier reports from Africa and Hungary. Later, workers in Jamaica in a study designed to investigate the hypoglycaemia of PEM were somewhat foiled by the absence of any primary nutritional hypoglycaemia. Hypoglycaemia was found only when PEM had been superimposed on congenital defect and it had a tendency to persist after recovery, (Kerr, Stevens, Robinson and Picou, 1973). Most workers agreed that when a low fasting blood glucose concentration does occur, it improves with recovery from malnutrition unless sufficient energy is not provided with the rehabilitation diet (Baig and Edozien, 1965; Hadden, 1967).

There is an impairment in glucose tolerance in patients suffering from kwashiorkor (Sloane, Teitz and Gilchrist, 1961; Baig and Edozien, 1965) and marasmus (Oxman, Maccioni, Zuniga, Spada and Monckeberg, 1968), although some reported that in marasmus GTT could still be within normal limits (Hadden, 1967; Bowie, 1964). Thus the blood glucose homeostatic mechanism seems to be disturbed in both directions, i.e. in diminished ability to deal with hypo- and hyperglycaemia.

C. PEM and Insulin Levels:

It has been shown in malnourished children that the fasting plasma insulin level is usually low (James and Coore, 1970; Milner, 1971), and that the rise of plasma insulin concentrations in response to intravenous glucose load is usually small or absent. Milner (1971) further could not show any increase in plasma insulin concentration after intravenous injection of glucagon. During recovery, insulin response to glucose is significantly improved, although it is still lower than in normal children (James and Coore, 1970).

However, the impairment in insulin sensitivity rather than the actual deficiency of insulin is probably the main contributor of poor glucose tolerance (Turner, 1966; Heard and Turner, 1967; Heard and Henry, 1969). Insulin sensitivity was measured in dogs either by injecting insulin alone (0.1 unit/kg Bw) or by injecting insulin (0.1 unit/kg Bw) together with glucose (0.4 g/kg Bw). Insulin sensitivity is defined here as the effectiveness of insulin in lowering blood glucose concentration, and is expressed as a percentage rate constant for the fall in glucose concen-

trition. Dogs fed with low protein diet showed abnormalities in carbohydrate metabolism, but glucose tolerance was correlated significantly with insulin sensitivity and not with circulating insulin levels (Heard and Henry, 1969).

CHAPTER VI
PURPOSE OF THE STUDY

There is no doubt, therefore, that dietary stress produces an impairment in glucose homeostasis, but the mechanism and the role of insulin and other related hormones may differ from one type of stress to another. It is particularly important to understand the extent to which changes in glucose homeostasis, insulin sensitivity, etc. are adaptive and protective to the 'stressed' organism. Almost inevitably phrases like 'impairment' in glucose tolerance or in insulin sensitivity will be used in this thesis, as in many other reports, to indicate a quantitative change, but it may not necessarily mean 'impairment' in the sense of being 'harmful'.

More information is needed on the complicated and sometimes obscure relationship between stress and insulin sensitivity. A study, therefore, was planned to investigate the effects of three types of physical stress on the role of insulin and other related hormones and substrates. This work concentrates on glucose homeostasis in patients undergoing surgery, in obesity and in old age.

PART II

GLUCOSE HOMEOSTASIS IN PATIENTS AFTER SURGERY

PART II

GLUCOSE HOMEOSTASIS IN PATIENTS AFTER SURGERY

CHAPTER I
INTRODUCTION

A. Trauma and Catabolism:

Surgery is a type of physical trauma which consists of a mechanical stress (the actual operation) and is then often followed by dietary restriction (nutritional stress), (see Part I, Chapter IV on stress). Because it is usually elective, it is also preceded by other stresses derived from the condition which needs the operation and creates anxiety. As will be seen later, nutritional status before an operation is, therefore, not necessarily normal. Severe trauma, as in surgery, is a negative balance of body protein associated with negative nitrogen balance, and this is reflected by the increase in urinary nitrogen excretion (Cuthbertson, 1964; Munro, 1964). However, a negative nitrogen balance could also be found in immobilized, otherwise healthy subjects (Schonholder, Hallskov and Olesen, 1954).

It is perhaps natural to assume that this period of negative nitrogen balance is due to increased catabolism, i.e. increased breakdown of protein. However, the loss of body nitrogen is associated with a fall in protein synthesis and no real evidence of an acute rise in the breakdown rates of body protein has been found in immobilized normal persons (Schonheyder, et. al., 1954), or in patients undergoing operations (O'Keefe, Sender and James, 1974; Crane, Picou, Smith and Waterlow, 1976). This change in synthesis may result from an altered flow of substrates (i.e. amino acids and high energy phosphates and/or specific change in the rate of initiation and elongation. Each of these changes is probably mediated by endocrine balance. The regulation of protein synthesis has been extensively reviewed by Munro (1970, 1976).

This part of the thesis concentrates on endocrine changes following surgery and in particular on the relationship in glucose homeostasis between insulin and its counterregulatory hormones, cortisol and glucagon (De Bodo and Altzuler, 1958; Ensinck and Williams, 1972). But these same hormones that effect carbohydrate metabolism also influence protein metabolism so that findings in one area usually have relevance to the other.

B. Anti-Insulin Hormones:

Anti-insulin hormones are defined as hormones which at physiological concentrations show anti-insulin effects. The anti-insulin effects could be in carbohydrate, fat or protein metabolism. However, this report limits itself only to hormones which have anti-insulin effects on carbohydrate metabolism. These hormones are: Adrenocorticotrophic hormone (ACTH), glucocorticoids (e.g. cortisol), glucagon, growth hormone and catecholamines (e.g. epinephrine).

Stress is usually associated with increased levels in blood of circulating ACTH (Cooper & Nelson, 1962), cortisol (Yates and Urquhart, 1962; Ross, Welborn, Johnston and Wright, 1966; Cuthbertson and Tilstone, 1969 , glucagon (Bloom, 1973; Lindsey, Santeusanio, Braaten, Faloona and Unger, 1974; Wilmore, Moyland, Pruitt, Linsey, Faloona and Unger, 1974; Russell, Walker and Bloom, 1975), growth hormone (Greenwood and Landon, 1966), and catecholamines (Walker, Zilelli, Reutter, Schoemaker, Friend and Moore, 1959). ACTH stimulates the secretion of adrenocortical hormones (e.g. cortisol), while mechanism of the actions of the other hormones in muscle, liver and adipose tissue is summarized in Table 4.

Table 4

The mechanism of action of some anti-insulin hormones in muscle, liver and adipose tissue. (Derived from Eminck and Williams, 1972 .

Hormones	Muscle	Liver	Adipose tissue
1. Epinephrine	1. Glycogenolysis 2. Inhibits glucose utilisation	1. Glycogenolysis 2. Gluconeogenesis	1. Glucose uptake 2. Lipolysis
2. Glucagon	1. Protein breakdown	1. Glycogenolysis 2. Gluconeogenesis	1. Lipolysis
3. Growth hormone	1. Protein synthesis 2. Inhibits glucose utilisation		1. Lipolysis 2. Inhibits glucose utilisation
4. Cortisol	1. Protein breakdown 2. Glycogenolysis 3. Inhibits glucose utilisation	1. Providing precursors for gluconeogenesis	1. Lipolysis

This report limits itself to the measurement of cortisol and its metabolites and glucagon. Epinephrine, ACTH and growth hormone were not measured.

C. Trauma and Endocrine Balance:

Increased circulating levels and excretion of cortisol are accepted as the usual consequence of many forms of stress (including surgery). Ross, et. al., 1966; Curthertson and Tilstone, 1969 , and recently it has become increasingly evident that the same is true of glucagon (Bloom, 1973; Lindsay, et. al., 1974; Wilmore, et. al., 1974; Russell, et. al., 1975). The situation with regard to insulin, seems at first glance, rather doubtful. Some have claimed that severe trauma results in depression of plasma insulin levels in relation to blood glucose values (Wilmore, et. al., 1974; Lindsay, et. al., 1974), while others reported elevated values and other signs of insulin resistance (Ross, et. al., 1966; Cuthbertson and Tilstone, 1969). However, the evidence suggests that during the acute phase, plasma insulin levels in relation to blood glucose values are indeed low (Allison, et. al., 1968; Wilmore, et. al., 1974; Lindsay, et. al., 1974), and they become elevated in the later phase of trauma (Ross, et. al., 1966; Allison et. al, 1968 .

Resolution of these problems has considerable practical importance in providing the rationale for effective dietary and possibly hormonal therapy after surgery and other forms of trauma. The present investigation seeks, therefore, to delineate the time and extent of hormonal and metabolic changes after surgery, to

attempt to correlate these with nitrogen balance and in particular, with the patient's insulinogenic capacity in a two hour glucose infusion test, carried out one day after the operation (day 1) and on 'recovery' (Chapter III, D. on glucose infusion.)

CHAPTER II
MATERIAL AND METHODS

A. Subjects:

1. Patients:

Eleven patients undergoing abdominal operations were studied. They were admitted to the surgical ward, University College Hospital, London. Written consent was obtained from each patient for his participation in this study. None of the patients was diabetic. Their mean age was 52 ± 3.4 years, mean height was 173 ± 2.3 cm and mean body weight was 68 ± 3.9 kg. The body weights ranged between 93 per cent and 118 per cent of the ideal weight for a given height and age (the mean was 94 ± 4.8 per cent). The ideal weight for a given height and age used for comparison was from the data of average weights of adults in Geigy Scientific Tables, 1970; based on the data of "Insured Persons in the United States" (Society of Actuaries, 1959), (Table 5).

A complete nitrogen balance was done on three of the eleven patients, while serial 24 hour urine collections were carried out on each patient. Fasting blood samples were taken from each patient during pre-operative, post-operative and 'recovery' periods. In addition to this, one day after the operation (day 1) and on 'recovery' (days 9 - 21), a two hour glucose infusion test was carried out on each patient. 'Recovery' is defined here as the time when the surgeons considered that the patients were fit enough to be sent home, and this varied from patient to patients.

2. Controls:

Four healthy young subjects were used as controls. Their mean age was 27 ± 2.3 years, mean height was 170 ± 6.3 cm, and mean body weight was 62 ±

Table 5

Sex, age, body weight and type of operation of patients who participated in this study

	Patient	Sex (M/F)	Age (years)	Weight (kg)	Height (cm)	% of ideal weight*	Type of operation
1.	HG	M	34	85	172	118	Proximal gastric vagotomy
2.	HT	F	41	50	168	74	Repair previous gastrectomy Roux-en-Y conversion
3.	NT	M	60	75	178	95	Vagotomy, pyloroplasty, fundoplication and dilatation of oesophagus
4.	MC	M	57	89	179	111	Prostate hypertrophy, prostatectomy
5.	MD	M	32	56	175	75	Spleenomegally, spleenectomy
6.	NB	F	50	74	158	118	Repair of previous gastrectomy
7.	BS	M	61	73	179	92	Repair of previous colostomy
8.	EC	M	65	61	178	77	Carcinoma colon, colectomy and colostomy
9.	CL	F	59	49	160	93	Proximal gastric vagotomy. Finney type pyloroplasty
10.	WD	M	53	70	179	87	Proximal gastric vagotomy
11.	WT	M	55	71	174	93	Proximal gastric vagotomy
Mean (± S.E.M.)			52 ± 3.4	68 ± 3.9	173 ± 2.3	94 ± 4.8	

*Compared with the data from Geigy Scientific Tables (1970)

4.2 kg. The body weights ranged between 86 per cent and 100 per cent of the ideal body weight for the given height and age (the mean was 92 ± 3.2 per cent), (Table 6). There was no significant difference between either the mean absolute body weights ($p > 0.05$) or the mean percentage ideal body weights of the controls and the patients (92 per cent and 94 per cent). These controls received the similar two hour glucose infusion test as given to the patients.

B. Nitrogen Balance Study:

Three patients participated in this study.

1. Sample preparation:

a. Food samples:

The patients were asked to eat or drink only food or liquid which was given by the hospital. The amount of food offered was recorded and protein content was calculated from Food Tables (McCance and Widdowson, 1960). Nitrogen content of the food was calculated from the protein content, using the equation: 1g nitrogen equal to 6.25 g protein. Any food which was left over was collected for each 24 hour period. Special plastic bags (weights known) were used for the food collection. The left over food was then weighed, mixed with water and was homogenized in a 'Kenwood' mixer. Then it was made to 500 ml with more water, and re-homogenized. Aliquots were put into universal containers and stored at -20° C until analysed for nitrogen.

Table 6

Sex, age, height and body weight of control subjects

	Subjects	Sex (M/F)	Age (years)	Height (cm)	Weight (kg)	% of ideal weight*
1.	PW	F	22	159	54	100
2.	WS	M	32	159	55	86
3.	PG	M	30	178	68	87
4.	GG	M	24	183	70	93
Mean (± S.E.M.)			27 ± 2.3	170 ± 6.31	62 ± 4.2	92 ± 3.2

*Compared with the data from Geigy Scientific Tables (1970)

b. Faecal samples:

Daily faecal excretions except for a few days following the operation when there were none, were also collected. The same plastic bags (weights known) were used for these collections. These bags fitted to special tin containers which enabled the patients to perform the collections easily. The faeces were also weighed, mixed with water and were homogenized in a 'Kenwood' mixer. They were also made to 500 ml with more water and re-homogenized. Aliquots were also put into universal containers and stored at -20° C until analyzed for nitrogen.

c. Urine samples:

Urine collections were done to all of the patients. Urine was preserved with 6N HCl (20 ml /24 hour collection). After measuring the volume of each 24 hour collection, samples of urine were put into universal containers and stored at -20° C until analyzed for nitrogen. Separate aliquots for steroid estimation, (see Section E, on analytical methods) had a few drops of chloroform added.

d. Calculations:

Aliquots of food and faeces and of urine were analyzed for nitrogen content. (See Section E on analytical methods)

The daily nitrogen balance was given as:

$ND - (N_R + N_F + N_U)$ g nitrogen

where: ND = total nitrogen (g) offered in 24 hours

N_R = total nitrogen content (g) of food residue and waste in 24 hours

N_F = total faecal nitrogen (g) in 24 hours

N_U = total urinary nitrogen.

ND was calculated from food tables, while N_R, N_F and N_U were obtained by direct analysis. Attempts were made to have duplicate meals, one was given to the patients and the other was analyzed for nitrogen content instead of getting the values of nitrogen from food tables. But these attempts proved to be too much time and energy consuming, and the results were more or less similar to the results done through food tables and did not give any more accuracy as it was first expected.

C. Blood Samples:

1. Collection of blood and separation and storage of plasma

Fasting blood samples were taken almost every morning on the first three patients, but in the others they were taken two days before the operation, the first three days after the operation, day 8 and on 'recovery'. Fasting blood was taken at 8.30 a.m. after the patients had been fasted from midnight. Patients who were still having intravenous fluids, had normal saline substituted for glucose at midnight.

For glucagon estimation, 4.5 ml of blood was added to a heparin tube containing 0.5 ml cold solution of Trasylol (10,000 KIU/ml Bayer) and after rapid centrifugation the supernatant was frozen immediately. The rest of the blood was put into another heparin tube and an aliquot of 0.05 ml was taken for blood glucose estimation. The tube was then centrifuged and the plasma separated. 0.05 ml of plasma was used for plasma glucose estimation and the rest was put into a polythene specimen tube and stored at -20° C until the day of estimation. Stored plasma samples were used later for estimations of plasma insulin, plasma NEFA, plasma amino acids and plasma cortisol.

2. Whole blood or plasma for glucose estimations

It has been reported that plasma glucose concentration is usually about 4 per cent higher than blood glucose (Ingram, Ingram, Turtle, Sturrock and Applegarth, 1971). Plasma is the 'carrier system' which carries glucose either from gastrointestinal tract or from the liver to various tissues, including the blood cells. However, most results are usually reported in terms of blood glucose (e.g. W.H.O. definition of diabetic, etc.). In the present work, both blood and plasma glucose were measured (see Section E, on analytical methods.) Although our values for plasma glucose were mostly higher than values for blood glucose, the difference was not always 4 per cent as reported by Ingram et. al. (1971). But we also found that with fasting values or in situations when the subjects were given insulin (see Part III and Part IV of this thesis) and the glucose concentration falls below fasting values, the blood glucose concentration is usually higher than that of plasma. These differences in the values of plasma and blood glucose did not give any significant difference to the results of the various tests. Another benefit of measuring

plasma glucose is that it could be used as a check if there was a technical error in the blood glucose estimation (e.g. using a wrong pipette, etc.), since when the blood is centrifuged and plasma separated, we were not able to repeat the blood glucose estimation. In this thesis, to avoid pointless duplication, plasma glucose values are not included in the results.

D. Glucose Infusion:

This test was used rather than the IvGTT used in the obese (Part III) and geriatric patients (Part IV) because it was thought to be the least burden to the patients, especially on the day immediately after the operation (day 1). And because continuous intravenous glucose infusion is the usual 'dietary regime' for most patients during the post-operative period, therefore, the substrates and hormonal changes would be measured in the most usual circumstances.

All glucose infusion tests were carried out in the morning. Fasting blood was taken at 8.30 a.m. as usual. A Halter Roller pump (Extra Corporeal Medical Specialities Ltd.) was used for infusing glucose through a butterfly needle inserted into an ante-cubital vein of one arm. The rate of glucose infusion was 0.35 g kg^{-1} h^{-1} (Reaven and Farquhar, 1969), and the test lasted for two hours. Another butterfly needle in the colateral ante-cubital vein was used for withdrawing blood samples. Normal saline was used to fill the latter butterfly needle to prevent the blood from clotting. Sometimes the blood did clot despite the regular flushing of the butterfly needle with saline, and we had to find another vein to insert another

needle. This was later solved by mixing heparin (Heparin Injection BP, 1000 units/ml) with the normal saline (2000 units heparin/500 ml saline). In preliminary work this concentration of heparin appeared to be the optimum for producing constant flow of blood without clotting, while still not affecting the NEFA concentration. Stronger solution of heparin (2000 units/20 ml saline) gave very high and erratic values for plasma NEFA concentration. (The values could be as high as 3000 μ mol/l to 4000 μ mol/l).

After the initial fasting blood sample had been taken, further blood samples were taken at 15', 30', 60', 90' and 120' after the start of the infusion. The blood samples were treated as described in the previous section (see Section C.1. in this chapter).

E. Analytical Methods:

1. Nitrogen (N):

a. Food and faeces:

Aliquots of 0.5 g of food and faecal homogenate were digested with 3 ml concentrated sulphuric acid and selenium catalyst in a Kjeldahl flask. Glass beads were used to prevent bumping. After the samples had cleared, digestion was continued for a further one hour. The samples were then allowed to cool and were diluted with distilled water to 100 ml. They were then analyzed for nitrogen by the

sodium phenate method for NH_3 in the Technicon autoanalyser, using $(NH_4)_2 SO_4$ as standards. The nitrogen content of the standards ranged between 5 µg/ml to 150 µg/ml. Samples which fell outside the range of the standards were redigested if the final nitrogen concentration was too low, and made to more appropriate volume or the final solutions were diluted, if the concentration was too high. Samples were run in the autoanalyser at a rate of 36 seconds sampling and 48 seconds washing (Technicon).

b. Urine:

Aliquots of 0.1 ml urine were digested with 0.5 ml concentrated sulphuric acid and selenium catalyst in a Kjeldahl flask. Glass beads were also used to prevent bumping. After the samples had cleared, digestion was continued for a further half hour. They were then allowed to cool and were diluted with distilled water to 10 ml. The rest of the procedure was similar to that of nitrogen analysis for food and faecal homogenate. From time to time, analysis for nitrogen content of random samples of food and faecal homogenate and urine were done manually using the Markham method (Wootton, 1964) to compare results with those done on autoanalyser.

2. Creatinine:

Creatinine was estimated in the urine. Urine samples were diluted with distilled water (1/20 dilution) and were put into the autoanalyser (Technicon). Creatinine solutions ranged between 1.0 mg/100 ml to 15.0 mg/100 ml were used as standards. Any samples which fell outside the range of the standards had the urine rediluted and the assay

repeated. Samples were run through the autoanalyser at a rate of 36 seconds sampling and 48 seconds washing (Technicon). Some urine samples chosen at random were analysed manually as a comparison to results obtained from autoanalyser.

3. Glucose:

Glucose in blood and plasma was measured enzymatically using the glucose oxidase Perid method and kit of Boehringer Corporation (London) Ltd. Aliquots of 0.05 ml of blood or plasma was mixed with 1.0 ml uranylacetate solution (160 mg/100 ml of normal saline) to precipitate the protein. After centrifugation, 200 µl of supernatant were mixed with 5 ml glucose oxidase solution. The blank (200 µl of water) and standard (200 µl of diluted Boehringer standard) were treated similarly. After standing 30 minutes at room temperature, the samples and the standard were read against the blank at 420 nm in a Unicam Spectrophotometer (Unicam SP600), using 10 mm glass cuvette. For the standard, the solution supplied with the Boehringer kit (100 mg glucose/100 ml) was diluted 1+1 with water.

4. NEFA:

The Boehringer kit for NEFA estimation was used (Boehringer Corporation, London Ltd.). Aliquots of 200 µl of plasma and standard (500 µmol/l) were mixed with 5 ml of chloroform and 1 ml of solution comprising a mixture of 0.27 M copper nitrate and 0.45 M triethanolamine buffer. For the blank, a tube containing all of the reagents but no plasma or standard was treated similarly. After 10 minutes shaking and 5 minutes centrifugation at 3000 RPM, the supernatant together with interfacial protein layer were removed by aspiration. A 2 ml aliquot of the remaining

chloroform extract was then added to 0.2 ml of 9 mM diethyl-dithiocarbamate. The samples and the standard were read against the blank in a Unicam spectrophotometer (Unicam SP600) at 436 nm, using 10 mm glass cuvette.

In the later part of this work, we observed that the reading of the standard in the spectraphotometer was getting lower and lower, resulting in apparent higher values of NEFA concentration in the plasma samples we were analysing. It was found out later that the standard supplied with the kit, contained less than the amount indicated in the label (500 µmol/l). We had to repeat some of our last few NEFA assays using palmitic acid (488 µmol/l) as the standard. A complaint was sent to Boehringer Corporation (London) Ltd. Their head office (Mannheim, West Germany) confirmed our findings. They apologised, promised to withdraw the faulty standard, and also promised us some compensation. They did send the compensation, unfortunately there were only few new NEFA kits. From then on, we always used palmitic acid (488 µmol/l) as the standard.

5. Insulin:

Plasma insulin was estimated by the radioimmuno assay method of Hales and Randle (1963), using the kit supplied by the Radiochemical Centre, Amersham, England. The method is based on a principle that the insulin in plasma and in standard solutions compete with the added radioactive insulin (^{125}I-insulin) for reaction with an antibody specific to insulin. The insoluble insulin-antibody complex which is formed, is filtered out and measured for radioactivity. The level of radioactivity in the filter paper is related in an inverse manner to the amount of insulin

present in plasma or standard solutions.

The amount of unlabelled insulin present in plasma is calculated as follows:

$$\frac{Co}{CT} = I\frac{1}{To} + 1 \quad \text{(Fig. 9)}$$

Where I = the concentration of unlabelled insulin

$\frac{1}{To}$ = the slope of the line

Co = the radioactivity of insulin-antibody complex when the concentration of unlabelled insulin is zero

Ci = the radioactivity of insulin-antibody complex when the concentration of unlabelled insulin is I

Co/CI is linearly related to I. In practice, the slope of the line is obtained by having a series of standard solutions made from human insulin standard provided by the kit. The standard solution ranged between 5 μunit/ml to 244 μunit/ml, and any samples which fell outside this range had to have the assay repeated. The plasma had to be diluted if the concentration was too high, or a larger amount of plasma sample was needed if the concentration was too low. An aliquot of 0.05 ml of plasma was used in this assay. In addition to this, a solution of unlabelled human insulin (40 μunit/ml) was made, put into a series of polythene tubes and stored at -20° Centigrade. This unlabelled human insulin solution was always included in each insulin assay and served as a quality control, and one batch of quality control overlapped with the next batch.

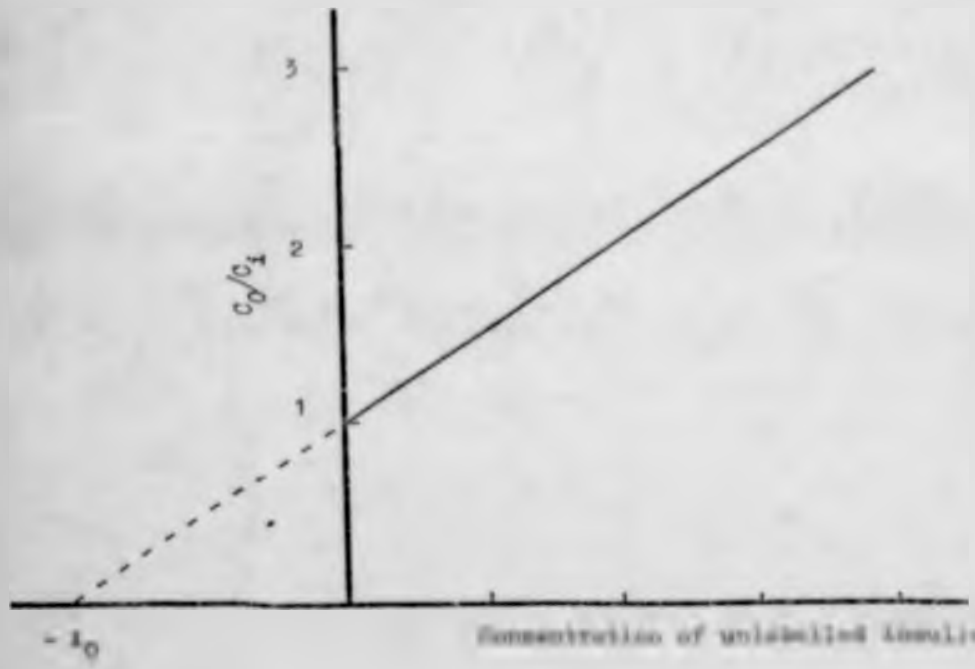
The counting of radioactivity was done using a well typed γ-scintillation counter (ECKO Electronics Ltd., England).

Figure 9. Radio-immuno assay of Insulin with Insulin-antibody Precipitate. Theoretical relationship between the ratio of the radioactivities in the insulin-antibody complex in the absence and presence of unlabelled insulin. (See details given in the text).

C_0= radioactivities in the insulin-antibody precipitate in the absence of unlabelled insulin.

C_1= radioactivities in the insulin-antibody precipitate in the presence of unlabelled insulin.

(from Hales and Randle, 1963) .


3
2
1
C_0/C_1
$-I_0$
Concentration of unlabelled insulin.

6. Plasma Amino Acids:

Plasma amino acids were estimated using the ninhydrin method (Spackman, Stein & Moore, 1958) in an amino acid analyser (Locarte, England).

7. Glucagon:

Plasma glucagon was measured by radioimmuno-assay using a pancreatic glucagon specific antiserum (Russell et. al., 1975).

8. Corticosteroids:

a. Plasma cortisol:

Plasma cortisol was measured by the competitive binding method of Few and Cashmore (1971).

b. Urinary free cortisol:

Urinary free cortisol was measured similarly to plasma cortisol but the extracted cortisol was purified by paper chromatography and losses monitored by the addition of ^{14}C-cortisol

c. Urinary 17-OH-corticosteroids:

These steroids were measured by the sodium borohydride reduction, sodium periodate oxidation method (Gray, Baron, Brooks and James, 1969).

F. Assessment of the Data:

Paired t test (Armitage, 1971) was used in assessing changes within the patients The ordinary student's t test was used when comparing data from the patients with those of the controls.

CHAPTER III

RESULTS

A. Nitrogen Balance:

A complete nitrogen balance study in three patients indicated the extent and duration of negative balance which was equivalent to about 50 g protein loss per day, for a period of 5 to 6 days after the operation. Since during the first part of this period the patients neither received food nor had any faecal losses, urinary (nitrogen) excretion adequately reflected the changes in the (negative) balance. However, this figure did not include the amount of blood transfusion (if any) or blood loss. The mean nitrogen balance in the three patients is shown in Fig. 10.

By day 10, a positive nitrogen balance had been re-established with a fall in urinary nitrogen excretion and an increase in food intake.

B. Urinary Nitrogen Excretion:

The 24 hour urinary nitrogen excretion showed an increase during the period following the operation. The mean values for eleven patients showed that the increases were significant compared to the mean pre-operative levels, on days 1, 2 and day 3 ($p < 0.05$, paired t test), and a significant decrease on day 6 ($p < 0.01$, paired t test) (Fig. 10).

C. Faecal Nitrogen Excretion:

During the days immediately following the operation, there was no faecal excretion. Table 7 shows the daily values of faecal nitrogen excretions in the three patients studied.

Figure 10. Mean (± SEM) 24 hour urinary nitrogen excretions in 11 patients (vertical lines), and mean nitrogen balance in 3 patients (vertical blocks) before and after operation:
There were significant increases (from preoperative values) in urinary nitrogen excretions on day 1, 2 and 3 ($p < 0.05$), and a significant decrease on day 6 ($p < 0.01$) (paired t test).

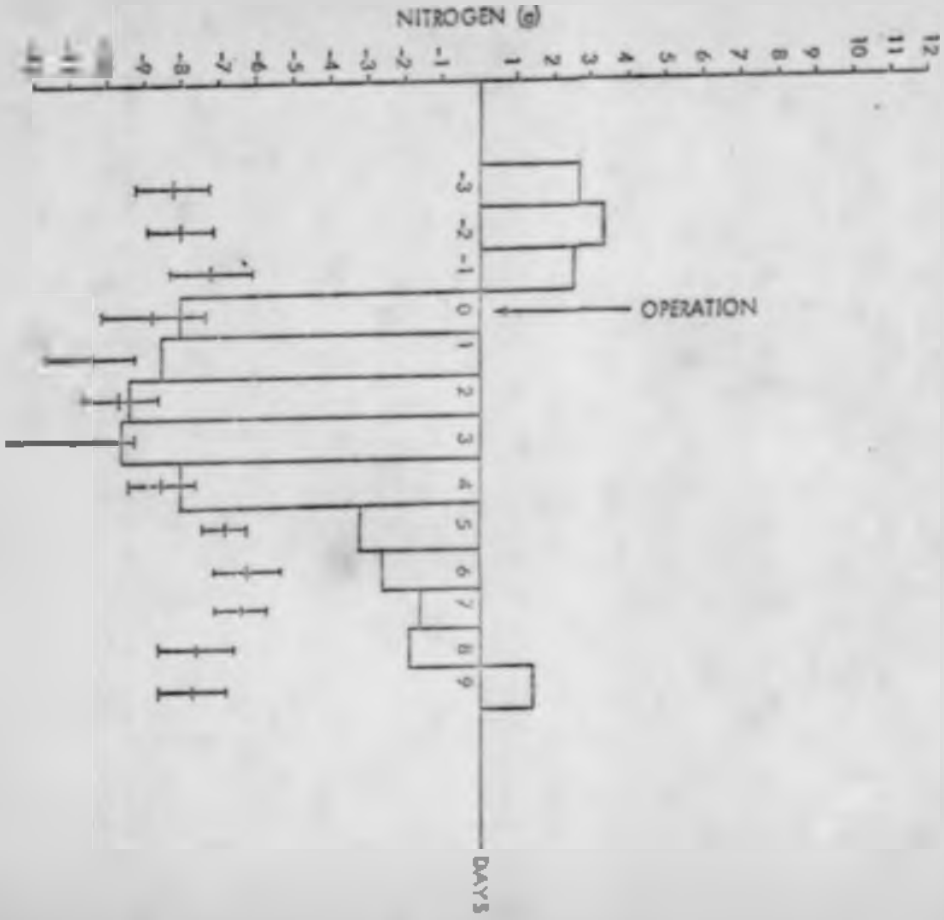

NITROGEN (g)
12 11 10 9 8 7 6 5 4 3 2 1 0 1 2 3 4 5 6 7 8 9 10 11 12
-3
-2
-1
0
1
2
3
4
5
6
7
8
9
OPERATION
DAYS

Table 7

Daily faecal nitrogen excretion in 3 patients (g)

Subjects	Day (g)												
	-3	-2	-1	Operation	1	2	3	4	5	6	7	8	9
HG	2.1	2.2	3.4	-	-	-	-	-	-	[illegible]	2.2	2.2	1.8
HT	3.8	6.3	0.4	-	-	-	-	-	-	3.4	1.4	0.1	0.2
NT		1.3	0.7	-	-	-	-	-	-	-	-	-	[illegible]

- = no faecal excretion

blank = not measured

D. Urinary Creatinine Excretion:

The 24-hour urinary creatinine excretion in eleven patients followed closely the pattern of urinary nitrogen loss. There were significant increases from the mean pre-operative levels on days 1, 2 and 3 ($p < 0.05$, paired t test), and significant decreases on days 5 and 6 ($p < 0.05$, paired t test). Fig. 11 shows the daily values of urinary creatinine excretion in the eleven patients studied.

E. The Relationship between Nitrogen Balance and Daily Hormonal and Substrate Values:

1. Plasma insulin:

The period of negative nitrogen balance coincided with increased fasting plasma insulin level, although it was only on day 1 that the difference between the values reached a significant level ($p < 0.05$, paired t test) (Table 8).

2. Plasma glucagon:

The fasting plasma glucagon level was also significantly raised from pre-operative levels in the first few days after the operation (paired t test) (Table 8).

3. Plasma cortisol

The fasting plasma cortisol level, like insulin, was significantly raised from pre-operative levels only on day 1 ($p < 0.05$, paired t test) (Table 8).

4. Urinary steroids:

The 24-hour excretion of urinary 17-OH-corticosteroids, however, was significantly raised for the first few days after the operation (paired t test) (Fig. 12), as also was the 24-hour urinary free cortisol excretion (paired t test) (Fig. 12).

Figure 11. Mean(+ SEM) 24 hour urinary creatinine excretions in 11 patients. There were significant increases (from preoperative values) on day 1, 2 and 3 ($p < 0.05$), and significant decreases on day 5 and 6 ($p < 0.01$ and $p < 0.05$ respectively).

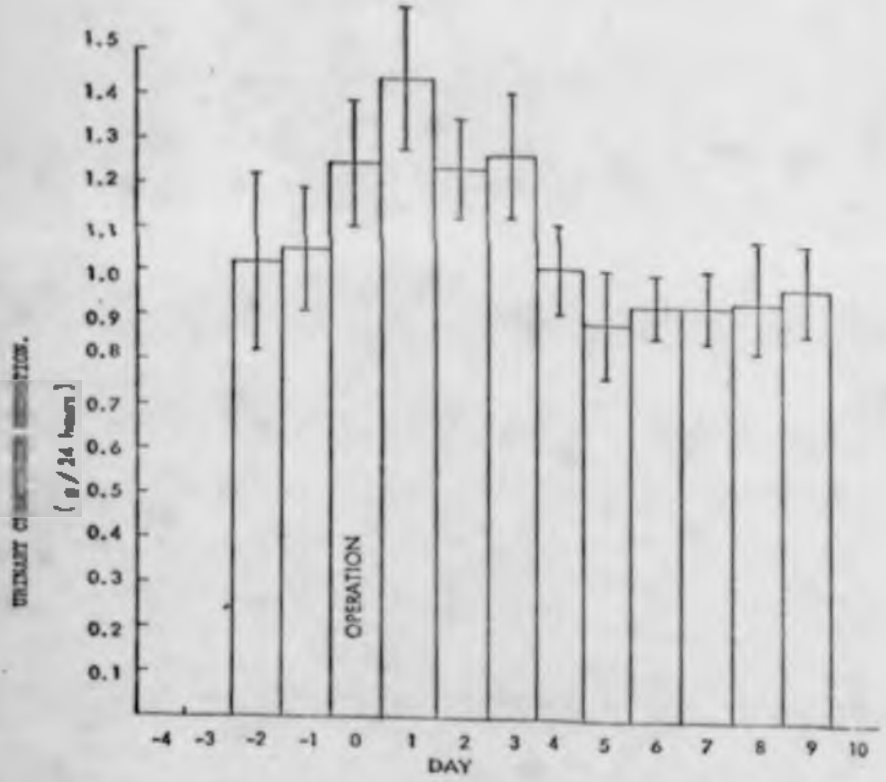

1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
OPERATION
-4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10
DAY

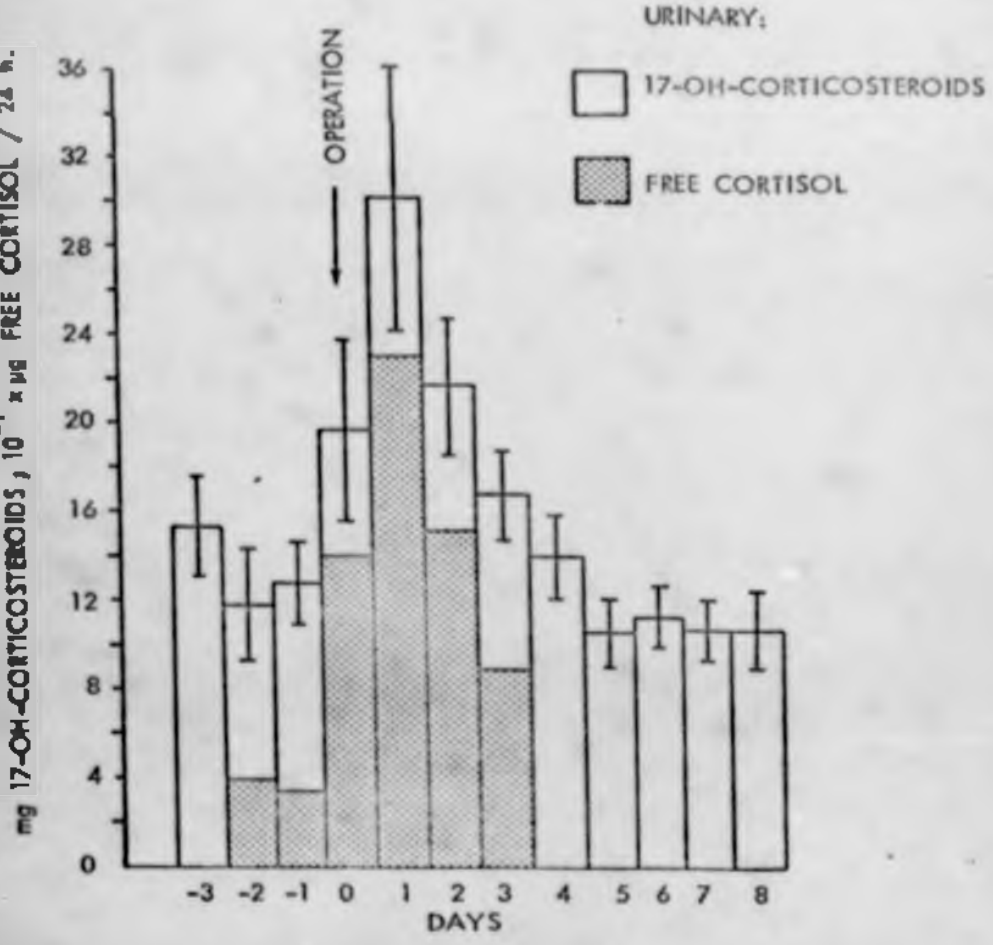
Table 8

Mean fasting levels of blood glucose, plasma insulin, plasma glucagon, plasma cortisol and NEFA in patients and controls (± SEM). Paired t test was used for comparing pre-operative values with the values on the days following the operation.

	No. of patients	Pre-operation	POST-OPERATION Day 1	Day 2	Day 3	Day 8	'Recovery'	Controls (n = 8)
Blood glucose (mmol/l)	10	5.3±0.14	5.9±0.23 *	5.8±0.37	5.6±0.30	5.6±0.15	5.1±0.15	5.1±0.35
Plasma insulin (uunit/ml)	10	16±0.8	25±2.2 **	24±4.0	18±3.1	20±2.5	16±1.8	15±2.5
Plasma glucagon (ng/l)	10	60±10.0	173±35.1		136±24.1*	64±19.6	66±16.3	35±12.1
Plasma cortisol (nmol/l)	9	359±26.5	486±57.1 *	282±30.1	339±18.2	386±36.2	315±19.6	306±13.5
NEFA (umol/l)	9	357±58	625±75 ***	432±54*	432±56*	468±82	459±103	500±197

Significant difference: *$p<0.05$; **$p<0.01$; ***$p<0.001$. (Paired t test)

Figure 12. Mean values of 24 hour urinary excretions of 17-OH-corticosteroids in 10 (± SEM), and free cortisol in 5 patients:
There were significant increases (from preoperative values) in urinary 17-OH-corticosteroids on day 1, 2 and 3 ($p < 0.05$), and in urinary free cortisol on day 1 and 2 ($p < 0.05$) (paired t test).


URINARY:
17-OH-CORTICOSTEROIDS
FREE CORTISOL
OPERATION
mg 17-OH-CORTICOSTEROIDS , 10^{-1} x µg FREE CORTISOL / 24 h.
36
32
28
24
20
16
12
8
4
0
-3 -2 -1 0 1 2 3 4 5 6 7 8
DAYS

5. Blood glucose:

The fasting blood glucose level, like insulin, was significantly raised from pre-operative level only on day 1 ($p < 0.05$, paired t test) (Table 8).

6. Plasma NEFA:

The fasting plasma NEFA concentration was significantly raised from pre-operative level during the first few days following the operation (paired t test) (Table 8).

7. Plasma amino acids:

There was a decrease in the levels of fasting total plasma amino acids. However, fasting levels of plasma leucine, iso-leucine and valine were raised in concentration (Table 9).

These substrate and hormonal changes in relation to nitrogen balance are shown in Fig. 13 for one patient (HG).

The overall picture is one of an acute rise in blood glucose, and in plasma insulin and cortisol on day 1 after the operation. This may be compared with the sustained changes lasting for about the period of the negative nitrogen balance, in plasma glucagon, in urinary glucorticoids excretion in plasma amino acid and NEFA concentrations.

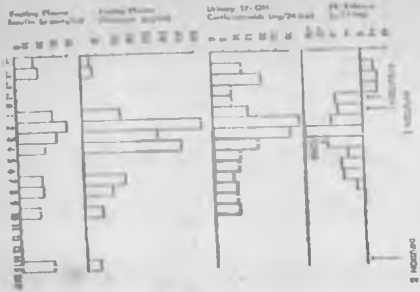
Table 9

Daily values of fasting plasma amino acids in patients and controls (± SEM). During the first few days after the operation, there was a decrease in the fasting levels of total plasma amino acids, but an increase in the fasting levels of isoleucine, leucine and valine.

Subjects	Pre-operation	Post-Operation Day 1	Day 2	Day 3	Day 4	Day 8	'Recovery'
		TOTAL PLASMA AMINO ACIDS (μmol/l)					
HT	2152	1545	1614	1431		2139	2412
NT	2581	2045	1973	1838	1810	2115	2675
MC	2391	2260	2222	2567	2884	2686	2801
MD	3678	2501			3108	1991	3037
NB	2333	2689					3469
Controls 2893 ± 175 (n = 4)							
		ISO-LEUCINE (μmol/l)					
HT	45	29	32	58		73	84
NT	54	46	62	71	83	75	74
MC	62	73	81	95	113	85	81
MD	83	73			113	65	92
NB	64	63					100
Controls 52 ± 10.1 (n = 4)							
		LEUCINE (μmol/l)					
HT	81	97	101	104		73	107
NT	133	147	163	166	174	150	109
MC	126	137	167	176	193	146	132
MD	148	167			222	119	185
NB	135	180					140
Controls 114 ± 11.8 (n = 4)							
		VALINE (μmol/l)					
HT	120	148	156	168		107	162
NT	205	207	219	210	223	211	159
MC	231	224	256	270	327	230	232
MD	259	276			342	207	286
NB	274	322					277
Controls 195 ± 17.7 (n = 4)							

Figure 13. Daily values before and after the operation for one patients (ME) showing: changes in nitrogen balance, in urinary 17-OH-corticosteroids excretion, and in fasting concentrations of glucagon, insulin, cortisol, glucose and NEFA in plasma.

DAY
FASTING NEFA (mmol/l)
FASTING PLASMA CORTISOL (µg/100 ml)
FASTING PLASMA GLUCOSE (mg/100ml)
OPERATION


Urinary 17-OH
Corticosteroids (mg/24 hr)

F. Substrate and Hormonal Changes during Glucose Infusion:

Except for the fasting levels ($p < 0.05$), there was no significant difference between the glucose values at any one time in infusion I (day 1) and at the same time in infusion II ('recovery'), but there was a trend to a higher 2-hour level on day 1 (12.08 m mol/l against 10.36 m mol/l). However, both curves were significantly higher than in normal subjects where the mean blood glucose levels reached a plateau at 6.07 m mol/l, Fig. 14).

Glucose infusion caused a substantial fall in plasma glucagon concentration (Fig. 14) but because of the higher fasting glucagon levels, plasma glucagon levels continued to be significantly higher during the infusion on day 1 than at similar times during the infusion done on 'recovery' ($p < 0.05$). Similarly, the mean glucagon curve during infusion on 'recovery' was higher than in controls, but the difference was not statistically significant ($p > 0.05$).

Infusion of glucose also decreased the levels of plasma NEFA, total plasma amino acids and plasma cortisol on both occasions in the patients and also in the controls (Table 10), but the changes in plasma cortisol during glucose infusion in controls were not significantly different from those found in normal subjects at rest (J.D. Few, unpublished data).

Plasma insulin levels during glucose infusion were significantly higher on day 1 than on 'recovery' both in respect to the fasting levels ($p < 0.001$) and the levels reached during glucose infusion, in which a significant difference was reached at 30 minutes ($p < 0.05$). At both times the patients had levels which were significantly higher than those of the normal controls. A significant difference between the patients'

Figure 14. Mean (± SEM) blood glucose, plasma insulin and plasma glucagon concentrations during 2 hour glucose infusion (0.55 g $Kg^{-1}h^{-1}$) in patients' infusion I (Δ), patients' infusion II (O) and infusion of controls (□).

Significance of differences :

Fasting values: See Table 8.

Glucose concentration: Infusion I x infusion II, non significant. Infusion I and II x controls , from 60' $p < 0.01$.

Insulin concentration: Infusion I x infusion II, from 30' $p < 0.05$. Infusion II x Controls, from 90' $p < 0.05$.

Glucagon concentration: Infusion I x infusion II, all times $p < 0.05$. Infusion II x controls, non significant.

The data were from 10 patients and 4 healthy control subjects. The paired t test was used for comparing values in patients' infusions I and II, and Student' t test for comparison of patients and control subjects.

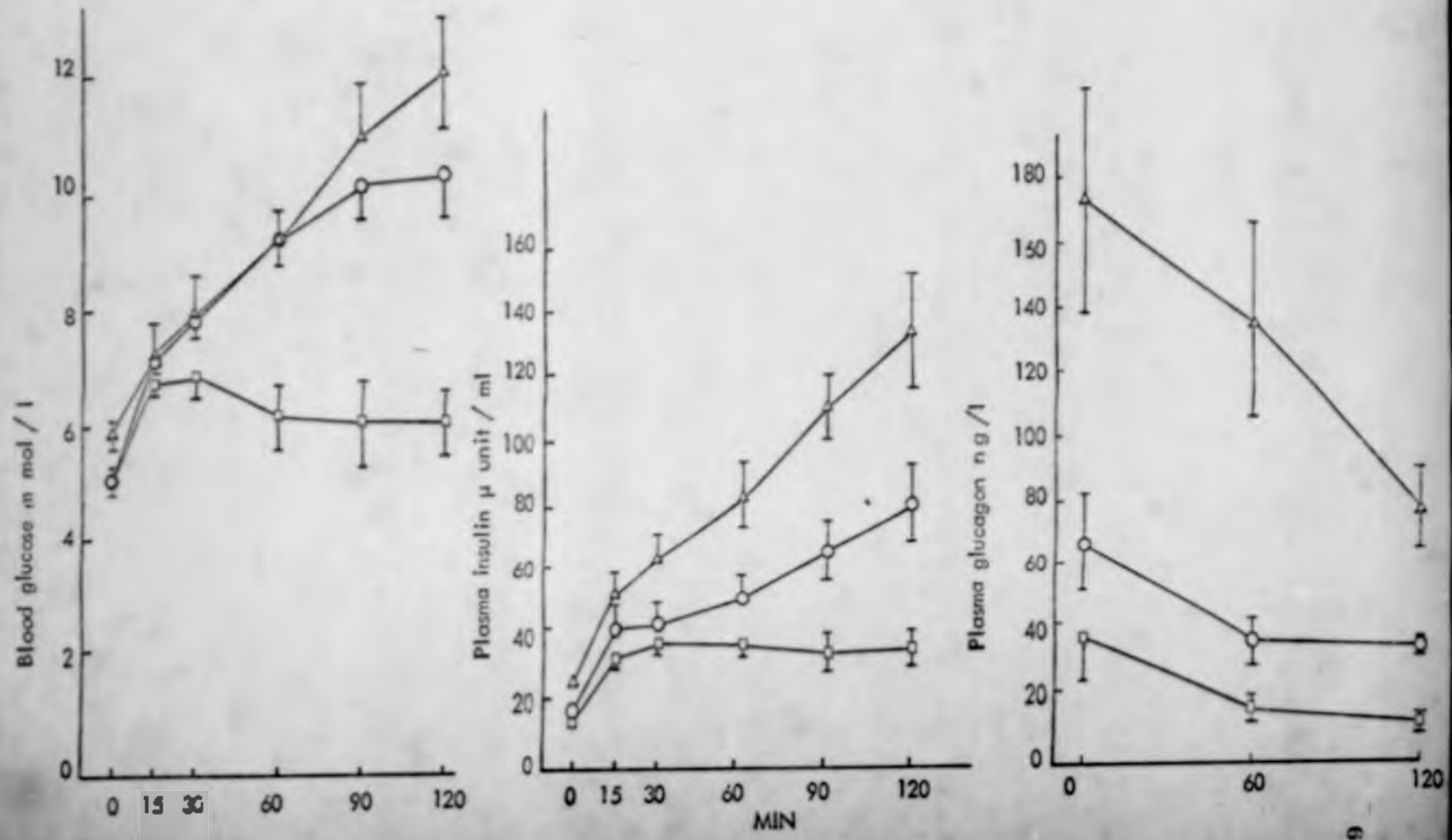

Blood glucose m mol / l
0
2
4
6
8
10
12
0 15 30 60 90 120
Plasma insulin μ unit / ml
0
20
40
60
80
100
120
140
160
0 15 30 60 90 120
MIN
Plasma glucagon n g /l
0
20
40
60
80
100
120
140
150
180
0 60 120

Table 10

The effect of 2 hour glucose infusion (0.35g kg^{-1} h^{-1}) on NEFA, total amino acids and cortisol levels in plasma of patients and controls (± SEM). Student's t test was used for comparing mean values between controls' and patients' infusion I (I) and between controls' and patients' infusion II (II). Paired t test was used for comparing values within individuals.

	Plasma NEFA (μmol/l)			Total plasma amino acids (μmol/l)			Plasma cortisol (nmol/l)		
	Fast		Post infusion	Fast		Post infusion	Fast		Post infusion
Infusion I	624 ± 75	***	132 ± 28	2208 ± 192	*	1701 ± 84	486 ± 57.1		403 ± 47.2
No. of patients		9		***	5	*		9	
Infusion II	459 ± 103	***	121 ± 36	2879 ± 179	*	2360 ± 186	315 ± 19.6	*	275 ± 29.3
				*(I)		*** (I)			**(I) *(II)
Controls	500 ± 197	***	208 ± 67	2893 ± 175	*	2408 ± 36	306 ± 13.5	*	160 ± 74.5
No. of controls		4			4			4	

Significant difference: *$p < .05$; **$p < .01$; ***$p < .001$

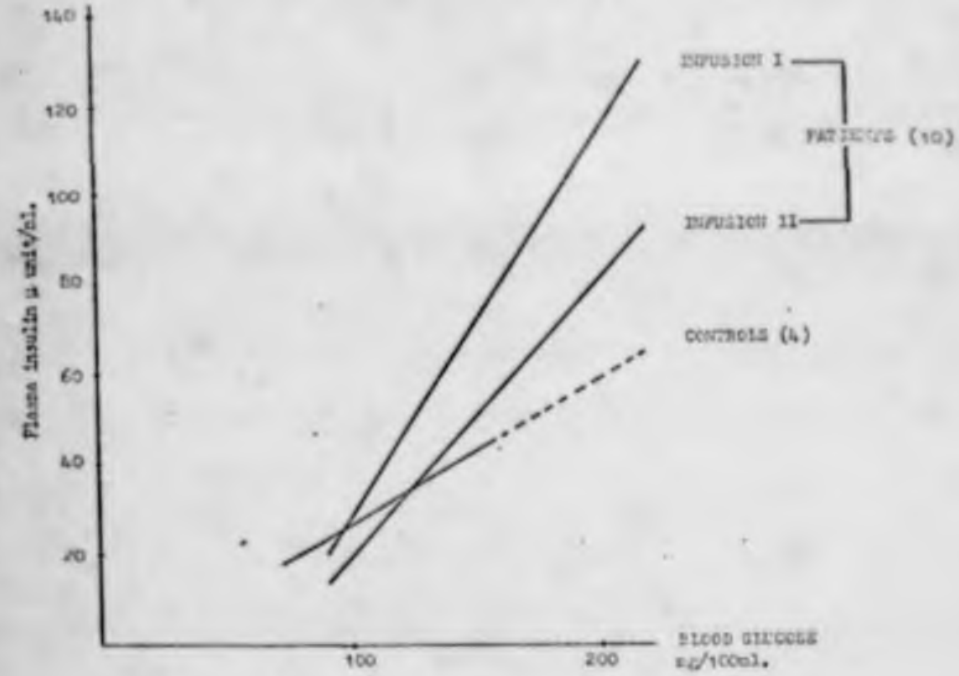
infusion I and controls' infusion was reached at 30 minutes ($p < 0.01$), and between patients' infusion II and controls' infusion at 90 minutes ($p < 0.05$) (Fig. 14).

Although the high levels of plasma insulin during the glucose infusion were associated with a higher level of blood glucose, the insulin levels tended to be higher for a given glucose value in the immediate post-operative period. Thus after 1 hour the mean blood glucose levels in the patients' two infusions were identical (Fig. 14) but the corresponding insulin values in the immediate post-operative state were almost twice those found in 'recovery'.

Fig. 15 shows the regression lines between plasma insulin and blood glucose concentrations during glucose infusion of controls and during infusion I and II of patients. A given glucose level tended to be associated with a higher insulin level in infusion I than in infusion II of the patients, and higher in infusion II of the patients than in control infusions.

Figure 15. Regression lines between plasma insulin and blood glucose concentrations during patients' infusion I, patients' infusion II and infusion of controls.

Data from 10 patients and 4 healthy control subjects.


140
120
100
80
60
40
20
Plasma insulin μ units/c.c.
100
200
BLOOD GLUCOSE
mg/100ml.
INFUSION I
PATIENTS (10)
INFUSION II
CONTROLS (4)

CHAPTER IV
DISCUSSION

When surgery is followed by a period of dietary deprivation, alleviated only by the use of intravenous glucose, patients obviously suffer from both acute protein and energy deficiency. This is manifested by negative nitrogen balance and an increase in the levels of those hormones and metabolites known to change in response to starvation.

Increased levels of glucagon, which promote rapid mobilization of fuel from carbohydrate, fat and protein (Foa, 1972 and of cortisol, which affects gluconeogenesis (De Bodo and Altzuler, 1958) are features of the body's response to fuel shortage. The increased output of urinary nitrogen in the face of a falling protein intake illustrates the effectiveness of energy mobilization and gluconeogenesis, which have a priority in the short term over protein retention.

The major difference between the effects of surgery and those of fasting in otherwise healthy subjects may lie in the insulin response. The immediate acute response to surgery is the same as to starvation, i.e. reduced levels of plasma insulin. After surgery this is followed by a period in which basal plasma insulin levels are raised (Lindsay, et. al., 1974) and we have found that glucose infusion results in an elevated insulin:glucose ratio compared with ratios found on recovery or in normal control subjects (Fig. 15). Therefore, the apparent discrepancies between reports of low insulin:glucose ratios and ours of high ratios, are indeed related to the time of observation.

During the initial acute phase of trauma, insulin secretion is probably blocked by the high circulating levels of catecholamines (Cerasi, Luft and Efendic, 1971). Our measurements were made, however, 24 hours after surgery at a time when the nutrient supply may well dominate the hormonal response.

The elevated levels of basal plasma insulin and high values for the insulin: glucose ratio in our patients can be interpreted as a compensation in insulin output as a result of peripheral insulin resistance (Porte, 1975). This resistance may be an innate response to trauma since patients after surgery when infused with amino acid solution alone, though having lower absolute levels of insulin and glucose, have the same insulin:glucose ratio as patients receiving parenteral glucose (Blackburn, Flatt, Clowes and O'Donnel, 1973). In the early stages after surgery, analgesia and the degree of surgical trauma certainly play a role in the metabolic response of hepatic and peripheral tissues (Long, Spencer, Kinney and Geiger, 1971; Wiklund and Jorfeldt, 1975). High circulating levels of catecholamines can induce lipolysis (Rosell, 1966), blockade the release of insulin (Cerasi et. al., 1971) and stimulate the output of glucose from the liver (Beam, Billing and Sherlock, 1951), appropriate analgesia in the post-operative period can reduce the circulating NEFA and glucose levels and the rate of release of glucose from the liver (Wiklund and Jorfeldt, 1975). Elevated levels of NEFA may themselves be expected to induce a reduction in the rate of glucose outflow from the circulation with the glucose intolerance and insulin resistance (Randle, Hales, Garland and Newsholme, 1963; Balasse and Neef, 1974) although our NEFA results are not particularly high. Thus, overproduction of glucose by the liver and slower uptake by the periphery may both tend to increase blood glucose levels and an altera-

tion in either process may be responsible for evidence of insulin resistance (Felig and Wahren, 1975).

This resistance seems to take some time to return to normal. There was no evidence of obesity in the patients, a condition which would explain a persistently low glucose tolerance and high insulin:glucose ratio (see Part II of this thesis). Furthermore, the age of the patients would lead one to expect lower insulin:glucose ratios (see Part IV of this thesis). The persisting increase in plasma glucagon in the patients studied in the 'recovery' phase argues against their hormonal status having returned completely to a pre-operative level despite being studied two weeks after surgery.

The demonstration of high circulating levels of glucagon after surgery is in keeping with the results of other studies of patients in the post-operative phase (Lindsey et al. 1974; Russell et. al., 1975). The elevation of glucagon persists despite the mildly raised blood glucose level but the glucose infusion led to a suppression of glucagon towards normal. This impaired suppression presumably reflects the resistance in the response of the α-cell to glucose and may be part of the more generalised insulin resistance at a cellular level (Samols, Tyler and Marks, 1972).

While glucagon itself may play an important role in the control of gluconeogenesis and glucose production, there is increasing evidence that its role in producing hyperglycaemia depends on insulin deficiency itself and the glucagon:insulin ratio as being of less importance in the pre-diabetic state (Sherwin, Fisher, Hendler and Felig, 1976). Thus enhanced hepatic glucose output after surgery may reflect either a direct short term effect of catecholamines on glycogenolysis or the combined

effects of insulin resistance and hyperglucagonaemia on gluconeogenesis. The additional role of enhanced corticosteroid secretion in stimulating gluconeogenesis probably operates over the 4-5 days after surgery as judged from the urinary data (Fig. 12).

It is not possible from the present evidence to quantitate the relative roles of hepatic overproduction of glucose and peripheral resistance to glucose uptake and oxidation despite the use of an infusion of glucose which might be expected to repress hepatic gluconeogenesis. Our infusion rate was approximately five times the basal glucose turnover rate in normal man (Bowen and Moorhouse, 1973) and it seems very unlikely in healthy controls that this quantity of glucose did not suppress gluconeogenesis (Madison, 1969). In favour of the suppression of hepatic glucose production in our patients, as well as in the controls during glucose infusion were the similar falls in glucagon, NEFA and amino acid levels, but we have no direct evidence that substrate supply for gluconeogenesis became rate limiting, or that the peripheral tissues were more or less sensitive to insulin than the hepatic cell.

A diminished protein synthetic rate post-operatively (O'Keefe et. al., 1974; Crane, et. al., 1976) might be the result of insulin resistance, but this aspect of protein metabolism requires further investigation. The net increase of protein breakdown is shown by the increased fasting plasma levels of branched-chain amino acids (Table 9). The rise of these amino acids, however, may not be specific to this phenomenon since prolonged starvation alone may also give a similar or even a greater rise (Adibi, 1968; Felig, Owen, Wahren and Cahill, 1969).

Therapeutic attempts to compensate for insulin resistance, assuming the resistance is undesirable, have sometimes taken the form of massive insulin administration (Hinton, Allison, Littlejohn and Lloyd, 1971). Insulin sensitivity may differ between various organs and tissues and even between different actions of insulin within the same tissue. This suggests caution in the use of insulin. Furthermore, it could even be argued that insulin resistance protects the subject from the less desirable feature of elevated insulin levels. An alternative approach would be to give parenteral amino acids rather than glucose (Blackburn, et. al., 1973). The lower levels of insulin induced by amino acid infusions would permit more ready mobilization of lipids and, therefore, allow the conservation of protein and glucose. Interestingly, plasma NEFA levels in our patients given glucose were not particularly high. If however, the development of insulin resistance places the patient at a disadvantage, this could be countered not only by giving insulin but also by blocking the release of insulin antagonists. It has been shown that surgery under morphine anaesthesia is not accompanied by the usual elevation of plasma cortisol and growth hormone concentration, and that the lack of adreno-cortical stimulation had no adverse clinical effect (George, Reier, Lanese and Rower, 1974).

It is shown, therefore, that after surgery, metabolic responses have some elements in common with starvation, but exacerbated and modified by hormonal stress responses. Similarly, there are resemblances with the diabetic, e.g. in hyperglucagonaemia, but in surgical patients the suppressibility of glucagon with glucose, possibly via elevated insulin levels, marked a clear distinction between the two states.

PART III

GLUCOSE HOMEOSTASIS IN OBESITY

CHAPTER I
INTRODUCTION

A. Definition:

Obesity is defined as a state in which an excessive amount of fat accumulates and where the body weight exceeds by at least 20 per cent the normal or desirable weight for a given height and age (i.e. 'ideal body weight') (Stern and Hirsch, 1972; Davidson, Passmore, Brock and Truswell, 1975). Others take 10 per cent above the ideal weight as the upper limit for normal weight, (Craddock, 1973). In the present work 'ideal' body weight for a given height and age was derived from the data of average weights of adults in Geigy Scientific Tables (1970). These are based on the data of insured persons in the United States (Society of Actuaries, 1959). Some may not agree with the use of relative excess of body weight as an indication of obesity, since this excess of body weight may not necessarily be fat (Lesser, Deutsch and Markofsky, 1971). But as a practical measure, most authorities accept the use of this parameter, since it is true to say that, in most cases (apart from a few pathological conditions, e.g. oedema) the higher the relative body weight, the higher the body's fat content (Garrow, 1974).

B. Problem of Obesity:

Millions of people in the developing world suffer and die from inadequate food intake, while ironically, millions of their counterparts in the western countries are in the opposite situation. They eat a lot and some even too much, and have too little physical exercise, which inevitably leads to obesity.

To some, obesity simply means an aesthetic problem, yet to many it comes as a serious health hazard. Obesity is known to be related to several diseases, e.g. diabetes (Joslin, 1921; Smith and Levine, 1964); hypertension (Marks, 1970); cardio-respiratory failure (Barlayne, 1958); and many others. Not suprisingly, therefore, obesity is also related to an increase in the rate of mortality and morbidity (Marks, 1970).

C. Aetiology of Obesity:

The question is inevitably asked whether obesity is always a simple problem of overeating and lack of physical exercise. The answer obviously would not be as simple as that.

Under normal conditions, the body is capable of regulating its energy balance quite accurately and the body weight of a healthy adult remains relatively steady. The human body has the ability to interrupt its food intake. Therefore, it is capable of having meals at certain times of the day, rather than as repeated small snacks, and thus it is able to do creative work during the day and have long intervals of sleep at night. All this is possible only because of the body's ability to store energy when it is available in excess of immediate requirements, and use it later when external energy supply falls below its requirements. However, interestingly in other higher primates such as gorillas and orang-utans, they spend most of their day time foraging for food and this is interrupted only by brief periods of rest and sex. This may be due to the type of food they eat rather than a major physiological difference the apes and man. The apes do not eat high energy density foods as humans do, therefore, they have to eat a lot to be able to meet their energy requirements.

For some curious reasons, our delicate energy balance can be disrupted resulting in an excessive amount of energy being stored, i.e. more than will be used in the interval before the next meal. This extra energy is stored mostly in the form of fat in adipose tissue. This may be the result of simple over eating, either caused by social pressure or other psychological stress; or may be due to something much more complicated and have an actual organic cause resulting in metabolic malfunctions. The latter could be of genetic, hypothalamic or endocrine origin (Mayer, 1957). This work does not attempt to investigate these complicated forms of obesity, although from the range of patients we studied, such forms of obesity cannot be excluded.

D. Insulin's Role in Obesity:

Excessive energy is stored mostly in adipose tissue as triglyceride. In fact 80 to 85 per cent of adipose tissue in an adult consists of triglyceride, (Craddock, 1973). In the presence of insulin, fat synthesis is enhanced, while when insulin is absent, glucose enters adipocytes less easily and the supply of lipogenic precursors is diminished. At the same time lipolysis in adipose tissue is accelerated.

It is known that obesity is usually related to insulin resistance (Franckson, Malaisse, Arnould, Rasio, Ooms, Balasse, Conrad and Bastenie, 1966; Chlouverakis, and White, 1969), and is associated with hyperinsulinaemia (Beck, Koumans, Winterling, Stein, Daughaday and Kipnis, 1964; Frankson et. al., 1966; Perley and Kipnis, 1966; Chiles and Tzagoumis, 1970). Insulin resistance could be defined here as the relative inability of insulin either endogenous or exogenous, to lower the blood glucose concentration. The glucose tolerance test in obesity may sometimes still be

within the normal limits (Perley and Kipnis, 1966) but most often it is impaired (Paullin and Sauls, 1922; Beck et. al., 1964; Perley and Kipnis, 1966; Chiles and Tzagoumis, 1970). Whether the degree of insulin resistance correlates with the degree of obesity and whether the amount of circulating insulin may in a way be the major factor in contributing to obesity, are being investigated in this work. This work concentrates mainly on the role of insulin in obese subjects faced by a glucose load, either oral or intravenous (iv), and compares them with the data collected from young normal adults. This work was not planned as an attempt to unravel the mysteries of obesity. Rather the studies were planned to extend the range of conditions exhibiting altered glucose tolerance, insulin sensitivity and plasma insulin levels. Insofar as the obesity is known to be associated with varying degrees of diminished glucose tolerance and increased insulin resistance and enhanced insulinogenic response to glucose, the biochemical signs of obesity are similar to those of the post-surgical patients. Yet such patients suffer from undernutrition while the obese struggle with overnutrition. It was hoped to find some clues why these very different nutritional states end up with a similar metabolic picture.

CHAPTER II
MATERIAL AND METHODS

A. Subjects:

1. Patients:

Twenty nine patients were studied (Table 11). Their mean age was 40 ± 2.9 years, their mean height was 165 ± 6.5 cm, their mean body weight was 109 ± 5.5 kg and they ranged from 115 per cent to 233 per cent of their ideal body weight (the mean was 166 ± 6.7 per cent). These patients were either from the obesity clinic, University College Hospital, or patients admitted from this clinic to the metabolic ward (Manson Ward, Hospital for Tropical Diseases), both of the University College Hospital Group. The patients were asked whether apart from their course of treatment, they would be willing to consider participating in our study. It was then explained in detail what kinds of tests were going to be involved in their study, and consent (oral or written) was obtained. None of the patients were known diabetic and all had fasting blood glucose concentrations less than 6.11 mmol/l (110mg/100 ml). 6.11 mmol/l is the upper limit for normal fasting blood glucose recommended by a working party appointed by the College of General Practitioners, England (1963). None of the patients had glycosuria.

The daily energy intake of the out-patients prior to the tests was not studied, but from information given by the patients it was estimated at between 8.4 and 12.6 MJ /day (2000 to 3000 Calories/day) including a reasonable carbohydrate intake. Patients who were admitted to the ward, received a diet of 8.4 MJ/day (2000 Calories/day) with a carbohydrate content of 200 g/day, for 3 to 4 days before they underwent

Table 11

List of Obese Patients

Subject	Sex	Age (years)	Height (cm)	Weight (kg)	% Ideal body weight*	Tests: Oral GTT (20 patients)	Tests: iv GTT and iv GITT (10 patients)	
CF	F	51	156	87	141	Yes	No	
CL	F	28	175	159	218	Yes	No	
OR	F	41	168	79	115	Yes	No	
BL	F	45	162	91	140	Yes	No	
PF	M	36	179	114	142	Yes	No	
DB	F	38	163	105	156	Yes	No	
SW	F	37	154	79	130	Yes	No	
DR	F	43	155	81	134	Yes	No	
SZ	F	17	157	71	139	Yes	No	
BK	F	48	156	132	218	Yes	No	
KM	F	68	154	124	202	Yes	No	Insulin not measured
CF	F	18	169	122	207	Yes	No	
RA	M	50	178	178	218	Yes	No	Insulin not measured
MC	M	27	169	132	192	Yes	Yes	

*Compared with the data from Geigy Scientific Tables (1970)

Subject	Sex	Age (years)	Height (cm)	Weight (kg)	% ideal body weight*	Oral GTT (20 patients)	iv GTT and iv GITT (10 patients)	
DR	F	33	166	110	175	Yes	Yes	
HG	F	21	173	135	213	Yes	Yes	
MR	F	17	163	90	160	Yes	Yes	Insulin not measured
MB	F	47	160	138	217	Yes	Yes	
BC	F	42	175	86	116	Yes	Yes	Insulin not measured
FH	F	22	162	75	122	Yes	Yes	
WT	F	14	161	94	177	No	Yes	
GR	M	20	176	180	233	No	Yes	
KY	F	27	161	107	179	No	Yes	
CW	F	53	162	80	119	No	Yes	
AD	F	68	158	79	123	No	Yes	
AR	M	45	168	120	164	No	Yes	
VR	F	53	156	96	156	No	Yes	
GG	F	51	155	100	164	No	Yes	Insulin not measured
PR	F	31	168	123	191	No	Yes	

*Compared with the data from Geigy Scientific Tables (1970)

any treatment or any further dietary restriction. The tests on these in-patients reported in this work were done during the end of this period.

2. Normal subjects:

Fourteen young non-obese normal subjects were used as controls (Table 12). Their mean age was 27.6 ± 1.3 years, their mean height was 169.2 ± 2.72 cm. their mean body weight was 61 ± 2.21 kg, and ranged between 85 per cent and 108 per cent of their ideal body weight (the mean was 94 ± 1.9 per cent). All the control subjects were told to eat sufficient food for at least three days prior to the test, including a reasonable carbohydrate intake.

B. Tests:

Most subjects, patients as well as the controls, received three kinds of test. Each was done in the morning.

1. Oral glucose tolerance test (oral GTT):

A butterfly needle attached to an antecubital vein on one arm was used for drawing blood samples. The fasting blood sample was taken at 9.30 a.m., after the subjects had been fasted from midnight. A drink of 50 g glucose in 250 ml water was given and serial blood samples were taken at 15', 30', 60', 90', 120' and 150' after the oral glucose. Heparin tubes were used to prevent the blood from clotting. An aliquot of 0.05 ml of blood was taken for blood glucose estimation. The blood tubes were centrifuged and the plasma separated. An aliquot of 0.05 ml of plasma was then used for plasma glucose estimation, and the rest of the plasma was stored. Plasma samples were stored at -20° C until analyzed for plasma insulin and NEFA (see Part II, Chapter II, Methods).

Table 12

List of Normal Subjects

Subjects	Sex	Age (years)	Height (cm)	Weight (kg)	% ideal body weight*	Tests: Oral GTT (13 subjects)	Tests: iv GTT and iv GITT (10 subjects)
SW	M	26	165	60	92	Yes	Yes
PG	M	30	178	68	90	Yes	Yes
MG	F	27	178	60	88	Yes	Yes
ED	F	25	165	62	108	Yes	Yes
DA	M	24	185	72	92	Yes	Yes
HP	F	24	158	53	100	Yes	Yes
JD	F	23	157	52	100	Yes	Yes
MS	F	22	160	51	100	Yes	Yes
WS	M	32	159	55	86	Yes	Yes
GG	M	24	180	70	92	Yes	No
CL	M	40	160	57	86	Yes	No
GC	M	30	180	78	96	Yes	No
SM	F	32	169	58	90	Yes	No
PB	M	27	168	55	81	No	Yes

*Compared with the [illegible] Scientific Tables (1970)

If the whole glucose (or insulin) curve in an oral GTT is thought of as being made up of 15 minute units (Fig. 16), then the 0' - 15' period is one unit, 15' - 30' is one unit, but 30' - 60', 60' - 90' etc. are each 2 units. The total number of 15 minute units is 10.

The average values of glucose or insulin concentrations during an oral GTT were, therefore calculated as:

Average value =

$$\frac{\frac{C_0 + C_{15}}{2} + \frac{C_{15} + C_{30}}{2} \times 2 + \frac{C_{30} + C_{60}}{2} \times 2 + \frac{C_{60} + C_{120}}{2} \times 2 + \frac{C_{120} + C_{150}}{2}}{10} =$$

m mol/l (Fig. 16)

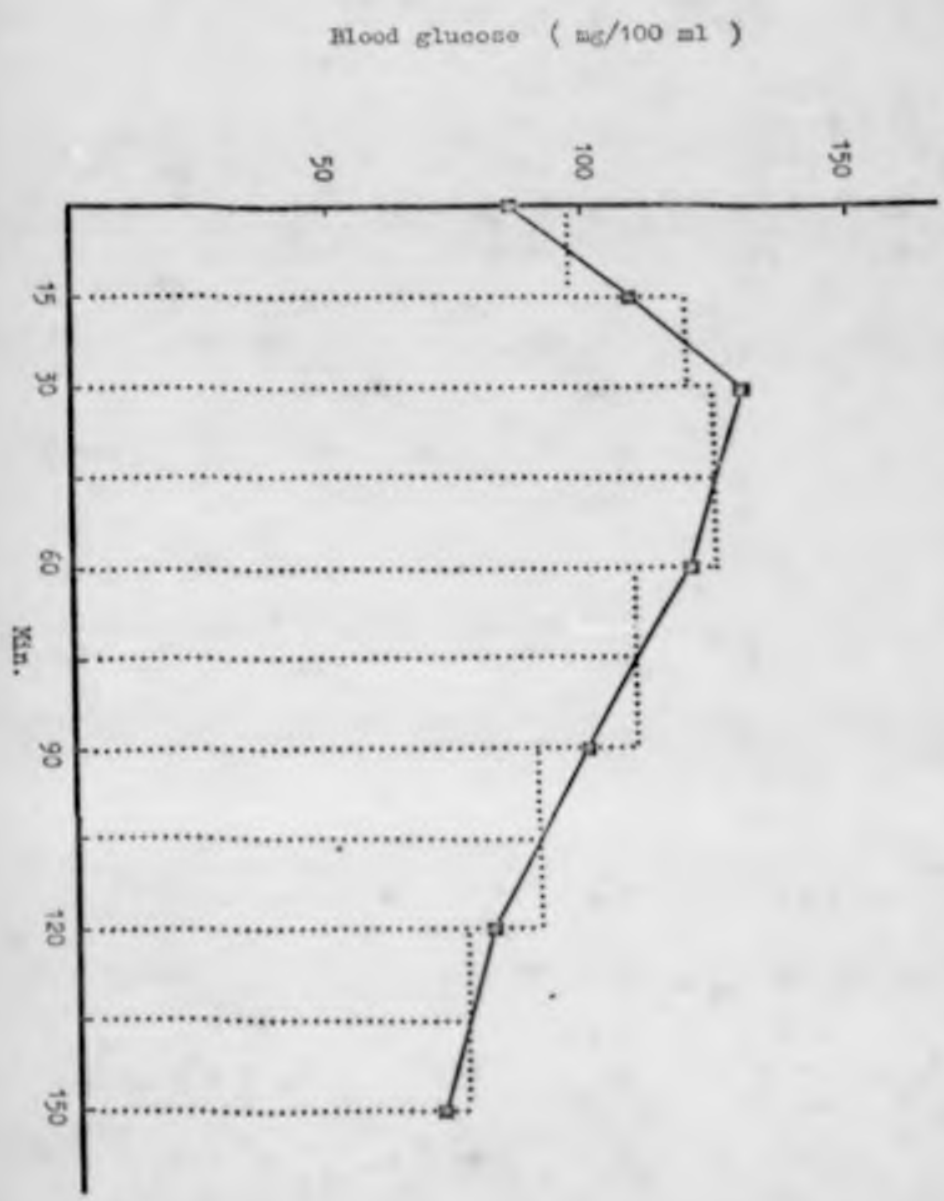
where C_0, C_{15}, C_{30}, C_{60}, C_{90}, C_{120}, and C_{150} were blood (or insulin) concentrations at 0', 15', 30', 60', 90', 120' and 150'.

The molar concentration of insulin was calculated from the concentration measured in the assay (μ unit/ml), on the basis of 1 unit of insulin being equivalent to 0.04167 mg (1 mg = 24 units) and the molecular weight of human insulin being 5807 (Scientific Tables, Geigy, 1970).

The insulin:glucose molar ratio during the oral GTT was calculated for each subject as follows:

$$\text{molar ratio} = \frac{\text{Average insulin value (m mol/l)}}{\text{Average glucose value (m mol/l)}}$$

Figure 15. Calculation of the average value of blood glucose concentration during oral glucose tolerance test (oral GTT).
(■) are observed concentrations of blood glucose at various times during the test.
(See details given in the text).


Blood glucose (mg/100 ml)
50
100
150
15
30
60
90
120
150
Min.

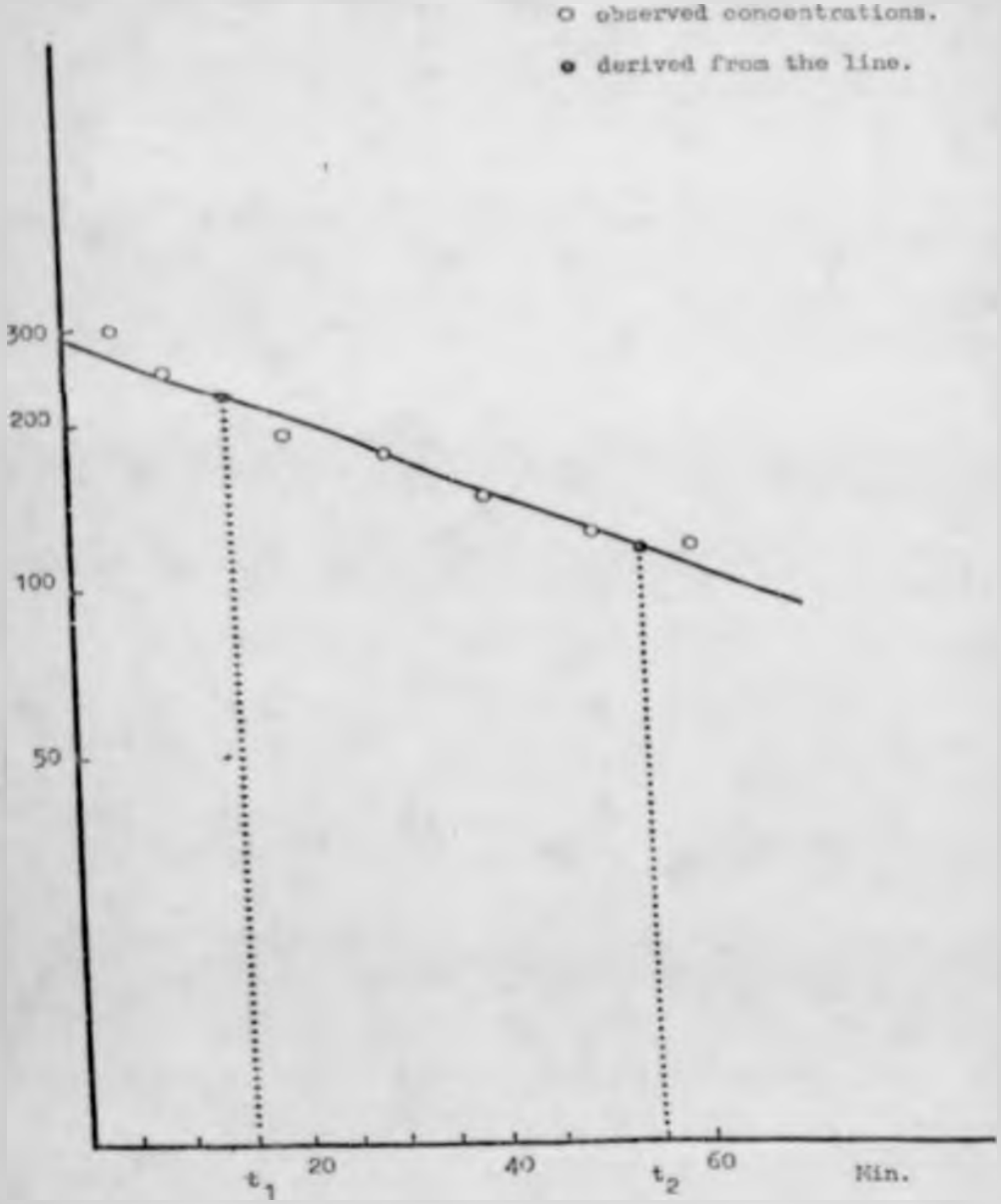
2. Intravenous glucose tolerance test (iv GTT).

This test was also done using a butterfly needle attached to an antecubital vein of one arm. A fasting blood sample was again taken at 9.30 a.m., and through the same needle a solution of 50 per cent glucose was injected. In the first obese patients an attempt was made to insert a butterfly needle in each arm, one for the injection of glucose and the other for blood sampling. However, it is difficult enough to find one suitable vein in grossly overweight subjects, without subjecting patients and operator to the added trauma of trying to do it twice. There was no evidence that the injected glucose in any way interfered with subsequent glucose sampling, especially as the tubing and needle were flushed in the normal saline after each operation (see Part II, Chapter II, Methods). The subjects received 0.33 g glucose/kg body weight (maximum 25g per person) (Franckson et. al., 1966). Glucose was injected within 4 to 5 minutes and serial blood samples were taken at 5', 10', 20', 30', 40', 50' and 60' after glucose injection. The procedure for handling blood samples was similar to that in the oral GTT. The blood glucose concentration (mg/100 ml) was plotted against time in minutes on Log-Linear graph paper and the best fitting straight line drawn through the points (Fig. 17). The percentage removal rate of glucose (K_G) was calculated from this line using the equation:

$$K_G = \frac{2.303\,(\log C_1 - \log C_2)}{t_2 - t_1} \times 100\%\ \text{min}^{-1}$$

where C_1 and C_2 were glucose concentrations read from the graph at any convenient times t_1 and t_2 minutes after glucose injection (Ikkos and Luft, 1957; Heard, 1966). The validity of this test will be discussed in Part V (General Discussion).

Figure 11. Calculation of the % removal rate of glucose during intravenous glucose tolerance test (iv GTT) (K_G value).
(See details given in the text).


o observed concentrations.
• derived from the line.
300
200
100
50
20
40
60
t_1
t_2
Min.

It was found that plasma NEFA concentration, during the iv GTT, decreased in the same way as did blood glucose concentration, so that when the plasma NEFA concentration (μ mol/l) was plotted against time on Log-Linear paper, a straight line could be drawn through these points also. From the best fitting line, the percentage removal rate of NEFA (K_F) was calculated using the same equation as for glucose.

3. Intravenous glucose insulin tolerance test (iv GITT):

This test was devised as a test of insulin sensitivity (with respect to blood glucose homeostasis), (Heard and Henry, 1969 a). The principle is that having derived a value for K_G from iv GTT described above, the test is immediately repeated, but on this occasion with insulin added to glucose at a concentration which would swamp the body's endogenous circulating insulin. The differences between subjects in the percentage removal rate of glucose in this test (K_{G+I}) cannot, therefore, be due to differences in plasma insulin concentration, and must be due to variations in responsiveness to insulin.

The validity of the iv GITT when carried out immediately after the iv GTT depends on the differences in results being due solely to the added insulin and not to any priming effect of the first glucose load on the disposal of the second. Conard (1955) has reported that if a second iv GTT is carried out immediately after the first, they give no difference in the K_G. Similar results have been reported by Soniols and Marks (1965), Heard and Henry (1969 a).

The procedure in the iv GITT was similar to that in the iv GTT. Subjects were given a second equal dose of glucose at the end of the first test, but this time insulin (Insulin BP, Wellcome, 20 units/ml) was added to the glucose (10 units of insulin in 50 ml of 50 per cent glucose; 0.133 units insulin/kg body weight). Therefore, the maximal dose of insulin per person was 10 units. Blood samples were taken at 10', 20', 30', 40', 50' and 60' after the glucose and insulin administration, and K_{G+I} was calculated in the same way as K_G. The procedure for handling blood samples was similar to that followed in the two previous tests.

C. Analytical Methods:

Analyses of blood or plasma glucose, plasma insulin and plasma NEFA were carried out according to the procedures reported in the previous study in surgical patients (see Part II, Chapter II, Analytical Methods.

D. Dietary Treatment

Dietary treatment, general ward management, clinical investigations and all tests other than oral GTT, iv GTT and iv GITT, were under the clinical control of Professor J.C. Waterlow, Dr. Andrew Tompkins, the late Dr. Peter Sender, Dr. Susan Ell, Dr. Graeme Glugston, Mrs. Inger O'Meara and Miss Elizabeth Roe (dieticians), and all of the nurses in Manson Ward, Hospital for Tropical Diseases.

For the first 3 to 4 days in the ward, all the hospitalized obese subjects received 8.4 MJ/day (2000 Calories/day) before they were submitted to any further dietary restrictions. The dietary restrictions were not always the same from patient to patient. It depended on the clinical situation and the ability of the patients to

cope with the treatment. Basically the restriction consisted of two parts:

1. Initial restriction:

The patients received a diet of either:

a. A non-protein diet of 1.3 MJ/day (300 Calories/day) as Hycal (63 per cent glucose syrup, Beecham Products, England) or as

b. A diet of 2.1 MJ/day (500 Calories/day) which contained 50g protein. The initial restriction was given for a period of 2 to 3 weeks.

2. Maintenance diet:

This diet followed the initial restriction and consisted of either:

a. A diet of 2.1 MJ/day (500 Calories/day) which contained 50 g protein, or as:

b. A diet of 3.4 MJ/day (800 Calories/day) which also contained 50 g protein, or:

c. A diet of 4.2 MJ/day (1000 Calories/day) which also contained 50 g protein.

Patients were sent home on this maintenance diet and were followed for periods of time which varied from patient to patient, depending on their co-operation.

An attempt to correlate the type of dietary restriction and weight loss, insulin sensitivity and weight loss in these patients is discussed in the latter part of this report.

E. Assessment of the Data:

The student's t test was used for comparing the patients data with those of the controls.

CHAPTER III
RESULTS

A. Oral GTT:

Oral GTT was done in 20 obese patients and in 13 normal control subjects (Tables 11, 12), 16 of these patients had their plasma insulin measured. To avoid unnecessary repetition, results reported below were only the results of 16 patients who had their insulin levels measured. There was no difference in the mean glucose curve between these 16 patients and the mean curve of all the 20 patients.

1. Blood glucose:

The mean fasting blood glucose concentration was 4.89 ± 0.16 m mol/l (88.1 ± 2.93 mg/100 ml) in the obese patients against 4.62 ± 0.16 m mol/l (83.2 ± 2.92 mg/100 ml) in controls (Table 13). The difference was not significant ($p > 0.05$). However, during the test the concentrations of blood glucose in the patients continued to rise after 30' and the peak was at 60', whereas the peak in the controls was at 30'. Blood glucose concentrations in the patients were higher than in control subjects, throughout the rest of the test, and the difference reached significant levels after 60' ($p < 0.05$) (Fig. 18A).

The average value for blood glucose concentration during the oral GTT was 6.73 ± 0.28 m mol/l (121.2 ± 5.10 mg/100 ml) in the obese patients against 5.71 ± 0.18 m mol/l (102.8 ± 3.25 mg/100 ml) in control subjects. The difference was significant ($p < 0.01$) (Table 13).

Figure 18. Oral GTT in 16 obese patients (O) and 13 normal control subjects (●).

A. Mean blood glucose values during oral GTT.
There were significant differences in the mean fasting values ($p < 0.05$); and in the mean values at 60', 90', 120' and 150' ($p < 0.05$) between the two groups.

B. Mean plasma insulin values during oral GTT.
There were significant differences in the mean fasting concentrations ($p < 0.05$); and in the mean values at 60', 90', 120' and 150' ($p < 0.05$) between the two groups.

C. Mean plasma NEFA values during oral GTT.
The differences between the mean values at various times during the test were not significant.
(mean values of 6 obese and 7 normal control subjects).

Blood glucose
(m mol/l)
2
4
6
8
A
0
30
60
90
120
150
Plasma insulin
(μ unit/ml)
20
40
60
80
100
B
Min.
0
30
60
90
120
150
Plasma NEFA
(μ mol/l)
500
1000
C
0
30
60
90
120
150

2. Plasma Insulin:

The mean fasting plasma insulin level was 21.4 ± 2.88 μunit/ml in the obese patients against 12.8 ± 1.31 μunit/ml in control subjects. The difference was significant ($p < 0.05$) (Table 13). During the test the levels of plasma insulin in the patients continued to rise after 30' and the peak was at 60', whereas the peak in the controls was at 30'. Plasma insulin levels in the patients were higher than in control subjects throughout the rest of the test and the difference reached significant levels after 60' ($p < 0.05$) (Fig. 18B).

The average value for plasma insulin concentration during oral GTT was 69.1 ± 10.63 μ unit/ml in the obese patients against 33.5 ± 3.51 μunit/ml in control subjects. The difference was significant ($p < 0.01$) (Table 13).

During the selection of patients, any obvious diabetic (fasting blood glucose concentration higher than 6.11 m mol/l) were excluded. (See Chapter IIA, Patients). WHO criteria for diabetes (1965) states that in normal oral GTT, the upper limit of 120 minute value for blood glucose concentration is 6.11 m mol/l (110 mg/100 ml). According to this criteria, among 16 obese patients studied, 7 were 'diabetic' and 8 were 'non-diabetic'. Therefore, we could divide the 16 obese patients into two groups. The 'non-diabetic' obese group did not show any significant differences in the mean blood glucose concentrations at various times during oral GTT compared to the normal control subjects, although they showed differences in mean plasma insulin concentrations. The differences between these two groups of obese patients and between these two groups and normal control subjects are summarized in Table 14.

2. Plasma Insulin:

The mean fasting plasma insulin level was 21.4 ± 2.88 μunit/ml in the obese patients against 12.8 ± 1.31 μunit/ml in control subjects. The difference was significant ($p < 0.05$) (Table 13). During the test the levels of plasma insulin in the patients continued to rise after 30' and the peak was at 60', whereas the peak in the controls was at 30'. Plasma insulin levels in the patients were higher than in control subjects throughout the rest of the test and the difference reached significant levels after 60' ($p < 0.05$) (Fig. 18).

The average value for plasma insulin concentration during oral GTT was 69.1 ± 10.65 μ unit/ml in the obese patients against 33.5 ± 3.51 μunit/ml in control subjects. The difference was significant ($p < 0.01$) (Table 13).

During the selection of patients, any obvious diabetic (fasting blood glucose concentration higher than 6.11 m mol/l) were excluded. (See Chapter IIA, Patients). WHO criteria for diabetes (1965) states that in normal oral GTT, the upper limit of 120 minute value for blood glucose concentration is 6.11 m mol/l (110 mg/100 ml). According to this criteria, among 16 obese patients studied, 7 were 'diabetic' and 8 were 'non-diabetic'. Therefore, we could divide the 16 obese patients into two groups. The 'non-diabetic' obese group did not show any significant differences in the mean blood glucose concentrations at various times during oral GTT compared to the normal control subjects, although they showed differences in mean plasma insulin concentrations. The differences between these two groups of obese patients and between these two groups and normal control subjects are summarized in Table 14.

Table 13

The mean values for fasting blood glucose and plasma insulin, average concentrations of blood glucose and plasma insulin, and insulin:glucose molar ratio during oral GTT, in patients and control subjects (± SEM). Number of observations in parenthesis.

	Mean fasting concentrations		Mean of the average value for 150 min.			10^6 x mean insulin: glucose molar ratio
	Blood glucose (m mol/l)	Plasma insulin (μ unit/ml)	Blood glucose (m mol/l)	Plasma insulin (μ unit/ml)	Plasma insulin (10^6 x m mol/l)	
Patients (16)	4.89 ± 2.93	21.4 ± 2.88	6.73 ± 0.28	69.1 ± 10.65	495.8 ± 76.4	75.3 ± 11.89
	*	*	**	**	**	*
Controls (13)	4.62 ± 0.16	12.8 ± 1.31	5.71 ± 0.18	33.5 ± 3.51	240.4 ± 25.2	42.2 ± 4.50

*$p < 0.05$; **$p < 0.01$; ns = not significant

Table 14

Mean blood glucose and plasma insulin concentrations during oral OTT in the diabetic class and non-diabetic obese patients and in normal subjects (SEM). Number of observations in parentheses.

Oral OTT

		Mean blood glucose concentrations (m mol/l)							Mean plasma insulin concentrations (μ u/ml)						
		0'	15'	30'	60'	90'	120'	150'	0'	15'	30'	60'	90'	120'	150'
I	Diabetic class (7)	5,51 ± 0.26	5,69 ± 0.32	7,03 ± 0.56	8,04 ± 0.58	8,59 ± 0.69	7,40 ± 0.31	6,14 ± 0.22	22,7 ± 3.85	38,3 ± 6.54	84,2 ± 9.11	76,4 ± 17.01	88,0 ± 19,34	69,3 ± 46,00	47,7 ± 17,65
	I ≠ II	-			-	**	***	—							
II	Non-diabetic obese (9)	4,66 ± 0.23	6,27 ± 0.30	7,28 ± 0.29	7,55 ± 0.56	6,22 ± 0.35	5,15 ± 0.27	4,60 ± 0.22	20,4 ± 4.38	82,2 ± 20,69	108,9 ± 24,76	105,9 ± 21,09	66,0 ± 19,17	46,3 ± 15,51	32,9 ± 9,35
	I ≠ III	**			***	***	***	**	**				***	**	*
	II ≠ III									*	*	**	*		*
III	Controls (12)	4,68 ± 0.16	6,15 ± 0.36	7,19 ± 0.30	6,40 ± 0.22	5,29 ± 0.29	4,48 ± 0.20	4,26 ± 0.19	12,8 ± 1.31	37,1 ± 5.39	56,2 ± 6.82	47,1 ± 6.97	27,8 ± 3.65	20,1 ± 2.94	14,8 ± 1.36

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Since there was no clear cut difference between the two groups of these obese patients, they will be reported in this work as one population.

The differences between non-diabetic and diabetic obese patients will be discussed later in Part V (General Discussion).

3. Insulin:glucose molar ratio:

The mean insulin:glucose molar ratio in the obese patients was (75.3 ± 11.89) $\times 10^{-6}$ against (42.2 ± 4.50) $\times 10^{-6}$ in control subjects. The difference was significant ($p < 0.05$) (Table 13).

4. Plasma NEFA:

There was a great range in the levels of plasma NEFA both in the obese patients and in the controls. This applied to fasting levels as well as the levels during the test. The mean fasting plasma NEFA level in the obese patients was 627 ± 120 μ mol/l against 590 ± 68 μ mol/l in control subjects. The difference was not significant ($p > 0.05$). In keeping with the elevated glucose and insulin values, there was a tendency to a lower 120' plasma NEFA value in the patients, but throughout the test, the difference in the mean plasma NEFA levels between the two groups was not significant ($p > 0.05$) (Fig. 18C).

5. Iv GTT and Iv GITT:

Iv GTT and Iv GITT were done in 16 obese patients and in 10 normal controls (Table 11 and Table 12).

1. K_G values:

The mean K_G values in the obese patients was 1.11 ± 0.18 %/minute against 1.81 ± 0.28 %/minute in control subjects. The difference was significant ($p < 0.05$) (Table 15).

There was no significant correlation between K_G and fasting plasma insulin values during iv GTT, in the obese patients nor in the control subjects (Fig. 19A). A higher K_G value was usually related to a lower fasting plasma insulin level.

There was also no significant correlation between K_G and peak plasma insulin values, in the obese patients nor in the control subjects (Fig. 19B). However, again higher K_G was usually related to a lower peak plasma insulin level.

2. Peak insulin levels during oral GTT and iv GTT:

There were 5 obese patients and 9 normal control subjects who had both oral GTT and iv GTT done.

The mean peak insulin levels during oral GTT were higher than the mean peak insulin levels during iv GTT both in patients and in the control subjects. The differences, however, were not significant ($p > 0.05$) (Table 16).

The mean peak insulin level during oral GTT in the obese patients was 160.6 ± 24.98 μ unit/ml against 72.0 ± 7.26 μ unit/ml in the control subjects. The difference was significant ($p < 0.01$) (Table 16).

Table 15

The mean values for K_G, K_{G+1} and K_F in the obese patients and in normal control subjects (± SEM). Number of observations in parenthesis.

	Mean Values		
	K_G (%/min)	K_{G+1} (%/min)	K_F (%/min)
Obese patients	1.11 ± 0.18 (16)	2.81 ± 0.35 (16)	2.86 ± 0.90 (6)
	*	*	ns
Controls	1.81 ± 0.28 (10)	4.55 ± 0.51 (10)	3.10 ± 0.73 (6)

*$p < 0.05$; ns = not significant

Figure 12 A. Correlation between K_0 and fasting plasma insulin values during iv GTT, in the obese patients (○) and normal control subjects (●).

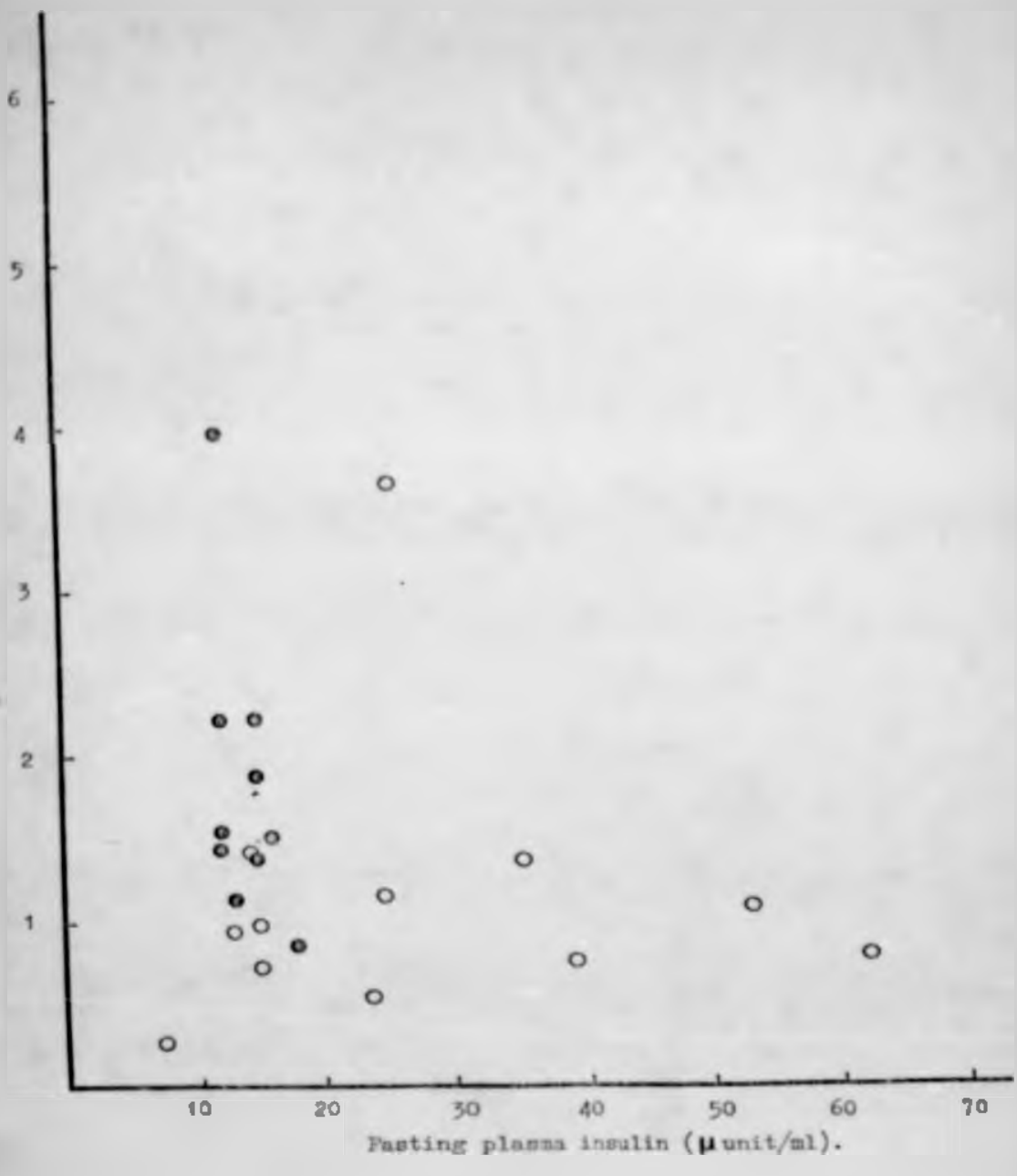
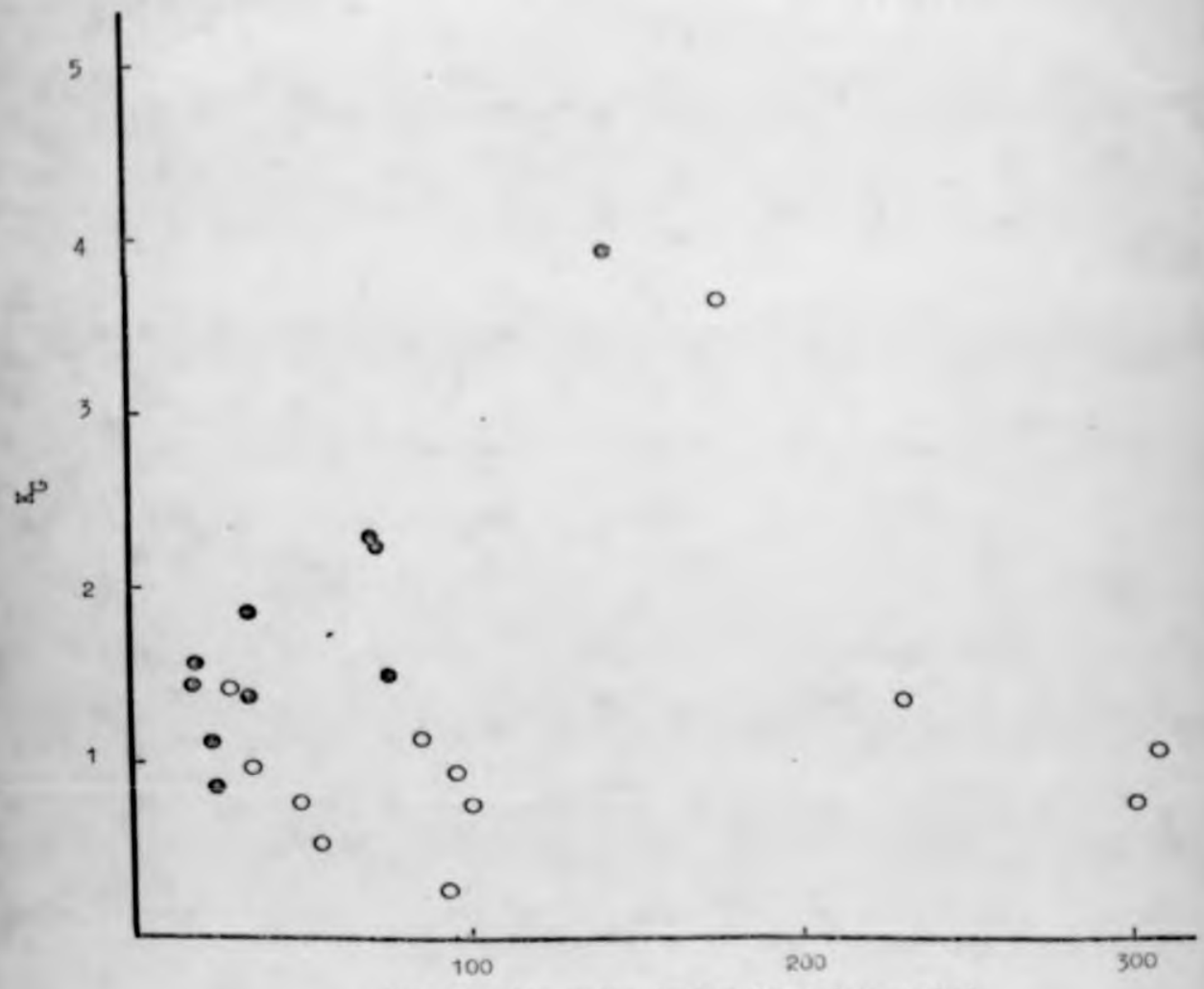

6
5
4
3
2
1
10
20
30
40
50
60
70
Fasting plasma insulin (μ unit/ml).

Figure 19 B. Correlation between K_g and peak plasma insulin values during iv GTT, in the obese patients (○) and normal control subjects (●).



Peak insulin value during ivGTT (μ unit/ml).

The mean peak insulin level during iv GTT in the obese patients was 138.4 ± 11.17 μ unit/ml against 54.0 ± 13.60 μ unit/ml in the control subjects. This difference was also significant ($p < 0.05$) (Table 16).

3. K_{G+1} values:

The mean K_{G+1} value in the obese patients was 2.81 ± 0.35 %/minute against 4.55 ± 0.51 %/minute in control subjects. The difference was significant ($p < 0.05$) (Table 15).

There was no correlation between K_{G+1} and fasting plasma insulin values in the obese patients nor in the control subjects (Fig. 20A). However, the lower K_{G+1} values were usually related to the higher fasting plasma insulin levels. This, however, is really just saying that lower K_{G+1} values and higher fasting plasma insulin levels are both characteristic of obesity.

There was also no correlation between K_{G+1} and peak plasma insulin values during iv GTT in the obese patients nor in the control subjects (Fig. 20B). A higher K_{G+1} was usually related to a lower peak plasma insulin level

There was a significant correlation between K_G and K_{G+1} values in the obese patients and in the control subjects ($p < 0.001$) (Fig. 21).

4. K_F values

The mean K_F value in the obese patient was 2.86 ± 0.90 %/minute against 3.10 ± 0.73 %/minute in the normal subjects. The difference was not significant (Table 15).

Table 16

The mean peak insulin levels (± SEM) during oral GTT and iv GTT in patients and normal control subjects. Number of observations in parenthesis.

	Mean Peak Insulin Level (μ unit/ml)			
	Oral GTT		Iv GTT	Ratio of oral: iv peak insulin
Obese Patients (5)	160.6 ± 24.98	ns	138.4 ± 11.17	1.16
	**		*	
Controls (9)	72.0 ± 7.26	ns	54.0 ± 13.60	1.33

* $p < 0.05$; ** $p < 0.01$; ns = not significant

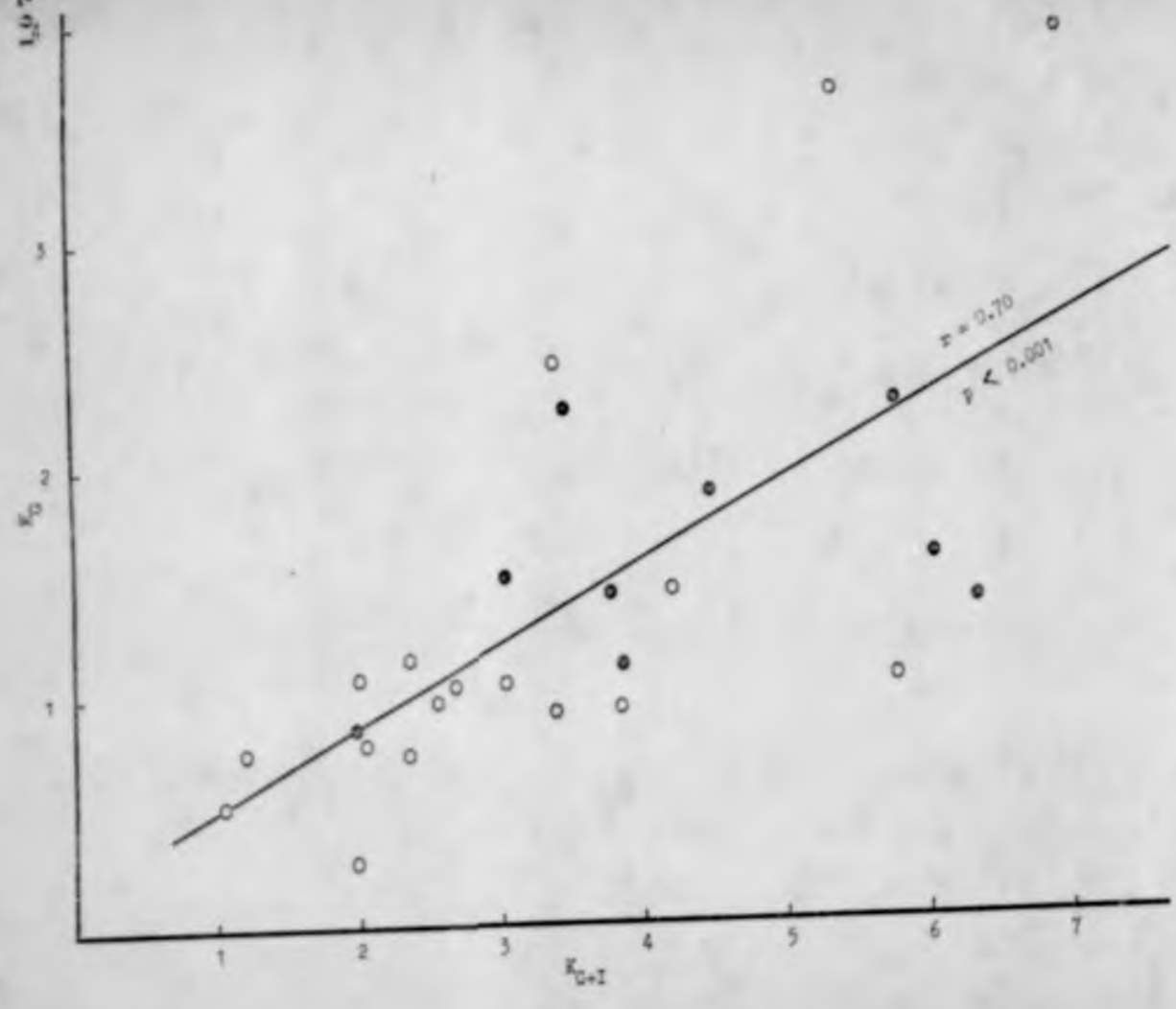
Figure 20 A. Correlation between the % removal rate of glucose during the intravenous glucose insulin tolerance test (iv GITT) ($K_{G.I}$ value) and fasting plasma insulin concentration, in the obese patients (▲) and normal control subjects (▲).


7
6
5
4
3
2
1
K_{G+I}
10
20
30
40
50
60
70
Fasting plasma insulin (μ unit/ml).

Figure 20 B. Correlation between $t_{\frac{1}{2}}$ and peak plasma insulin values during iv GTT, in the obese patients (▲) and normal control subjects (△).

Peak insulin value during iv GTT (μunit/ml).

Figure 11. Regression line between the values of K_Q and K_{Cr-T} in the combined group of obese patients (○) and normal control subjects (●).


r = 0.70
p < 0.001
1
2
3
4
5
6
7

C. Correlation between Biochemical Results and Degree of Obesity:

1. Blood glucose:

Since subjects with a fasting blood glucose greater than 6.11 m mol/l (110 mg/100 ml) were excluded from this study, the range in the fasting blood glucose concentrations in patients as well as in controls was very narrow, lying between 2.39 m mol/l (61 mg/100 ml) and 6.05 m mol/l (109 mg/100 ml). In contrast to this, there was a very wide spread in the range of % ideal body weight in these subjects. The % ideal weights varied between 85 per cent (the lowest of the controls) and 233 per cent (the highest of the obese group) (Fig. 22A). There was no correlation between fasting blood glucose values and body weight as % ideal body weight.

2. Plasma insulin:

There was a positive correlation between the logarithm of the value of fasting plasma insulin concentration and % ideal body weight ($p < 0.001$). (Fig. 22B).

There was no statistical correlation between the peak plasma insulin concentration during oral GTT (or the logarithms of these values) and % ideal body weight. However, the lower values of peak plasma insulin concentration during oral GTT were usually related to lower values of % of ideal body weight. If we take 160 per cent of ideal body weight as a dividing line, the obese patients and normal control subjects could be divided into two groups. One group was made up of the subjects who had weights less than 160 per cent of their ideal body weight. Among 22 subjects (13 normal and 9 obese) in this group, all except 5 (1 normal and 4 obese) had peak plasma insulin concentrations less than 100 μ units/ml. The other group consisted of subjects

FIGURE IV.1 Correlation between fasting blood glucose concentration and % ideal body weight in the obese patients (○) and normal control subjects (●).

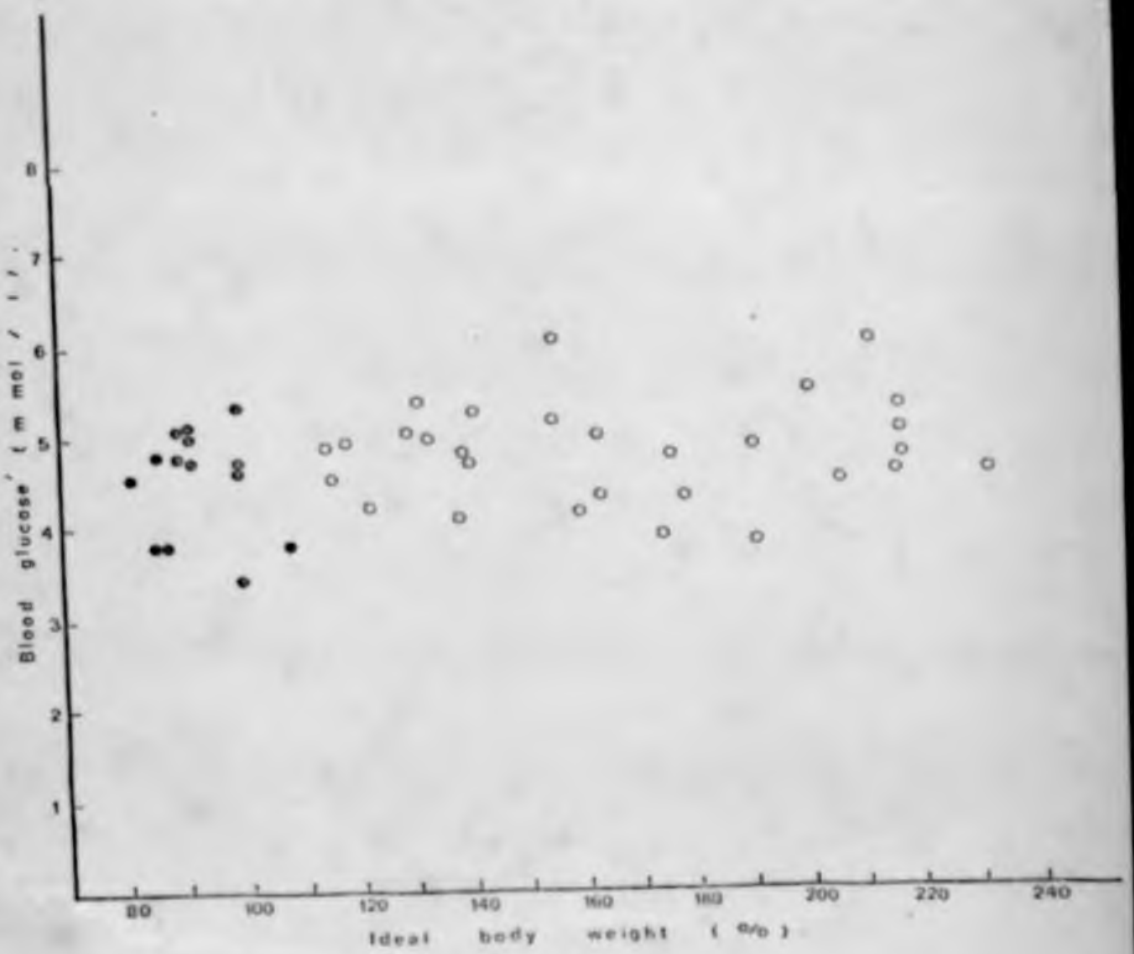
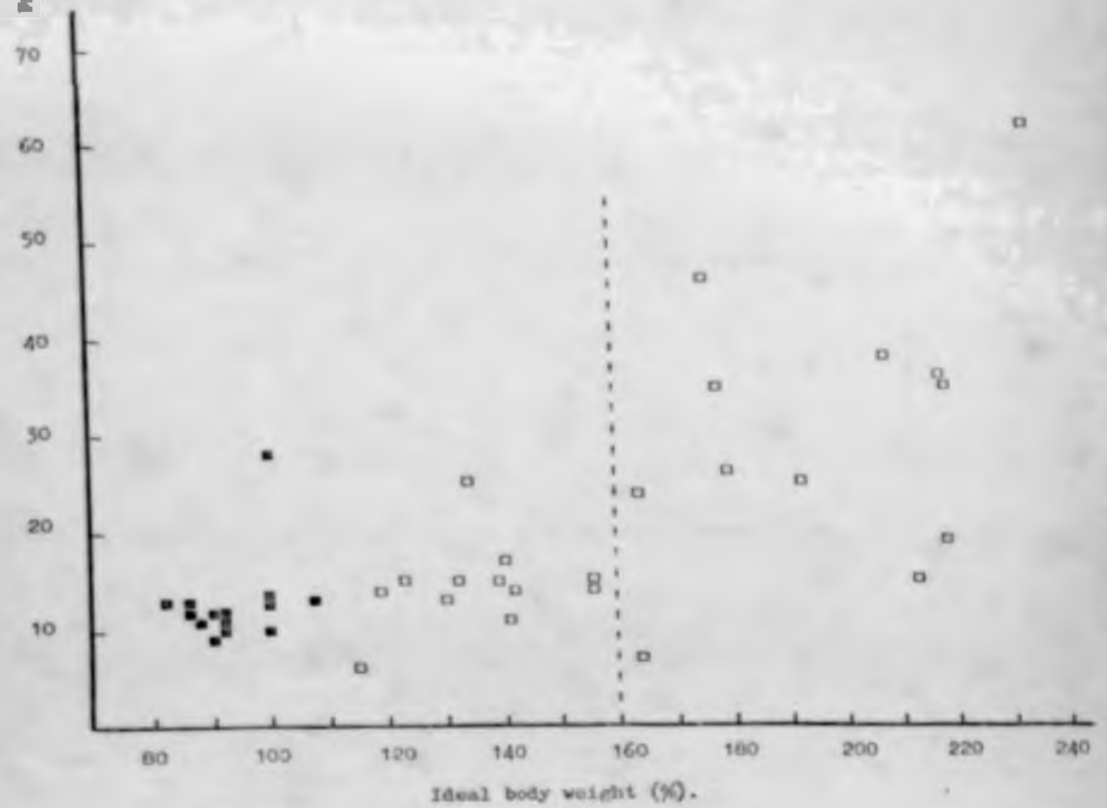

Blood glucose (m mol / l)
1
2
3
4
5
6
7
8
80
100
120
140
160
180
200
220
240
Ideal body weight (%)

Figure 22 B. Correlation between fasting plasma insulin concentration and % ideal body weight in the obese patients (□) and normal control subjects (■).


Fasting plasma insulin (pmol/l).
70
60
50
40
30
20
10
80
100
120
140
160
180
200
220
240
Ideal body weight (%).

who all had weights higher than 160 per cent of their ideal body weight. Among 7 subjects (all obese) in this group, all except one had peak plasma insulin concentrations higher than 100 μ units/ml. However, only 5 out of 9 obese subjects who had weights less than 160 per cent ideal body weight, had low peak insulin concentrations as found in the normal control subjects (Fig. 23A).

There was no statistical correlation between the peak plasma insulin concentration during iv GTT and % of ideal body weight. However, here again, the lower values of peak plasma insulin during iv GTT were usually related to lower values of % ideal body weight. And if once more we take 160 per cent ideal body weight as a dividing line, there were 14 (10 normal and 4 obese) whose body weight was less than 160 per cent of their ideal body weight, and all except 2 (1 normal and 1 obese) had peak plasma insulin concentrations less than 80 μ units/ml. However, 4 of the obese who had weights less than 160 per cent ideal weight, 2 still had low peak plasma insulin concentrations as in normal control subjects. There were 9 others subjects (all obese) who had body weights higher than 160 per cent ideal body weight, and all except 1 had had peak plasma insulin concentration higher than 80 μ units/ml. We chose 80 μ units/ml instead of 100 μ units/ml, because usually there is a lower peak plasma insulin value for iv GTT compared to oral GTT, either in obese or in normal control subjects (Fig. 23B).

2. K_G values

There was no statistical correlation between K_G value during iv GTT and % ideal body weight. However, the lower values of K_G were usually related to higher values of % ideal body weight. If, here again, we take 160 per cent ideal body weight as a dividing line, 15 subjects (10 normal and 5 obese) who had their weights less than 160

Figure 23 A. Correlation between peak plasma insulin value during oral GTT and % ideal body weight in the obese patients (□) and normal control subjects (■).

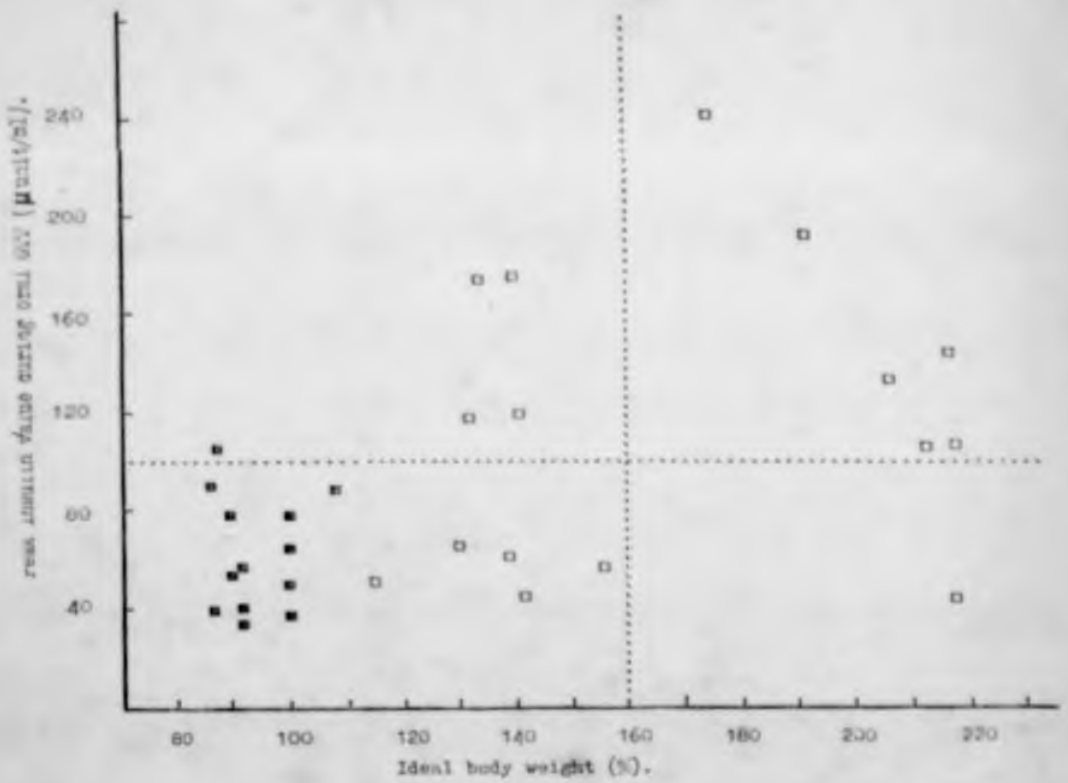
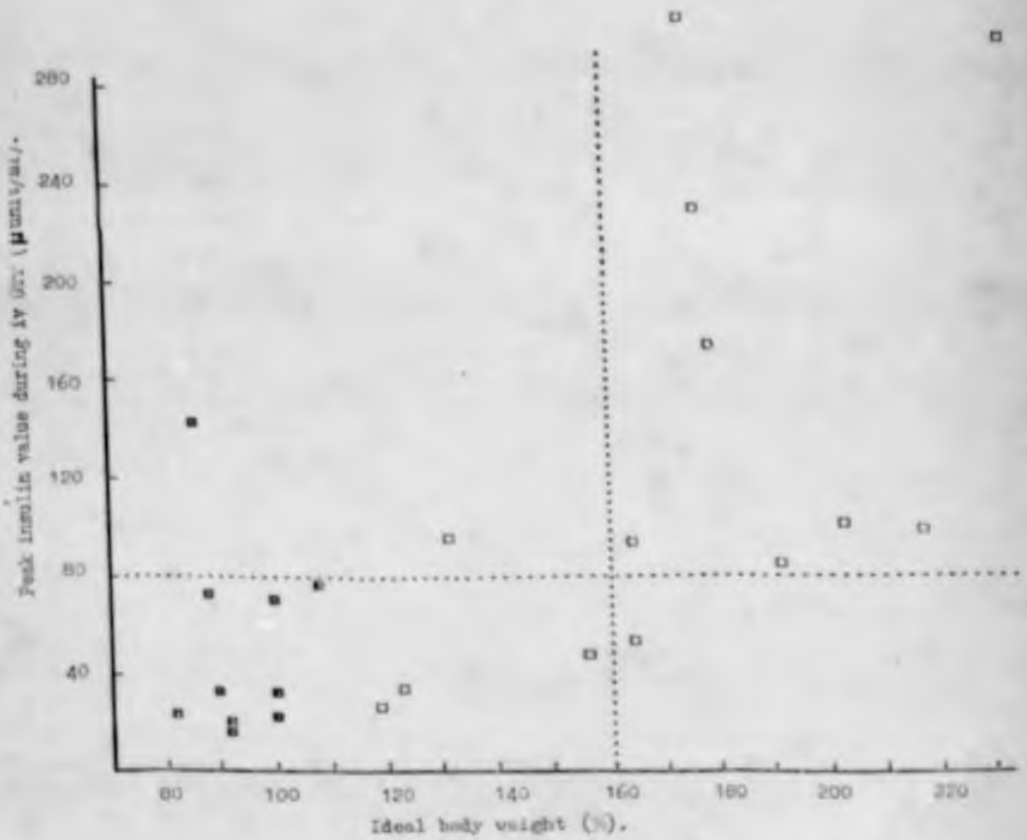

240
200
160
120
80
40
60
100
120
140
160
180
200
220
Ideal body weight (%).

Figure 23 B. Correlation between peak plasma insulin value during iv GTT and % ideal body weight in the obese patients (□) and normal control subjects (●).


280
240
200
160
120
80
40
80
100
120
140
160
180
200
220
Ideal body weight (%).

per cent their ideal body weight, 9 (8 normal and 1 obese) had K_G values higher than 1.20 %/min and only 6, (2 normal and 4 obese) had K_G values lower than 1.20 %/min, 5 of the obese subjects who had weights less than 160 per cent ideal body weight, only 1 had K_G value higher than 1.20 %/min, as in normal control subjects. And among 11 subjects who had their weights equal or higher than 160 per cent their ideal body weight (all obese), all except 2 had K_G values lower than 1.20 %/min. (Fig. 24).

4. K_{G+I} values:

There was no statistical correlation between K_{G+I} value during iv GTT and % ideal body weight. However, the lower values of K_{G+I} were usually related to higher values of % ideal body weight. If, here also, we take 160 per cent of ideal body weight as a dividing line, 15 subjects (10 normal and 5 obese) who had weights less than 160 per cent their ideal body weight, 10 (8 normal and 2 obese) had K_{G+I} values higher and only 5 (2 normal and 3 obese) had K_{G+I} values lower than 3.20%/min. However, only 2 out of 5 obese subjects who had weights less than 160 per cent ideal body weight had K_{G+I} values higher than 3.20%/min as in normal control subjects. 11 subjects had their weight higher than 160 per cent their ideal body weight (all obese). All except 3 had K_{G+I} values less than 3.20%/min. (Fig. 25).

D. The Effect of Dietary Restriction on Weight Loss in the Obese Patients:

As mentioned above, dietary management of patients was related firstly to the need of the patients and secondly to those of the investigations a. the investigation of protein turnover by Professor J.C. Waterlow, Dr. P. Sender, Dr. Susan Ell,

Figure 24. Correlation between K_g value and % ideal body weight in the obese patients (O) and normal control subjects (●).

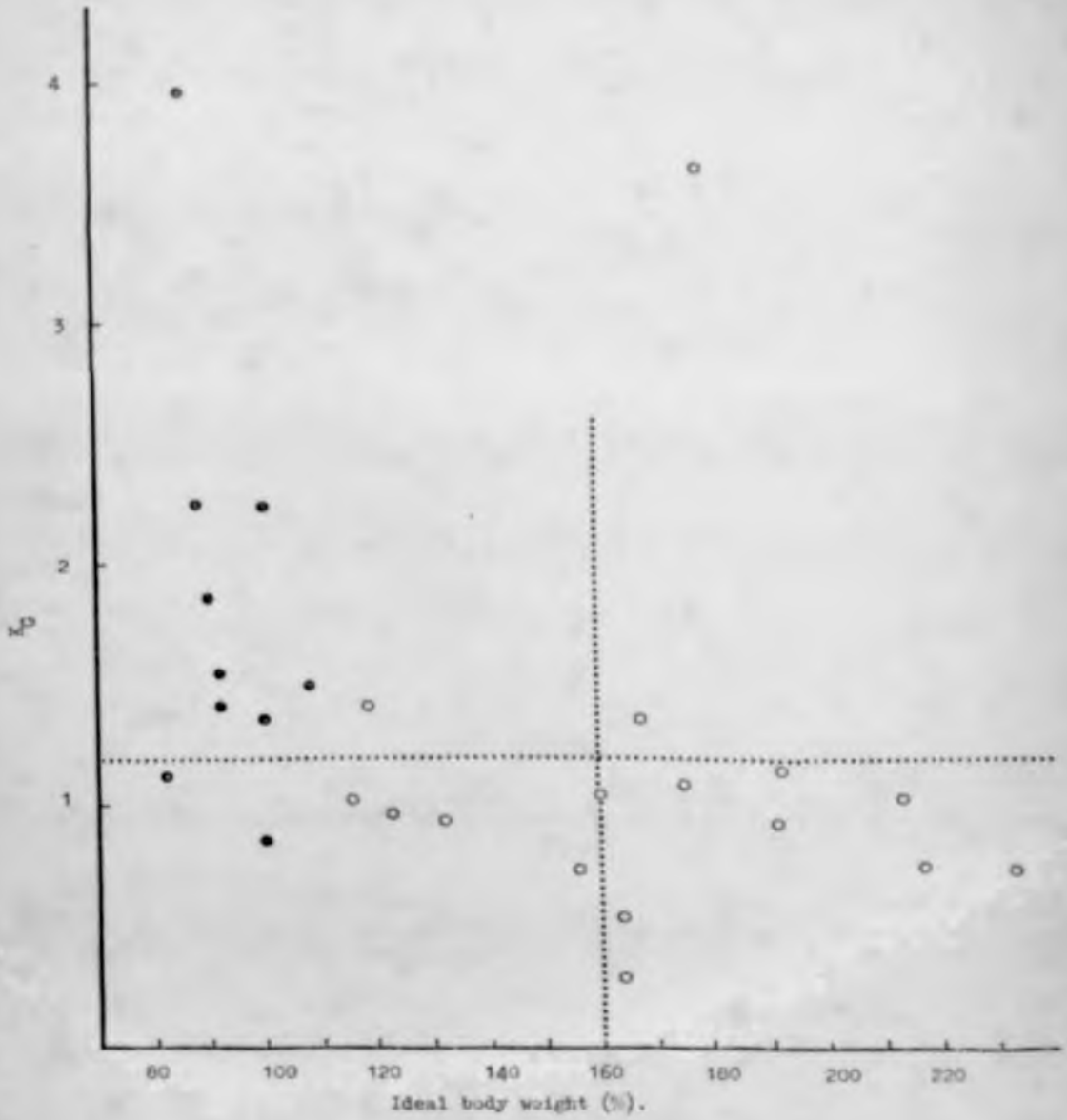

4
3
2
1
K_D
60
100
120
140
160
180
200
220
Ideal body weight (%).

Figure 25. Correlation between K_{G+I} value and % ideal body weight in the obese patients (○) and normal control subjects (●).

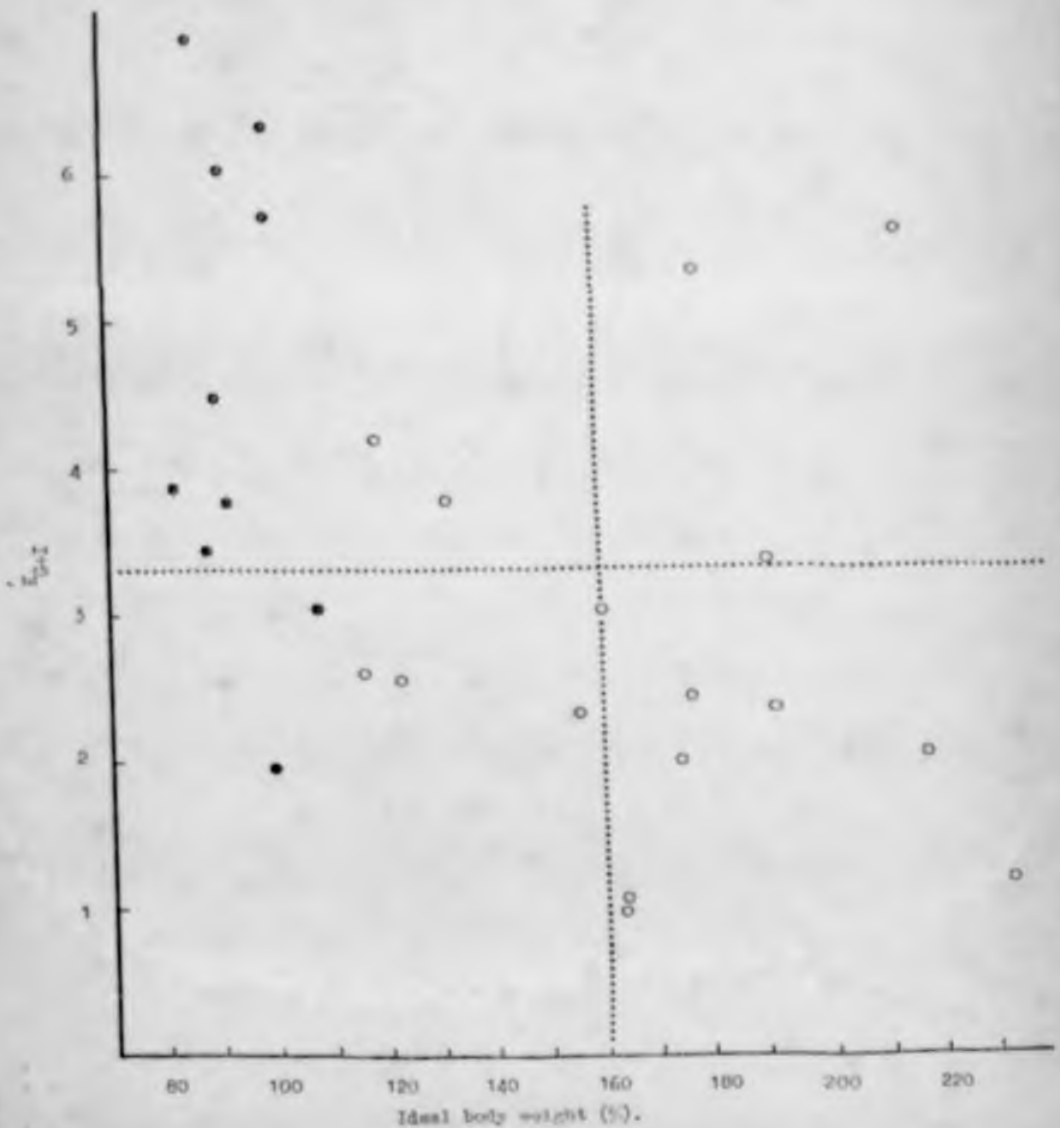
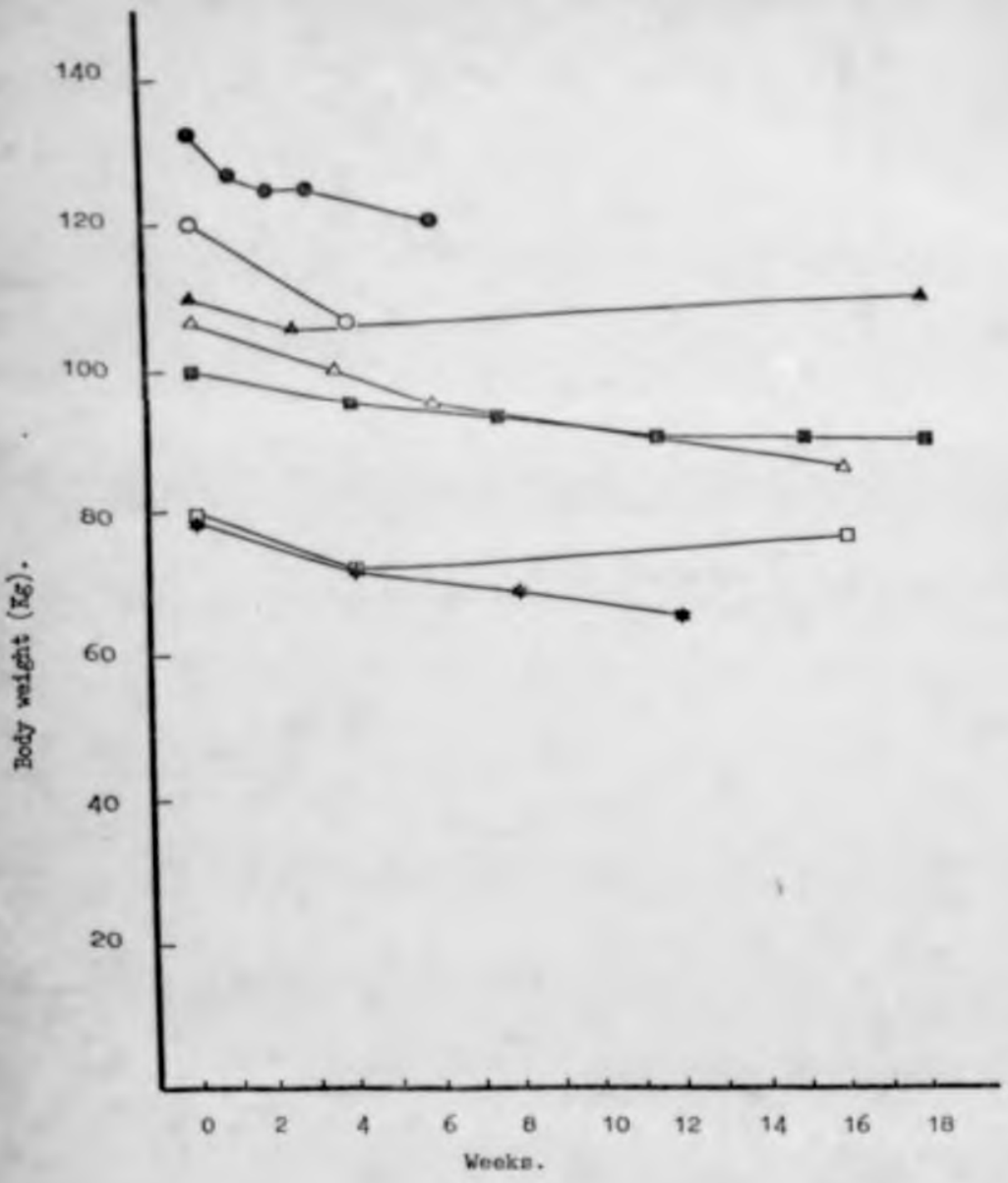

6
5
4
3
2
1
80
100
120
140
160
180
200
220
Ideal body weight (%).

Figure 26. Weight loss during dietary restriction in some obese patients.

(●) MC.
(○) AR.
(▲) DR.
(△) KY.
(■) GG.
(□) CW.
(●) AB.


140
120
100
80
60
40
20
Body weights (Kg).
0
2
4
6
8
10
12
14
16
18
Weeks.

Dr. P. Garlick and Dr. G. Clugston (using radioactive tyrosine and leucine), and for this reason drug therapy was not usually employed except for a very small number who received thyroxine; b. our study of glucose homeostasis.

There was no statistical correlation between the weight loss in the patients (kg/day) and the type of diet during dietary restriction. There was also no statistical correlation between weight loss and K_G or K_{G+1} values. There was also no statistical correlation between weight loss and fasting values of plasma insulin or peak plasma insulin during oral GTT and iv GTT (Table 17).

Fig. 26 shows body weight changes during dietary treatment in some patients showing different patterns of weight loss.

TABLE 17.

The effect of various dietary restrictions in relation to K_G, K_{G+I} and plasma insulin values in some obese patients. The patients' numbers are the same as those given in Table 11.

Patients	Age (years)	K_G, K_{G+I} (%/min)	INITIAL DIET MJ/day	Duration (days)	Weight loss (Kg/day)	MAINTENANCE DIET MJ/day	Duration (days)	Weight loss (Kg/day)	INITIAL WEIGHT Kg	% ideal weight*	INSULIN (μunit/ml) Fast	Peak in oral GTT	Peak in ivGTT
23. KY	37	3.65 5.35	1.3	26	0.27	4.2	90	0.14	107	178	26		175
24. CW	53	1.41 4.20	1.3	33	0.21	2.1	33	0.11	80	119	15		28
25. AD	68	0.97 2.56	1.3	27	0.22	2.1	73	-0.05	79	123	15		34
26. AR	45	0.54 1.04	1.3	30	0.43	ILEO-JEJUNAL BY-PASS			120	164	24		55
27. VR	53	0.74 2.31	1.3	14	1.14	2.1	17	0.18	96	156	15		48
14. MC	29	1.15 2.34	1.3	18	0.44	4.2	18	0.22	133	192	25	193	86
15. DR	33	1.09 2.00	1.3	10	0.40	4.2	153	-0.03	110	175	46	241	308
17. MR	17	1.05 3.01	2.1	13	0.46	Became overweight again soon after discharge.			90	160	Not measured		
18. MB	47	0.76 2.04	2.1	30	0.37	2.1	30	0.13	138	217	36	144	100
19. BC	42	1.03 2.67	2.1	20	0.20	2.5	30	0	86	116	Not measured		
20. FH	22	0.94 3.75	2.1	30	0.23				75	132	13	118	96
28. GG	51	0.29 0.97	3.3	30	0				100	164	7		94
29. PR	31	0.93 3.38	3.3	30	0				123	191	Not measured		
16. HG	21	1.04 5.68	3.3	20	0				135	213	15	107	102
6. DB	58	Not measured	3.3	60	0.07	3.3	45	0.04	105	156	14	57	

*Compared with the data from Geigy Scientific Tables, 1970.

CHAPTER IV
DISCUSSION

The results show the familiar findings of an impairment in glucose tolerance in obesity (Paullin and Saul, 1922; Beck et. al. 1964; Perley and Kipnis, 1966; Chiles and Tzagournis, 1970; Rabinowitz, 1970), and that this impairment is usually accompanied by hyperinsulinaemia.

A decrease in insulin sensitivity in respect to carbohydrate metabolism was present in these patients as shown by high blood glucose in the presence of high plasma insulin concentrations during the oral GTT (Fig. 18A; Fig. 18B and Table 13). Similarly, the iv GTT in the obese patients showed that lower K_G values were usually related to higher insulin concentrations (Fig. 19A and 19B).

During iv GTT, the obese patients showed a significantly lower mean value of K_{G+1} than did the normal controls ($p < 0.01$), (Table 15). The value of K_{G+1} is a measure of insulin sensitivity (in respect to carbohydrate metabolism), (Franckson et. al. 1966; Heard and Henry, 1969 a). Glucose tolerance (K_G value) was significantly correlated to insulin sensitivity ($p < 0.001$) but not to the actual concentration of plasma insulin (Fig. 21, Fig. 19A and Fig. 19B). These results confirm similar findings reported in malnourished dogs on the significance of insulin sensitivity rather than insulin concentration in determining glucose tolerance (Turner, 1966; Heard and Turner, 1967; Heard and Henry, 1969 a).

'Insulin sensitivity' or 'Insulin resistance' are terms which have usually been used in the context of carbohydrate metabolism, (Franckson et. al. 1966; Heard and Turner, 1967; Heard and Henry, 1969 a').

Insulin resistance in respect to fat metabolism probably also occurred in the obese patients. Most reports show that obese subjects have slightly elevated plasma NEFA. In the present small series, however, there were no significant differences between the obese patients and control subjects in the values of fasting plasma NEFA (Fig. 18C), nor in the fall of plasma NEFA concentrations during oral GTT (Fig. 18C) and iv GTT (Kg; Table 15). In all cases, however, the observed 'normal' values of NEFA in the obese subjects were accompanied by higher concentrations of plasma insulin (Fig. 18B); implying an overall resistance of fat metabolism to insulin.

Insulin inhibits lipolysis (Kipnis, 1965); on the other hand, insulin promoted glucose uptake and lipogenesis (Jeanrenaud and Renold, 1969). Either will result in the fall in plasma NEFA concentration. Only a very low level of insulin is needed to inhibit lipolysis, and once this level has been exceeded (Kipnis, 1965) any further effect of insulin on NEFA levels would be expected to operate via enhanced glucose uptake and reesterification of NEFA. The insulin resistance reflected in NEFA: insulin relationships might therefore be in large part another measure of insulin resistance in respect to glucose metabolism. Kipnis (1965) also showed that growth hormone administration resulted in an apparent dissociation between muscle and adipose tissue in response to insulin, and he discussed the question of whether this is due to different organ sensitivity to insulin or different responses of glucose uptake and inhibition of lipolysis.

Passmore, Malkiejohn, Dewar and Thow (1955) observed that weight gain in thin young men was less than expected when they were subjected to an overfeeding for 14 days. Whereas overfeeding in two fat young women resulted in a greater weight gain than that which was found in these thin men (Passmore, Swindal and El Din, 1963). Not only are obese people still susceptible to increased energy intake, but they also seemed to have lost the capacity to burn the extra energy in the same way as thin people do. It is interesting to note that Rolly (1921), more than fifty years ago showed that the specific dynamic action of food stuff was reduced to practically zero after the development of obesity. 'Specific dynamic action' is the term used to describe the effect of food in raising the metabolic rate above the value when fasting.

All obese patients must at some time eat in excess of their requirements in order to tip the energy balance and put on weight. This early stage could be described as an 'active' obesity, and reflects a 'normal' subject who eats to excess. He may, therefore, still have a normal insulin sensitivity (in respect of carbohydrate and fat). 'Active' obesity is represented here by the similarity between controls and some obese subjects under 160 per cent ideal body weight in the values of fasting insulin (Fig. 22B) and peak insulin during oral GTT (Fig. 23A) and iv GTT (Fig. 23B). Some of these patients, however, had started to have higher values of fasting insulin, peak insulin during oral GTT and iv GTT. Interestingly, most of them had already shown a decrease in iv glucose tolerance (K_G, Fig. 24) and insulin sensitivity (K_{G+I}, Fig. 25). This group may represent a transition period before they go into a further stage, i.e. 'passive' obesity. 'Passive' obesity could be represented here by

the obese subjects above 160 per cent ideal body weight showing predominant pictures of high fasting insulin concentrations (Fig. 22B), high peak insulin during oral GTT (Fig. 23A) and iv GTT (Fig. 23B), low K_G values (Fig. 24), and low insulin sensitivity (K_{G+I}) (Fig. 25).

Some apparently 'normal' subjects showed very high values for K_G and K_{G+I} (Figs. 21, 24 and 25). It is speculated that these subjects may be in an early phase of 'active' obesity. This speculation is based on the similar pattern of changes in K_G and K_{G+I} found in experimental animals fed diets deficient in protein (Heard and Turner, 1967; Heard and Stewart, 1971). In all cases, early signs of hypersensitivity to insulin were later followed by an impairment of glucose tolerance and diminished insulin sensitivity. However, such speculation requires further investigation.

Our results are in line with the view that 'gorging' should be avoided, especially if the diet is rich in carbohydrate. 'Gorging' leads to a greater increase in lipogenesis (Gwinup, Byron, Roush, Kruger and Hanwi, 1973; Bray, 1972). This effect would be accentuated in those who are already obese and in whom any carbohydrate intake (oral or iv) leads to very high levels of plasma insulin (Table 12, Figs. 18B, 23A, and 23B).

It is very difficult to assess the effect of dietary restriction on body weight in these patients. It depends more on the willpower to stick to the diet and the mental attitude of the individual. Some followed the diet restriction properly, some did not, resulting in an inability to show any weight loss. As the patients primarily came for treatment, the type of diet may, therefore, differ from subject to subject.

None of the biochemical parameters measured in this work showed any predictive value in respect of effectiveness of dietary therapy.

This may seem surprising if obesity is to be regarded not as a 'disease', but rather as a 'gift'. The genetic development of obesity is not at all well understood in man. The genotype for obesity was assumed to be present since early human population (Montagu, 1966). To ancient people in the paleolithic times, or perhaps even at present, in areas where food could sometimes be scarce, the ability to become obese, would have provided greater reserves of food supply upon which to draw in times of scarcity.

PART IV

GLUCOSE HOMEOSTASIS

IN

OLD AGE

CHAPTER I
INTRODUCTION

A. Ageing Process:

In the past century, man's life expectancy has increased considerably (Department of Health and Social Security, England, 1976), through the advancing knowledge of modern medicine. This has been in two main areas: in preventive medicine, through improvement of the environmental sanitation, better management not only in maternal and child health care, but also the health of the population in general; and in the field of curative medicine, especially following the discovery of antibiotics. While the medical successes are considered a good thing, the results expose us to different kinds of new problems. The increasing number of old people in the community emphasizes the importance of seeking better knowledge of ageing processes, and better attitudes in caring for and understanding old people.

The word 'ageing' means to 'grow old' (Oxford Concise English Dictionary). However, in biological studies, this term could have many different connotations. Bellamy (1970) gave a definition of ageing as a "decline in the ability of the homeostatic system of the body to cope with fluctuations in the external world". In the process of growing old, there is a steady decline of physiological functions, in conjunction with a decline in anatomical, biochemical and hormonal features and performance (Norris and Shock, 1966). A decrease in physical activity with or without painful feelings in the bones and joints, a decrease in food intake, emotional disturbances and many other physical or psychological inadequacies show that old age and stress are closely associated. Old age, therefore, is not merely the passing of

years, but many malfunctions added together become a generalized picture of old age, and, whatever the original cause, some elements of stress either physical or psychological or both are always present in old age (see Part I, Chapter IV, Stress).

8. Ageing and Carbohydrate Metabolism

Increasing age has always been associated with a decline in glucose tolerance, either oral GTT (Grant, 1960; Burch and O'Meally, 1967; Chlouverakis, Jarrett and Keen, 1967), or the iv GTT (Streeten, Gerstein, Marmor and Daisy, 1965; Franckson et. al. 1966; Cockford, Herbeck and Williams, 1966). The decline in iv glucose tolerance is associated with a decrease in insulin production (Cockford et. al. 1966). However, in the oral GTT, some have claimed that the prolonged hyperglycaemia resulted in higher levels of plasma insulin (Chlouverakis et. al. 1967; Andres, Pozefsky, Swerdloff and Tobin, 1970).

It is important to try to decide whether we should apply the same criteria in diagnosing and treating diabetes in the elderly as are used for young adults. Most authorities would suggest making 'allowances' for age (WHO Expert Committee on diabetes mellitus, 1965; Andres, 1973). This seems reasonable if only because elderly people probably have diminished food intake and sometimes have impaired absorptive capacity (Webster and Leeming, 1975), and, therefore, do not, under normal circumstances, load their systems to the same extent as young people. In tolerance tests on the other hand, the same load is given to young and old alike.

Andres (1973), in his review claimed that impaired glucose tolerance (intravenous test) in the elderly was due, not to impaired sensitivity to insulin, but to

deficient insulin production. However, he also acknowledged that there was not yet any clear consensus between various workers about plasma insulin levels during the oral GTT.

In all the other situations we have studied, insulin sensitivity played as large a part (if not more) in determining glucose tolerance as did insulin levels. Therefore, we sought to differentiate between insulin levels and insulin effectiveness in the elderly.

Because of the gastro-intestinal tract changes in old people (Agate, 1963), including possible impairment in intestinal insulinogenic capacity, and also the inconclusive reports on insulin response during oral GTT, it was also desirable to compare the effects of oral and iv glucose loads on blood glucose and plasma insulin levels.

The decrease in glucose tolerance in old age could be due either to the decreased uptake of glucose by the liver and peripheral tissues, or due to the inability of insulin to stop liver gluconeogenesis. Therefore, we also compared the gluconeogenic capacity of the liver between the geriatric patients and normal control subjects. This was carried out by intravenous injection of l-alanine (Wise, Handler and Felig, 1973; Mestyan, Schultz and Harvath, 1974) and measuring the rise in blood glucose concentration, the changes in plasma insulin and glucagon, and the disappearance of injected l-alanine from the circulation. The % removal rate of injected l-alanine (K_A) was calculated the same way as these calculations for K_G and K_{G+I}. (See Part III, Chapter II, Methods).

C. Intravenous l-alanine Tolerance Test and a Measure of Gluconeogenesis:

Gluconeogenesis is very difficult to measure. A number of different 'tolerance' tests have been used in which substrates capable of serving as glucose precursors have been administered. These include dihydroxyacetone or glycerol (Fernandes, Koster, Grose and Sargedrager, 1974), fructose (Pagliara, Karl, Keating, Brown and Kipnis, 1972), pyruvate (Moorhouse, 1964), Lactate (Kreisberg, Pennington and Boshell, 1970) and alanine (Felig, Marliss, Owen and Cahill, 1969; Wise et. al. 1972; Mestyán et. al. 1974). Alanine is of special importance. It has been suggested that alanine uptake by the liver amounts to 50 per cent of the total amino acid uptake by this organ (Felig, Wahren, 1971). This is accounted for by the fact that alanine is also released by the muscles in amounts far exceeding the alanine content of muscle protein. Protein contains only 5 to 7 per cent alanine (Kominz, Hough, Symond and Laki, 1954), yet alanine accounts for about 30 per cent of total amino acids released from muscle (London, Foley and Webb, 1965).

This production of alanine requires pyruvate to serve as an amino group acceptor in alanine amino transferase reactions. Pyruvate is derived from glucose by glycolysis in muscle. In the liver, pyruvate is regenerated from alanine, and then serves as a gluconeogenic precursor to replenish the glucose removed from the blood by muscle and other tissues, and so completes the 'glucose-alanine cycle' (Felig, 1973). The success of this process, therefore, depends upon the availability of glucose precursors and the efficiency of the liver in converting alanine into glucose.

Alanine is known to promote both gluconeogenesis and glycogenolysis through stimulation of glucagon production (Wise, Hendler and Felig, 1972). Thus, the rise in blood glucose concentrations after intravenous alanine administration cannot be attributed to gluconeogenesis alone, unless liver glycogen stores have been depleted by fasting. Ideally, the subject should be fasted 24 to 48 hours to be sure of more or less complete exhaustion of liver glycogen stores (Hultman and Nilsson, 1971). However, such a procedure was not possible on geriatric patients. With this limitation taken into account, geriatric patients and normal control subjects were given an intravenous alanine test after fasting for 8 hours from midnight.

CHAPTER II
MATERIAL AND METHODS

A. Subjects:

1. Patients:

Patients from the geriatric wards, St. Pancras Hospital, were studied. They were asked whether they would be willing to consider participating and the kinds of tests involved were explained in detail. Consent (oral or written) was thus obtained from each patient and control subject participating in the study. These patients were presented to us as non-diabetics, and any subjects who had a fasting blood glucose concentration higher than 6.11 m mol/l (110 mg/100 ml) (working party, College of General Practitioners, 1963) were excluded. Their medical records showed that none of these patients had any glycosuria, and all received normal hospital diet.

The first three tests (oral GTT, iv GTT and iv GITT) were carried out on 23 patients (Table 18). Their mean age was 79 ± 1.1 years, their mean body weight was 56 ± 2.2 kg. For three to four days before the tests, each of them received an extra 150 g carbohydrate/day. This was to ensure that all the subjects had a reasonable carbohydrate intake before the tests were done. This extra carbohydrate was in the form of 150 g/600 ml solution, consisting of 50 per cent orange juice and 50 per cent Calorean (Scientific Hospital Supplies Ltd., England).

Unfortunately, some of the patients were not able to participate in the fourth test, the intravenous (iv) alanine tolerance test. They were either unwilling or were

Table 18

List of geriatric patients who participated in oral GTT, iv GTT and iv GITT

Subject	Sex	Age (years)	Body weight (kg)	Previous diseases
GV	F	88	50	Gangrene of the toes, amputation
WT	F	87	51	Myocardial infarct
CH	M	74	69	Atherosclerosis
OV	M	83	69	Myocardial infarct
VL	M	83	60	Left hemiplegia
CR	M	74	73	Prostatectomy
ED	F	77	71	Kyphosis
EL	F	81	50	Rheumatoid arthritis
PS	F	77	60	Myocardial infarct
SH	F	76	50	Mitral stenosis
FD	M	73	62	Myocardial infarct
BJ	F	78	57	C.V.A., right hemiplegia
BX	F	82	43	Dementia
OT	M	79	37	Dementia
FL	F	82	47	C.V.A.
MR	M	78	67	C.V.A., paraplegia
EN	M	86	61	Prostate hypertrophy
MG	F	74	62	Cervical spondylitis
MC	M	78	55	C.V.A., right hemiplegia
PG	M	77	53	Mild left ventricular failure
DW	F	73	67	Dermoid tumour in the pelvis
PN	F	78	34	Carcinoma cervix
ST	M	68	49	C.V.A., right hemiplegia

unable to do so because of their clinical conditions. Therefore, a few more patients had to be added to make up a reasonable number. Fig. 19 shows the list of 8 geriatric patients who had the iv alanine tolerance test. Their mean age was 78 ± 1.6 years and their mean body weight was 52 ± 3.9 kg.

Due to the advanced age of all geriatric patients studied, we were unable to get a reliable measurement of their heights, thus also unable to calculate their % ideal body weight, and these measurements were not included in this report.

2. Normal subjects:

Normal control subjects for the oral GTT, iv GTT and iv GITT, were the same 14 young, non-obese subjects used as controls in the study on obesity (see Part III, Chapter II, Normal Subjects), (Table 12).

As with the patients, so also with the control subjects; some of the original volunteers were unwilling or not available to participate in further tests. More new volunteers were, therefore, recruited to make a reasonable number of control subjects for the iv alanine tolerance test.Table 20 shows the list of 8 control subjects for the iv alanine tolerance test. Their mean age was 28 ± 1.5 years, their mean height was 172 ± 3.5 cm, their mean weight was 65 ± 4.2 kg, and their mean %

Table 19

List of geriatric patients who participated in the intravenous alanine tolerance test

Subject	Sex	Age (years)	Body weight (kg)
JG	M	78	67
FL	F	82	47
EL	F	81	46
CL	F	82	41
AD	F	77	56
HE	M	71	63
BJ	F	78	57
JL	F	71	35

Table 20

List of control subjects who participated in the intravenous alanine tolerance test

Subject	Sex	Age (years)	Height (cm)	Weight (kg)	% ideal weight*
AT	M	34	181	76	94
SM	F	32	169	58	90
RA	M	31	170	70	97
JL	M	27	188	83	99
WS	M	32	159	55	86
SB	F	24	165	54	95
VP	M	23	178	74	102
CN	F	24	162	52	95

*Compared with the data from Geigy Scientific Tables (1970)

ideal body weight was 95 ± 1.8%.

B. The Tests:

1. Oral GTT, iv GTT and iv GITT:

The methods and procedures used in these tests, the calculation of the average glucose or insulin concentrations and insulin:glucose molar ratio during oral GTT; the calculation of K_G and K_F in the iv GTT, and K_{G+1} in iv GITT, have all been described (see Part III, Chapter II, Methods).

Although all geriatric patients showed low values of K_G and K_{G+1}, a few of the first patients tested showed hypoglycaemic signs a few minutes after the end of the iv GITT. For that reason, all later patients received an extra 20 - 30 ml of 50 per cent glucose solution intravenously at the end of the iv GITT, and the nursing staff was asked to provide food and drink as soon as possible.

2. Intravenous alanine tolerance test (iv ATT):

This test was devised as a measure of the gluconeogenic capacity of the liver. The subjects were fasted from midnight and the test was carried out in the morning.

A butterfly needle attached to an antecubital vein of one arm was used for drawing blood sample. Two fasting blood samples (10 minutes apart) were taken at about 9.30 a.m. Through the same needle, a solution of 10 per cent L-alanine (0.15 g/kg body weight) (Wise et. al. 1973) was injected. The alanine solution (see section B.3. below) was injected within 5 minutes, and serial blood samples through the same needle, were taken at 5', 10', 20', 30', 60' 90' and 120' after

the injection. The procedure for handling blood samples was similar to those in the oral GTT, iv GTT or iv GITT. Except that for glucagon estimation, that 4.5 ml of blood was also taken and added to another heparin tube containing 0.5 ml cold solution of Trasylol (10,000 KIU/ml, Bayer) and after immediate centrifugation the supernatant was frozen rapidly in solid CO_2 and stored at -20° C until the day of analysis.

Although none of the subjects complained of having any clinical complications from this test, a few of them however, experienced a slight stomach discomfort for several minutes following the injection of alanine.

3. 10% l- alanine solution:

Alanine solutions were made by dissolving sterile l-alanine (Kabi-Vitrum Pharmaceutical, Sweden) in sterile distilled water as a 10% solution. This solution was buffered to a ph between 7.0 and 7.3 with sodium hydroxide. It was then passed through a millipore filter and autoclaved at 120° C for 10 minutes (Mastyan et. al., 1974).

This alanine solution was made by the Department of Pharmacy, University College Hospital. We attempted to make our own solutions, but the Hospital refused to allow us to do the test unless the solution was made by the Hospital's own Pharmacy and from time to time the quality control of the solution was checked.

C. Analytical Methods:

Analysis for blood or plasma glucose, plasma NEFA, plasma insulin and plasma glucagon were carried out according to the procedures reported in the previous study (see Part II, Chapter II, Methods'. Plasma alanine was analysed by an enzymatic

method, using l-alanine dehydrogenase (Reilly, 1975) with reagents supplied by the Boehringer Corporation, London Ltd.

D. Assessment of the Data:

Student's t test was used for comparing the patients data with those of the controls.

CHAPTER III
RESULTS

A. Oral GTT:

The oral GTT was carried out in 23 geriatric patients and in 13 normal control subjects (Tables 12 and 23).

1. Blood glucose:

The mean blood glucose concentration was 4.92 ± 0.11 m mol/l (88.6 ± 2.02 mg/100 mg) in the geriatric patients against 4.62 ± 0.16 m mol/l (83.2 ± 2.92 mg/100 ml) in control subjects (Table 21, Fig. 27). The difference was not significant ($p > 0.05$), but during the test the concentrations of blood glucose in the patients continued to rise after 30' and the peak was at 90', whereas the peak in the controls was at 30'. Blood glucose concentrations in the patients were higher than in control subjects throughout the rest of the test. And the difference reached significant levels after 60' ($p < 0.01$, and from 90' $p < 0.001$) (Fig. 27A).

The average value for blood glucose concentration during the oral GTT was 7.72 ± 0.28 m mol/l (139.1 ± 5.05 mg/100 ml) in the geriatric patients against 5.71 ± 0.18 m mol/l (102.8 ± 3.25 mg/100 ml) in control subjects. The difference was significant ($p < 0.01$) (Table 21).

2. Plasma insulin:

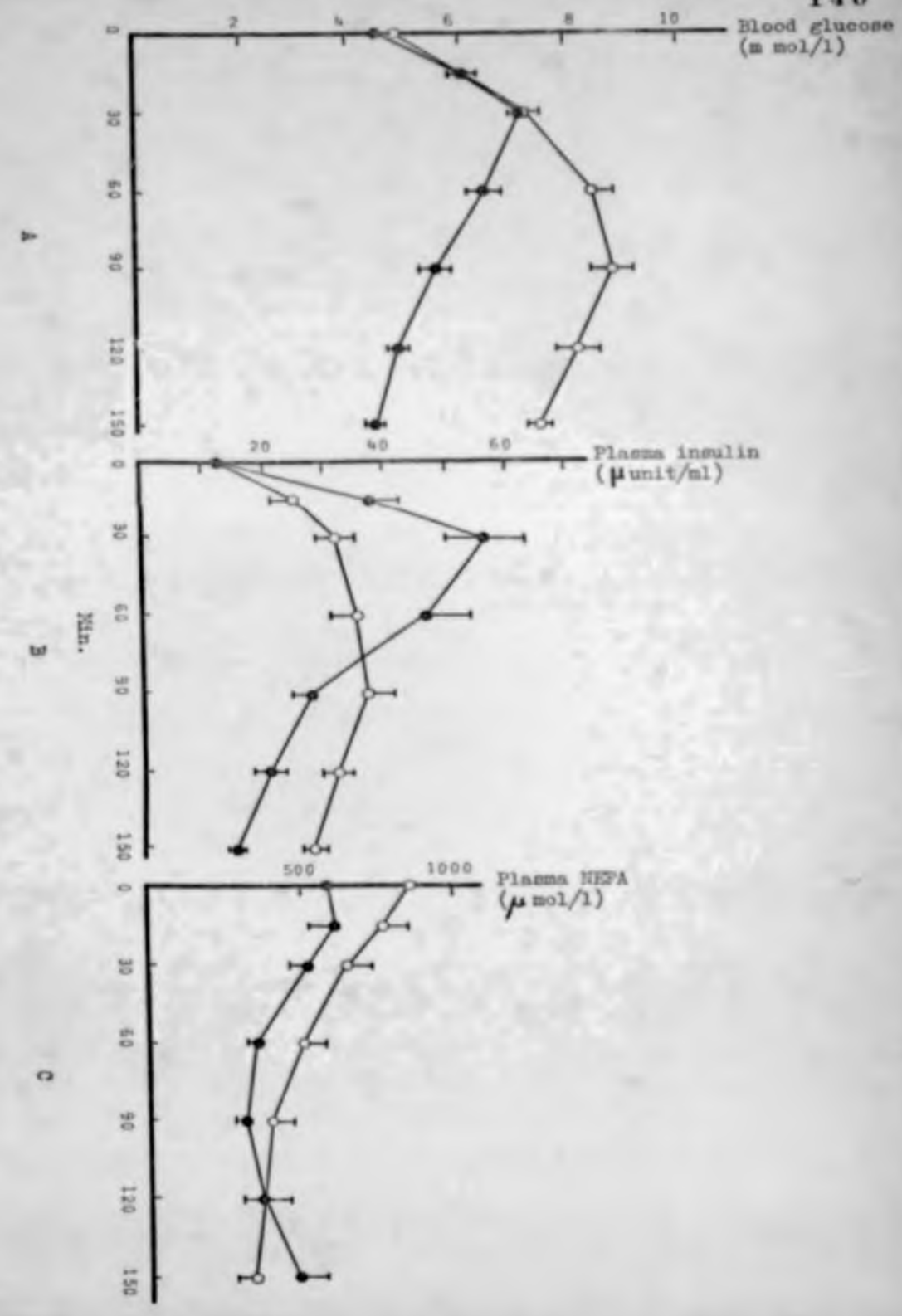
The mean fasting plasma insulin concentration was 12.3 ± 0.54 µunit/ml in the geriatric patients against 12.8 ± 1.31 µunit/ml in control subjects. The difference was not significant ($p > 0.05$) (Table 21). During the test the concentrations

Figure II. Oral GTT in 25 geriatric patients (O) and 13 normal control subjects (●).

A. Mean blood glucose values during oral GTT.
There were significant differences in the mean values at 60' ($p < 0.01$), and at 90', 120' and 150' ($p < 0.001$) between the two groups.

B. Mean plasma insulin values during oral GTT.
There were significant differences in the mean values at 30' ($p < 0.01$), at 120' ($p < 0.05$) and at 150' ($p < 0.01$) between these two groups.

C. Mean plasma NEFA values during oral GTT.
Except for the mean fasting levels ($p < 0.05$), the differences in the mean levels at various times during the test between the two groups were not significant.
(mean values of 12 geriatric and 7 normal control subjects).


Blood glucose
(m mol/l)
Plasma insulin
(μunit/ml)
Plasma NEFA
(μmol/l)
Min.
A
B
C

of plasma insulin in the geriatric patients did not rise as high as in the control subjects, but continued to rise after 30', and the peak was at 90'. Whereas the peak in the controls was at 30'. Plasma insulin concentrations in the patients were lower than in control subjects, up to 60' (significant difference at 30', $p < 0.01$). But, while the insulin concentration after 60' decreased in the control subjects, the insulin concentration in the patients remained high, and was significantly greater than in the controls at 120' and 150' ($p < 0.05$ and $p < 0.001$ respectively). (Fig. 27B).

The average value for plasma insulin concentration during oral GTT was 31.6 ± 2.52 μ unit/ml in the geriatric patients against 32.5 ± 3.51 μ unit/ml in control subjects. The difference was not significant ($p > 0.05$) (Table 21).

As also in the study of obesity (Part III, Chapter II, Patients) any overt diabetics (fasting blood glucose higher than 6.11 m mol/l) were excluded during the initial selection of patients. WHO (1965) further gave as a criterion for a 'normal' oral GTT, that the 120' blood glucose value should be less than 6.11 m mol/l (110 mg/100 ml) and gave the lower limit for diabetes as a 120' value of 7.22 m mol/l (130 mg/100 ml). Having regard to the advanced age (mean 79 ± 1.1 years) of these patients we arbitrarily chose 7.77 m mol/l (140 mg/100 ml) for an upper limit for 'non-diabetic' geriatric subjects. According to this new criterion, among 23 geriatric patients studied, 15 could be classified as 'diabetic' and 8 as 'non-diabetic' geriatrics (Table 22). Fortuitously, the dividing line of 7.77 m mol/l corresponded closely with the mean

Table 21

The mean values for fasting blood glucose and plasma insulin, average concentrations of blood glucose and plasma insulin, and insulin:glucose molar ratio during oral GTT, in geriatric patients and control subjects (± SEM). Number of observations in parenthesis.

	Mean fasting concentrations		Mean of the average value for 120 min.			10^6 x mean insulin: glucose molar ratio
	Blood glucose (m mol/l)	Plasma insulin (μ unit/ml)	Blood glucose (m mol/l)	Plasma insulin (μ unit/ml)	Plasma insulin (10^6 x m mol/l)	
Patients (23)	4.92 ± 0.11 ns	12.3 ± 0.54 ns	7.72 ± 0.28 **	31.6 ± 2.52 ns	227.0 ± 18.1 ns	29.8 ± 2.27 **
Controls (13)	4.62 ± 0.16	12.8 ± 1.31	5.71 ± 0.18	33.5 ± 3.51	240.4 ± 25.2	42.2 ± 4.50

* $p < 0.05$ ** $p < 0.01$ ns = not significant

120' blood glucose value for all 23 patients (8.12 m mol/l).

3. Insulin:glucose molar ratio:

The mean insulin:glucose molar ratio in the geriatric patients was (29.8 ± 2.27) x 10^{-6} against (42.2 ± 4.50) x 10^{-6} in control subjects. The difference was significant ($p = 0.01$) (Table 21).

4. Plasma NEFA:

There was a great range in the levels of plasma NEFA both in the geriatric patients and in control subjects. This applied to the fasting concentrations as well as to the concentrations during the test. The mean fasting plasma NEFA concentration in the geriatric patients was 866 ± 86 µ mol/l against 590 ± 68 µ mol/l in control subjects. The difference was significant ($p < 0.05$). Although the plasma insulin concentrations during the oral GTT were lower in the geriatric patients, there were no significant differences in the mean NEFA concentrations at any time during the test between the geriatric patients and the normal controls. The NEFA curve during the oral GTT in the patients mirrored their sluggish insulin curve, showing a delayed and prolonged reaction. Thus at 150' the mean NEFA concentration was lower in the patients than in the controls, since in the latter the NEFA concentration had started to rise again (Fig. 27C).

B. Iv GTT and Iv GITT:

The Iv GTT and Iv GITT were carried out in 23 geriatric patients and in 10 normal controls (Tables 23 and 12).

1. K_G values:

The mean K_G value in the geriatric patients was 0.98 ± 0.09 %/min against

Table 22

Mean blood glucose and plasma insulin concentrations during oral GTT in the 'diabetic' and 'non-diabetic' geriatrics and in normal control subjects (± SEM). Number of observations in parentheses.

Oral GTT

		Mean blood glucose concentrations (m mol/l)							Mean plasma insulin concentrations (μ unit/ml)						
		0'	15'	30'	60'	90'	120'	150'	0'	15'	30'	60'	90'	120'	150'
I	'Diabetic' geriatrics (15)	5.07 ± 0.15	6.37 ± 0.22	7.66 ± 0.28	9.24 ± 0.49	9.74 ± 0.52	9.07 ± 0.46	8.31 ± 0.43	12.9 ± 0.72	21.5 ± 2.27	31.9 ± 4.64	37.1 ± 5.28	35.7 ± 4.03	34.1 ± 4.01	28.8 ± 2.46
	I x II			*	**	**	***	**							
II	'Non-diabetic' geriatrics (8)	4.63 ± 0.08	5.77 ± 0.35	6.43 ± 0.44	7.08 ± 0.40	7.04 ± 0.37	6.35 ± 0.27	5.78 ± 0.38	12.4 ± 0.82	31.4 ± 10.18	32.0 ± 4.92	32.1 ± 5.37	40.0 ± 11.60	28.1 ± 2.45	25.9 ± 3.96
	I x III				***	***	***	***		**	**			*	***
	II x III					**	***	**			*				**
III	Control subjects	4.62 ± 0.16	6.15 ± 0.36	7.19 ± 0.30	6.40 ± 0.32	5.50 ± 0.29	4.81 ± 0.20	4.36 ± 0.19	12.8 ± 1.31	37.3 ± 5.39	56.3 ± 6.82	47.1 ± 6.97	27.8 ± 3.65	20.6 ± 2.94	14.8 ± 1.34

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

1.81 ± 0.28%/min in the control subjects. The difference was significant ($p < 0.001$) (Table 23).

There was no statistical correlation between K_G and fasting plasma insulin values during iv GTT in the combined group of geriatric patients and the normal controls (Fig. 28A).

There was also no correlation between K_G and peak plasma insulin values, in the geriatric patients and control subjects (Fig. 28B). However, a higher K_G was usually related to a lower peak plasma insulin concentration.

2. Peak insulin concentrations during oral GTT and iv GTT:

There were 23 geriatric and 9 normal control subjects who had both oral and iv GTT done.

The mean peak insulin concentrations during the oral GTT were higher than the mean peak insulin concentrations during iv GTT both in the geriatric patients and in the control subjects. The differences, however, were not significant ($p > 0.05$) (Table 24). Whereas in the control subjects the peak insulin concentrations during the oral GTT were always higher than the peak during the iv GTT, in the geriatric patients however, 8 out of 23 patients had peak insulin concentrations during the iv GTT higher than the peak during the oral GTT. This suggested that in these 8 geriatric patients there was some degree of small intestinal malabsorption. The mean peak insulin concentration during the oral test in the geriatric patients was 48.0 ± 5.34 μ unit/ml against 72.0 ± 7.26 μ unit/ml in the control subjects. The difference was significant ($p < 0.05$) (Table 24).

Table 23

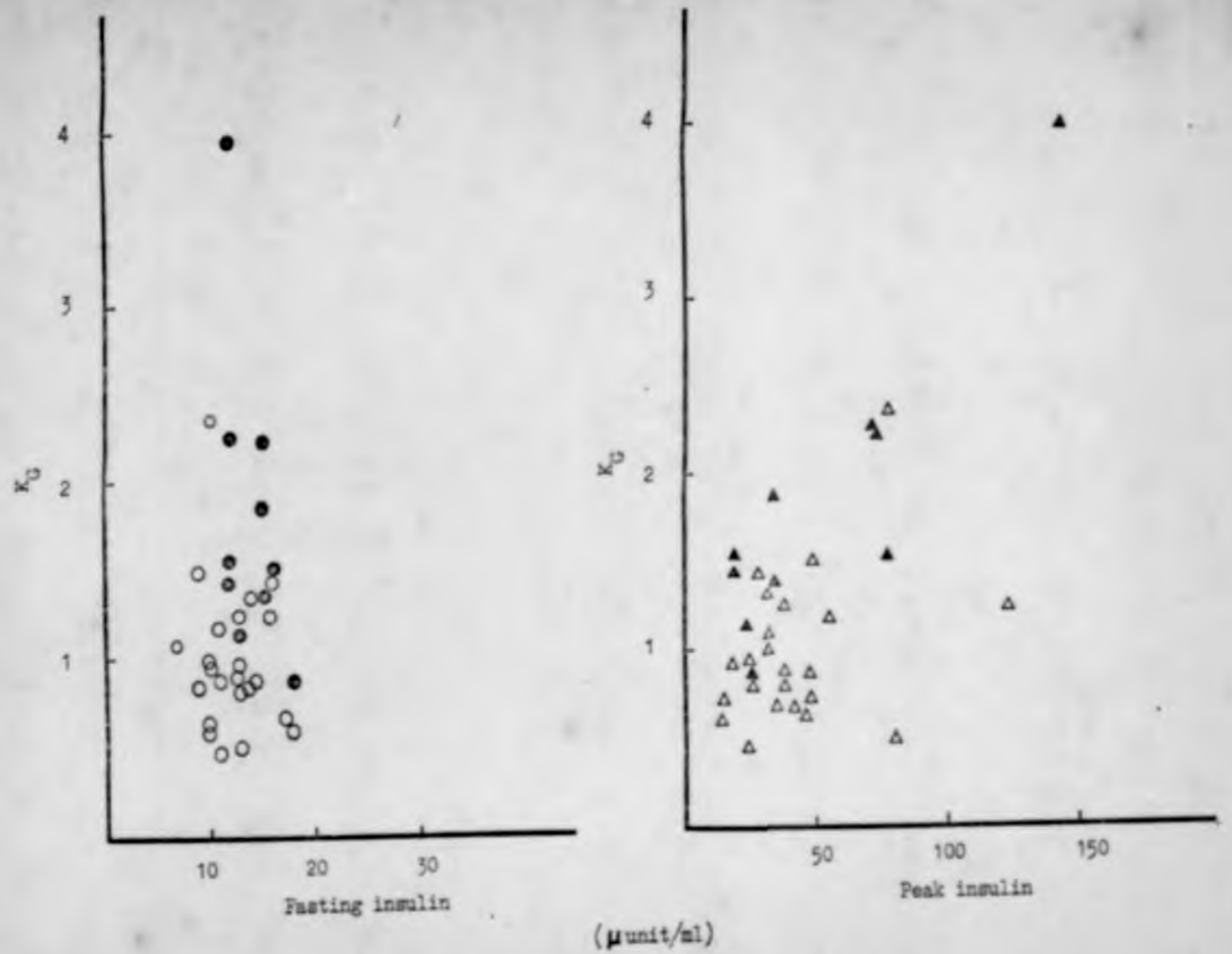
The mean values for K_G, K_{G+I}, K_F in the geriatric patients and in normal control subjects (± SEM). Number of observations in parenthesis.

	Mean Values		
	K_G (%/min)	K_{G+I} (%/min)	K_F (%/min)
Geriatric patients	0.98 ± 0.09 (23)	2.29 ± 0.16 (23)	2.21 ± 0.34 (10)
	***	***	ns
Control subjects	1.81 ± 0.28 (10)	4.55 ± 0.51 (10)	3.10 ± 0.73 (6)

*** $p < 0.001$; ns = not significant

Figures 28 A. Correlation K_G and fasting plasma insulin values during iv GTT in the geriatric patients (○) and normal control subjects (●).

28 B. Correlation between K_G and peak plasma insulin values during iv GTT in the geriatric patients (△) and normal control subjects (▲).


4
3
2
1
K_D
10
20
30
Fasting insulin
4
3
2
1
K_D
50
100
150
Peak insulin
(μunit/ml)

The mean peak insulin concentration during iv GTT in the geriatric patients was 41.6 ± 5.13 μ unit/ml against 54.0 ± 13.60 μ unit/ml in the control subjects. This difference was not significant ($p > 0.05$) (Table 24).

3. K_{G+I} values:

The mean K_{G+I} value in the geriatric patients was 2.29 ± 0.16%/minute against 4.55 ± 0.51 %/minute in the control subjects. The difference was significant ($p < 0.001$) (Table 25).

There was no correlation between K_{G+I} and fasting plasma insulin values in the geriatric patients nor in control subjects (Fig. 29 A).

There was also no correlation between K_{G+I} and peak plasma insulin values during iv GTT in the geriatric patients nor in control subjects (Fig. 29 B).

There was a highly significant correlation between K_g and K_{G+I} values in the combined geriatric patients and normal control subjects. ($r = 0.71$, $p < 0.001$) (Fig. 30).

4. K_g values :

The mean K_g value in the geriatric patients was 2.21 ± 0.34 %/minute against 3.10 ± 0.73 %/minute in the normal control subjects. The difference was not significant (Table 25)

Table 24

The mean peak insulin concentrations during oral GTT and iv GTT (± SEM), in geriatric patients and normal control subjects. Number of observations in parenthesis.

	Mean Peak Insulin Concentrations (μ unit/ml)			Ratio of oral : iv peak insulin
	Oral GTT		Iv GTT	
Geriatric patients (23)	48.0 ± 5.34	ns	41.6 ± 5.13	1.15
Controls (9)	72.0 ± 7.36	ns	34.0 ± 13.60	1.39

* $p < 0.05$; ns = not significant

Figures 29 A. Correlation between K_{G+I} and fasting plasma insulin values during iv GTT in the geriatric patients (O) and normal control subjects (●).

29 B. Correlation between K_{G+I} and peak plasma insulin values during iv GTT in the geriatric patients (△) and normal control subjects (▲).

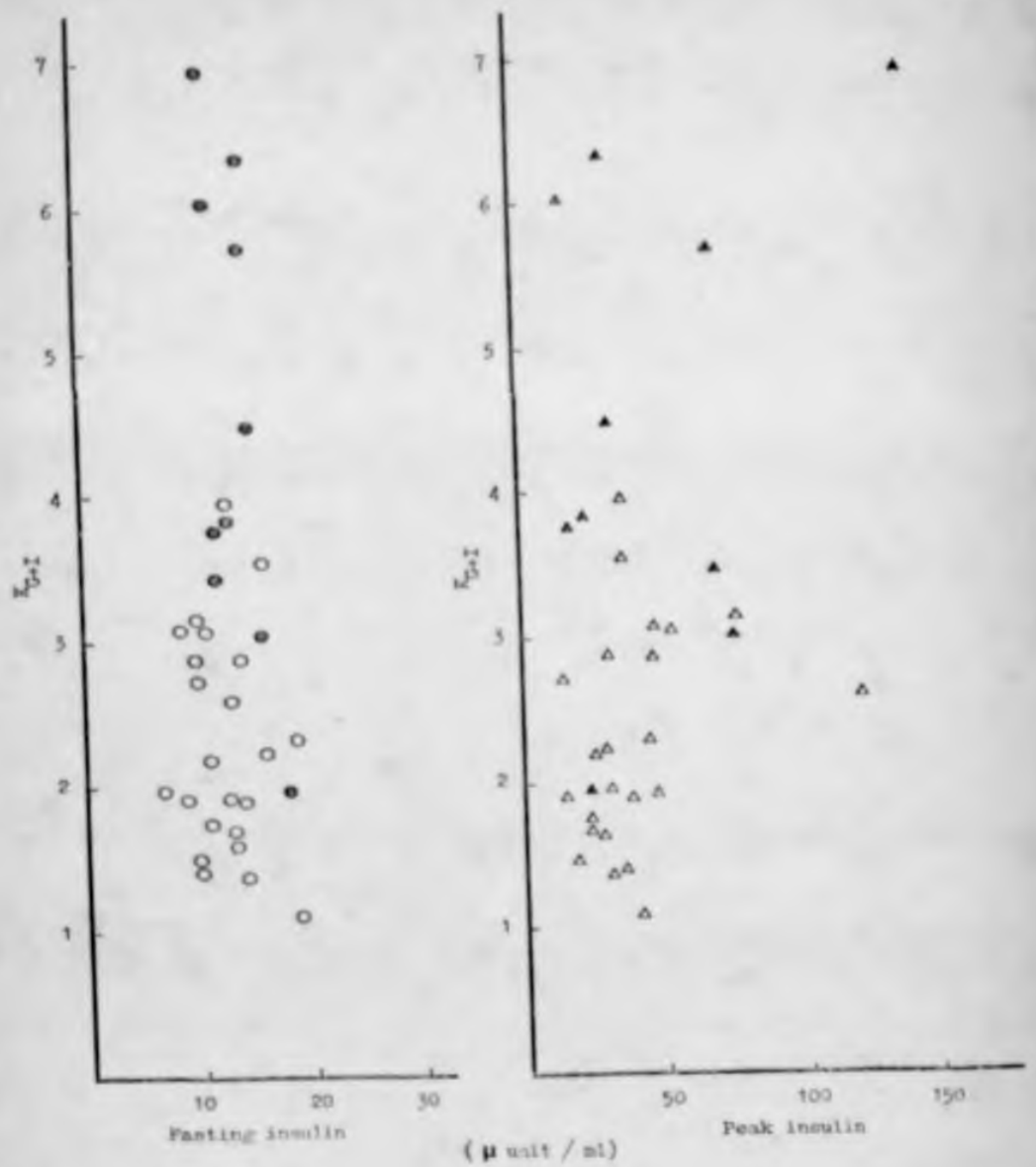
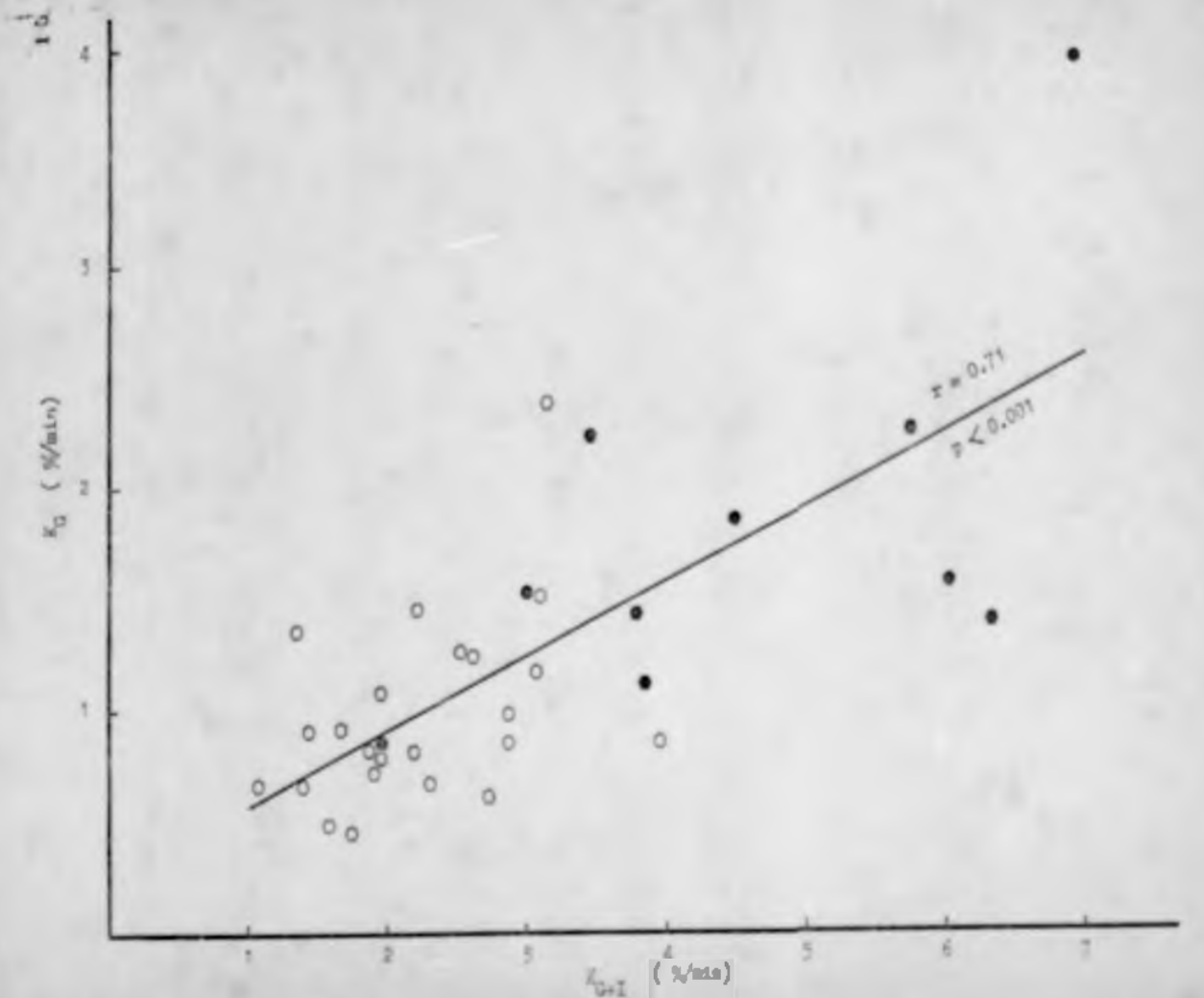

7
6
5
4
3
2
1
10
20
30
Fasting insulin
50
100
150
Peak insulin
(μ unit / ml)

Figure 32. Regression line between the values of K_C and K_{C+I} in the combined group of geriatric patients (O) and normal control subjects (●).


$\dot{V}_O$ (%/min)
$\dot{V}_{O+I}$ (%/min)
r = 0.71
P < 0.001

C. Iv Alanine Tolerance Test (Iv ATT):

1. Blood glucose:

The mean fasting blood glucose concentration was 4.77 ± 0.17 m mol/l (85.9 ± 3.02 mg/100 ml) in the geriatric patients against 4.92 ± 0.07 m mol/l (88.6 ± 1.32 mg/100 ml) in the control subjects (Fig. 31A). The difference was not significant ($p > 0.05$). During the test in the geriatric patients, blood glucose concentration continued to rise after 10' and reached a plateau only at 30', whereas the blood glucose concentration in the control subjects only rose and reached a peak at 10' before falling again. Blood glucose concentrations in the geriatric patients were significantly higher than in the control subjects from 30' onwards ($p < 0.01$) (Fig. 31A).

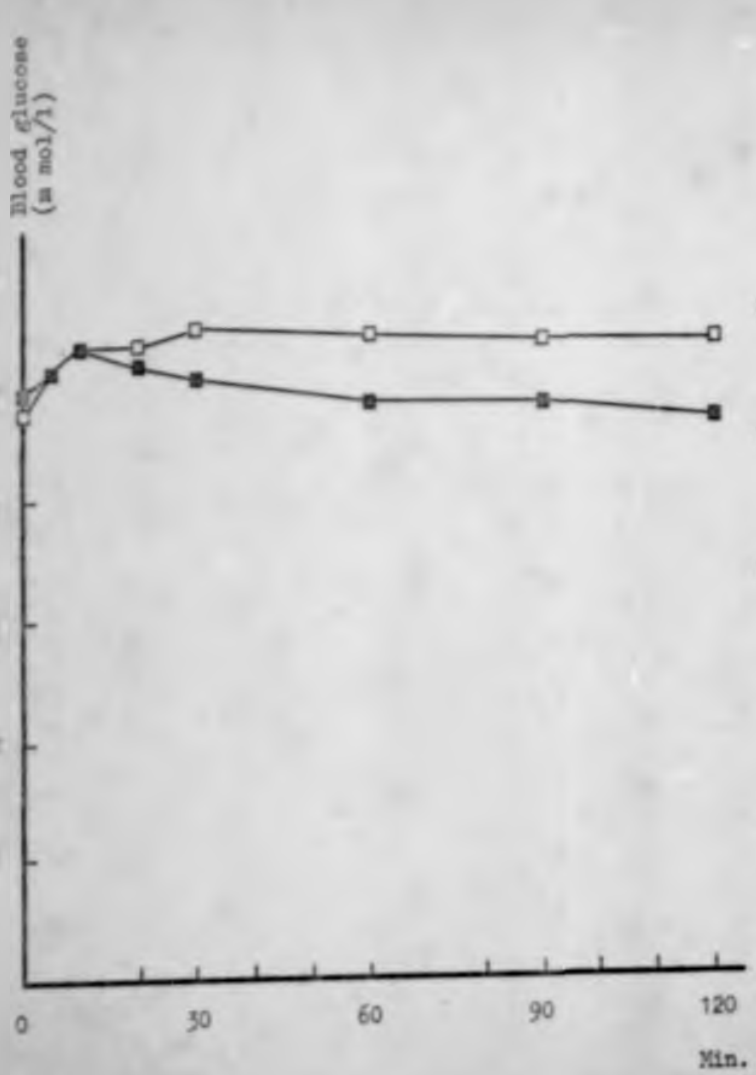
2. Plasma insulin:

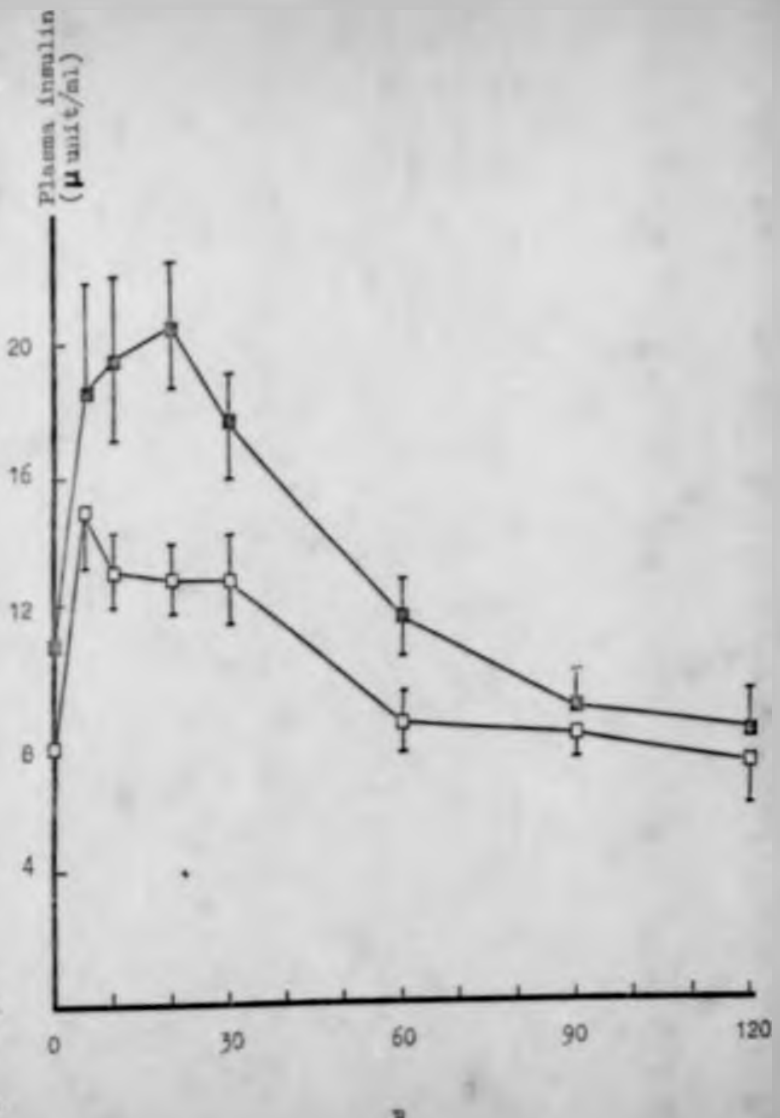
The mean fasting plasma insulin concentration was 7.8 ± 0.86 μ unit/ml in the geriatric patients against 10.9 ± 1.22 μ unit/ml in the control subjects. The difference was significant ($p < 0.01$) (Fig. 31B). During the test the levels of plasma insulin in the control subjects continued to rise and the peak was at 30', whereas the peak in the geriatric patients was at 5'. Throughout the test the plasma insulin concentrations in the control subjects were always higher than in the geriatric patients. The differences were significant at 10' and 20', ($p < 0.001$), at 30'

Figure 31. Intravenous Alanine tolerance test (0.15 g l-alanine/Kg BW) in 8 geriatric patients (□) and 8 normal control subjects (■).

A. Mean blood glucose concentration following the iv injection of alanine.
There was no significant difference in the mean fasting blood glucose levels, but in the geriatric patients blood glucose was raised and remained elevated throughout the test. The difference in the mean levels reached significant level after 30' ($p < 0.01$).

B. Mean plasma insulin values at various times during the test.
There was a significant difference in the fasting values between the two groups ($p < 0.01$); at 10' and 20' ($p < 0.001$), at 30' and 60' ($p < 0.01$).


Blood glucose
(m mol/l)
0
30
60
90
120
Min.
A


Plasma insulin
(μ unit/ml)
4
8
12
16
20
0
30
60
90
120
B

and 60' ($p < 0.01$) (Fig. 31B).

3. Plasma alanine:

The mean fasting plasma alanine concentration was 398 ± 64 μ mol/l in the geriatric patients against 387 ± 62 μ mol/l in the control subjects. The difference was not significant (Table 25).

The peak plasma alanine concentration was 5536 ± 362 μ mol/l in the geriatric patients against 5456 ± 661 μ mol/l in the control subjects. Both were at 5' after an injection of l-alanine and the difference was not significant (Table 25).

The mean % removal rate of alanine (K_A) was 2.93 ± 0.18 %/min in the geriatric patients against 5.31 ± 0.71 %/min in the control subjects. The difference was highly significant ($p < 0.001$) (Table 25).

4. Plasma glucagon:

Radioimmune assay of glucagon is carried out in Dr. S.R. Bloom's laboratory, The Royal Post Graduate Medical School, London. At present, they are having difficulty with the assay technique, and at the time of writing this thesis, results of plasma glucagon levels during the iv alanine test have not been received. The glucagon results cannot therefore be included in this report, but it is hoped to add them as a separate appendix as soon as they are available.

Table 25

The mean fasting and peak concentrations and the mean percentage removal rate of plasma alanine (K_A) during iv alanine tolerance test (± SEM), in the geriatric patients and in the control subjects. Number of observations in parenthesis.

	Plasma alanine (μ mol/l)		K_A (%/min)
	Mean fasting concentration	Mean peak concentration (at 5')	
Geriatric patients (4)	398 ± 64	5536 ± 362	2.93 ± 0.18
	ns	ns	***
Control subjects (4)	387 ± 62	5456 ± 661	5.31 ± 0.71

* $p < 0.05$; ns = not significant

CHAPTER IV
DISCUSSION

Our results show the familiar findings of an impairment in oral GTT in old age (Brant, 1960; Burch and O'Meally, 1967; Chlouverakis, et. al., 1967; Smith and Hall, 1973) (Fig. 27A). Insulin secretion showed a sluggish response to the glucose load, with a delayed peak at 90' (Fig. 27B). The peak of plasma insulin corresponded with the peak of the glucose curve. There was no significant difference in the mean fasting concentration and the mean average concentration of plasma insulin during the oral GTT, between the geriatric patients and normal control subjects. However, the insulin:glucose molar ratio was significantly lower in the geriatric patients ($p < 0.01$) (Table 21).

This work has demonstrated that looking only at a single time for insulin during the oral GTT, e.g. the 60' value as reported by Chlouverakis, et. al. (1967), could be very misleading, since at that stage (60') insulin concentration in geriatric patients was still rising (peak at 90') whereas in the control subjects it was in the process of going down (peak at 30'). Therefore, at this time (60'), there was an apparently higher mean plasma insulin value in the geriatric subjects (Fig. 27B). Our results are in line with those reported by Smith and Hall (1973), where they also had serial measurements of plasma insulin during the oral GTT.

It is interesting to note that the decrease in the level of plasma NEFA during the oral GTT was not significantly different between the geriatric patients and normal controls (Fig. 27C), despite the fact that the geriatric patients had a delayed insulin curve. This suggests that insulin levels in the geriatric patients were still

able to prevent NEFA release from adipose tissue. Plasma NEFA concentrations, like those of plasma insulin also showed a sluggish response to glucose administration in the elderly.

Our results also confirmed earlier findings that old people have an impairment in iv GTT (Streeten, et. al. 1965; Franckson et. al., 1966; Cockford. et. al., 1966). There were significant differences in the mean K_G ($p < 0.001$) and $K_G + I$ ($p < 0.001$), but not in the mean KF values, between the geriatric patients and the normal controls (Table 23). This may suggest that old age affects carbohydrate more than fat metabolism (represented by anti-lipolytic action of insulin).

The patients had suffered at variable times before the test from the usual range of clinical conditions found in geriatric wards, e.g. cardiovascular accident (C.V.A.), myocardial infarct, gangrene, etc. Some still had the results of their previous illnesses such as hemiplegia, paraplegia, kyphosis, etc., at the time of the test (Table 18). However, all were in reasonably 'good health'. None of these previous illnesses (e.g. myocardial infarct), show any correlation with the results of glucose tolerance. This was seemingly surprising for ischaemic cardiovascular diseases show correlation with impaired glucose homeostasis in the 50 to 60 year old adults (Wahlberg and Thomason, 1968). Possibly in the younger people, low K_G values as other indices of impaired glucose homeostasis may be signs associated with those more prone to heart attacks. But, by the advanced age of the patients in this work, all seem to have reached such a degree of impairment, that differences due to factors other than age are difficult to distinguish.

Drug treatments received by these patients (if any) were discontinued for at least 12 hours before the test.

The mean peak insulin level during the oral GTT was significantly lower ($p < 0.05$) in the geriatric patients compared with normal control subjects. The mean peak insulin level during the iv GTT was also lower in the elderly but not significantly so ($p > 0.05$) (Table 24). These findings of low peak levels of insulin response to glucose load, appear to support the hypothesis of Andres (1973), who gave prime importance to the decreased β-cell response to glucose stimulus in contributing to the impairment of carbohydrate metabolism in old age.

As in the study in obesity, the results for geriatric patients show that insulin sensitivity (K_{G+1} value) is a more important factor in determining glucose tolerance than the actual plasma insulin concentration. This is shown first by the highly significant correlation between K_G and K_{G+1} values in the combined group of geriatric patients and normal control subjects ($r = 0.71$, $p < 0.001$) (Fig. 30), and secondly by the fact that there was no statistical correlation between K_G and fasting or peak plasma insulin values during the iv GTT (Figs. 28A and Fig. 28B). Nor did sensitivity to insulin relate to basal or peak insulin levels during the iv GTT, since there was no statistical correlation between K_{G+1} and fasting or peak insulin values during iv GTT (Figs. 29A and 29B).

Glucose absorption by the small intestine (during oral GTT) must also play a part in determining the shape of the curve, Webster and Leeming (1975), using a modified

xylose tolerance test and the method of Roe and Rice (1948) for determining xylose concentration, showed that 26 per cent of geriatric subjects showed some degree of malabsorption. If this is true, the delayed entry of glucose into the circulation would add its effect to those of other factors responsible for prolonged hyperglycaemia, and increase its severity (Fig. 27A). Hepatic glucose production is the most obvious non-dietary candidate for the cause of delayed hyperglycaemia in the oral GTT. It was hoped that this could be assessed by l-alanine administration, but in order to separate the hepatic from gut factors, alanine had to be given intravenously.

Injection of l-alanine produced a significantly higher concentration of blood glucose in the geriatric patients than in control subjects (Fig. 31A). It seemed that in the geriatric patients, plasma insulin (which was low compared to normal control subjects) was unable to suppress gluconeogenesis by the liver (Fig. 31B). Therefore, the glucose level remained high, whereas in the control subjects it went down after 10'. Unfortunately glucagon values during this test are not yet available at the time of writing.

Glycogen content in the liver must have been low if not depleted altogether (at least in the geriatrics) as shown by low fasting values of blood glucose in both groups (Fig. 31A), (Hultman and Nilsson, 1971), so that the new glucose must in large part be due to the conversion of alanine rather than glycogenolysis in the liver.

The fact that fasting must have been reasonably adequate is shown also by the fact that alanine produced, even in control subjects, only a modest increase in insulin secretion (Fig. 31B). If the glucagon results had been available, they would

probably have shown a marked rise (Unger, 1972). Only if glucose is available does alanine have a marked stimulation effect on the β-cell and under such circumstances the effect on glucagon release is minimal.

Within the limitations of this simple test, l-alanine injections seemed to indicate some inability of endogenous insulin to suppress glucose production by the liver in the geriatric subjects. However, the alanine results on their own do not really prove that the higher glucose values in the geriatric patients are not simply due to the shortage of insulin.

As in the study in obesity, the geriatric patients were also divided into two groups based on 120' blood glucose value during oral GTT. The 'diabetic' group has 120' blood glucose value higher than 7.77 m mol/l (140 mg/100ml) and the 'non-diabetic' group values lower than 7.77 m mol/l. Fortuitously, the dividing line (7.77 m mol/l), which had been arbitrarily chosen, corresponded closely with the mean 120' blood glucose value for all 23 patients (8.12 m mol/l) and with the 50th percentile value for the 120' blood glucose value of 80 year olds given by Andres (1973). The degree of impairment in oral GTT in these two groups was not related to the degree of hypoinsulinaemia (Table 22). In fact, the average insulin values during oral GTT were almost identical for the 'diabetic' and 'non-diabetic' groups, each being slightly less than for normal controls. The only difference lay in the shape of the curves. This confirms the argument advanced earlier for the geriatric patients as a whole, that insulin deficiency was not the main cause of impaired glucose tolerance.

The question really is, if the elderly do indeed produce less insulin in response to a glucose challenge than young people, does insulin 'deficiency' constitute a limiting factor in glucose homeostasis? Another way of looking at this problem is to pick only control subjects who had low insulin:glucose molar ratio during oral GTT (low-ratio controls). In these low-ratio controls (LRC) ($n = 5$), their mean insulin:glucose molar ratio was 27.8 ± 1.33 against 29.8 ± 2.29, in the elderly ($p > 0.05$). The mean average plasma insulin concentration during oral GTT in the LRC was 20.8 ± 0.55 μunit/ml against 31.6 ± 2.52 μunit/ml in the geriatric patients. The difference was also not significant. However, the LRC, despite the fact that they had slightly lower mean value of plasma insulin, were able to manage to have a significantly lower mean value (= 'normal') of average blood glucose concentration during the oral GTT. This mean value was 5.38 ± 0.25 m mol/l in these LRC against 7.72 ± 0.28 m mol/l in the geriatric patients. The difference was highly significant ($p < 0.001$) (Table 26). These results again show that hypoinsulinaemia was not the main cause of impaired glucose tolerance in the elderly.

Our results show significant differences in carbohydrate metabolism between old and young people, and we are convinced that the liver plays an important role. Future work should attempt to define this role more clearly. For instance, if a tracer dose of labelled glucose accompanied the test load, measurements of specific activity of plasma glucose at various times during the test would throw some more light on

Table 26

The mean values for average concentrations of blood glucose and plasma insulin, and insulin:glucose molar ratio during oral GTT; in controls, 5 controls with low insulin glucose molar ratio (low-ratio controls) and in geriatric patients (± SEM). Number of observations in parenthesis.

		Mean of the average value for 150 min.		10^6 x mean insulin: glucose molar ratio
		Blood glucose (m mol/l)	Plasma insulin (μ unit/ml)	
I	Controls (13)	5.71 ± 0.18	33.5 ± 3.51	42.2 ± 4.50
	I x II	ns	ns	ns
	I x III	**	ns	**
II	Low-Ratio Controls (5)	5.38 ± 0.25	20.8 ± 0.55	27.8 ± 1.33
	II x III	***	ns	ns
III	Geriatric patients	7.72 ± 0.28	31.6 ± 2.52	29.8 ± 2.27

** $p < 0.01$; *** $p < 0.001$; ns = not significant

the significance of liver gluconeogenesis.

As with carbohydrate metabolism, it is not surprising that protein synthesis and protein turnover is also reduced in the elderly (Young, Steffee, Pencharz, Winterer and Schrimshaw, 1975; D.J. Millward, personal communication). Our results show that added alanine disappeared significantly more slowly (low K_A) from the blood of the elderly than from normal young people ($p < 0.001$; Table 25). This may indicate that transfer rates for amino acids like alanine become diminished in old age. Dr. D.J. Millward (personal communication) reports that infusion of labelled amino acids takes considerably longer to reach plateau levels in old rats than in young rats. Possibly, impaired glucose tolerance may even be a part of this general phenomenon.

PART V

GENERAL DISCUSSION

CHAPTER I
INTRODUCTION

The common thread running through this work is the role of insulin in glucose homeostasis in three different situations, each of which may be regarded as presenting some degree of stress viz. surgery, obesity and old age. Each of these topics has already been discussed individually in some detail. The object of the present discussion is to comment briefly on a few general points which have escaped earlier reference or which are of general importance across the whole area of this investigation.

CHAPTER II
METHODS

A. Choice of Subjects:

Patients participating in the study of glucose homeostasis after surgery were chosen by the surgical staff, Department of Surgery, University College Hospital, London (Head: Professor C.G. Clark), based on our specification (see Part II, Chapter II, Subjects).

This study was planned in such a way that each patient would be his own control by comparing tests carried out on day 1 post-operatively, and on 'recovery'. Four young normal subjects were also included in the study and were referred to as 'controls'. However, they could not serve as 'controls' in the pure sense, because, although their mean body weights and mean % ideal body weight were not significantly different from those of the patients, they were significantly younger. The mean age of the normal subjects was 27 ± 2.3 years against 52 ± 3.4 years, in the patients ($P < 0.01$). (See Part II, Chapter II, Subjects). If work with glucose infusion were to continue, it might be useful in the future to recruit more volunteers to make up the number, and extend the age range, so that it covers the age range of the patients. However, in the present work, any differences between the surgical patients and the 'control' subjects could, therefore, not only be due to the effect of surgery and food deprivation, but also the effect of age.

If age (52 years) were a significant factor in determining the results of glucose infusion, one would expect at least a trend towards impaired rather than heightened insulin secretion in response to glucose (compare geriatric patients, Part IV).

Therefore, the effect of age in the surgical study is likely to have been minimal.

In the choice of patients in the study in obesity, we had 29 obese subjects who attended the Nutrition Clinic, University College Hospital and willing to participate in our study, but not all agreed to have the three tests carried out on them. Some agreed to having the oral GTT while the others agreed only to participate in the iv GTT and iv GITT (these two tests were carried out one after another on the same morning). And only 7 subjects agreed to have all three tests carried out (Table 11).

It is hoped that in future work a larger number of obese patients will be studied, so that they can be classified not only according to % ideal body weight alone (as in the present study), but to be extended to the possible classification of patients by combined age and % ideal body weight.

Fortunately, in the study in the elderly, we were able to persuade the volunteers (geriatric patients) to participate in all of the three tests (oral GTT, iv GTT and iv GITT). Basically, most of these patients were lonely, and they loved to have someone show an interest in them. In general they were less concerned about the trauma (pain) of the needle used during the test, compared to the attitudes of the surgical and obese patients, and even more, the healthy controls.

However, we also faced difficulties when we asked the elderly patients to participate in the iv alanine tolerance test. This may be due to the fact, apart from some of the patients not being available, the iv alanine tolerance test gave a slight stomach discomfort to some, for 10 to 30 minutes after the injection of l-alanine.

As this became known, the difficulty in obtaining volunteers increased, not only in the elderly but also among potential 'control' subjects.

B. Choice of Test:

It would be ideal if we could have the same test for all three studies (surgery, obesity and old age). However, each test has its own merits and disadvantages.

1. Glucose infusion test:

This test was considered the best for surgical patients, because it represents only a minor change (a pump instead of the usual infusion drips) in the method of infusing glucose which most patients received as usual post-operative 'dietary' therapy. It was, therefore, of minimal burden to these immediate post-operative patients (day 1), and included those who participated in this study and most of them did not seem to mind this change. However, during the 'recovery' period the presence of the operator together with his infusion set and infusion pump was not a welcome sight.

The amount of glucose infused (0.35 g kg^{-1} h^{-1}) would certainly have suppressed hepatic glucose output (Madison, 1969). Therefore, when a plateau of blood glucose concentration had been reached, we could discount the hepatic factor, and the rate of infusion at this stage represents the peripheral glucose uptake, unless blood glucose concentration exceeds the renal barrier, in which case some would be lost through urine.

Since the rate of infusion was the same on all occasions, so also was the total glucose uptake. Now total glucose uptake = concentration x fractional rate

of uptake (Franckson, et. al. 1966). Therefore, those patients who showed the highest plateau concentrations of blood glucose (day 1, post-operatively) must have had the lowest fractional rates of glucose removal (equivalent to the lowest K_G values).

We also tried similar glucose infusions on some obese and geriatric patients. They also shared the view of those recovering surgical patients (during the 'recovery' period), of not being keen on having this test carried out. This, together with other technical problems such as the difficulty in getting two nice veins, one in each arm, in some obese subjects, meant that there seemed no good reason to persist with glucose infusion as opposed to the other tests.

2. Oral GTT:

This test is straightforward and has not been associated with any complaints of discomfort from patients or subjects. Its main obvious advantage is that it is physiological, in that the glucose enters by mouth and is subject to the full range of factors before it reaches the tissues. Its main disadvantage is that one is presented with a curve or a set of results rather than a single answer. This may necessitate quite difficult value judgements, e.g. in deciding the weight to be attached to fasting or 120' blood glucose values in diagnosing 'diabetes'. (Working Party, Royal College of General Practitioners, 1963, WHO, 1965).

The fact that the oral GTT has been so widely used under fairly standardised conditions in a large variety of subjects, makes it a valuable reference test in comparing data from one source with another.

3. Iv GTT:

The test is relatively unfamiliar to most clinicians compared with the oral GTT; and its advantages are the short duration of the test (60 minutes) and the fact that the test has a result which can be expressed as a single figure (K_G). This permits statistical comparison between individuals or groups and between the same subject at different periods of time. Moreover, since it bypasses the gut, the Iv GTT eliminates the complication of having to consider the absorption and gut insulinogenic factors. However, it also has some disadvantages.

Lafferty, Giddings and Mangnall (1975) argued that % disappearance rates (K_G values) for glucose were without value and absolute removal rates alone have any meaning. It can, however, be argued that 'normality' in absolute glucose removal rates alone tells us very little about the state of the subjects. For instance, in the earlier discussion (B.I.) on surgical patients, it was pointed out that all had the same absolute glucose removal rates, but because blood glucose concentrations differed, K_G values must have differed similarly (but inversely). The K_G values gave a measure of insulin sensitivity (they significantly correlate with K_{G+I} values; Table 27), and tell us that subjects differ in this respect. Absolute blood glucose and insulin levels reveal the extent and manner of adaptation to this changing sensitivity to insulin. It is in fact difficult to see how absolute glucose removal rates could be impaired, unless there was a severe failure in the β cell or wastage of glucose via the urine.

4. Iv GITT:

This test is extensively used in animals (Turner, 1966; Heard and Turner,

Table 27

Correlation between K_G and K_{G+1} values in control subjects, obese and geriatric patients, and in the combined three groups.

	No.	Regression Line	r	P
Control subjects	10	$y = 0.33x + 0.32$	0.60	> 0.05
Obese patients	16	$y = 0.34x + 0.15$	0.65	< 0.01
Geriatric patients	23	$y = 0.23x + 0.44$	0.43	< 0.05
Combined group	49	$y = 0.33x + 0.21$	0.70	< 0.001

1967; Heard and Henry, 1969, a, b) resembles others which have been used in man (e.g. Franckson, et. al., 1966) and is in part, identical to that of Silverstone, Bronfenbrener, Shock and Yiengst, (1957). The key difference is that in the present test the iv GITT should be coupled with iv GTT and measurements of plasma insulin, to give simultaneous assessment of glucose tolerance and insulin status and sensitivity. The iv GITT is then, a good measure of insulin sensitivity (Table 27).

The administration of a relatively large dose of exogenous insulin in this test should certainly switch off glucose output from the liver, and, therefore, any differences in the KG + I values can only be due to the differences in peripheral tissue responsiveness.

The 10' values of plasma insulin concentration after the injection of glucose and insulin, could be as high as 150 to 300 μ unit/ml in the normal subjects, and even higher in some of the obese patients.

C. Skinfold Measurements:

Skinfold measurements were carried out in some obese, elderly and control subjects, as a measure of their nutritional status. These measurements, however, were so unreliable in the obese, especially the extremely obese (reproducibility very poor). They seemed to have better reproducibility in the elderly, but the present data for comparison (Durnin and Womersley, 1974) did not cover very old subjects (the highest was 50 + years). The measurements of the elderly subjects (mean age 79 years) if compared with their data, gave apparently lower values of body fat content. This may be due to the fact that in the very old the elasticity of the skin had become very poor, and the measurements gave a false, lower reading of skinfold thickness.

CHAPTER III
STUDY IN SURGICAL PATIENTS

A. Insulin Resistance:

The patients showed a 'temporary' insulin resistance, manifested in infusion I, by high plateau values for blood glucose (i.e. low K_G values; see Chapter II, B.1), in the presence of levels of insulin which were higher than normal. In infusion II blood glucose plateau concentrations were almost the same as in infusion I, and therefore K_G values had not changed significantly, but plasma insulin values were lower. Therefore, presumably insulin resistance had diminished, but had not returned to normal (compare with young 'control' subjects).

In infusion I, the development of insulin resistance was probably due to the high circulating levels of cortisol and glucagon and probably other anti-insulin hormones (e.g. catecholamines and growth hormone).

B. Significance of Insulin Therapy:

As discussed earlier, insulin administration has been advocated and practised to overcome the insulin resistance of trauma (Hinton, et. al., 1971) (see Part II, Chapter IV). It must be acknowledged that insulin treatment is probably necessary for very serious trauma, like burns. The very high catabolic state in burns would deplete glycogen stores and enhance lipolysis. This increase in lipolysis causes raised levels of NEFA, which were shown to cause massive deposition of fat in the liver (Felgelson, Pfaff, Karman and Stienberg, 1961). On the other hand in moderate or minor types of surgery (or other physical trauma), lipolysis is probably desirable, to enable the body to use its energy reserves of fat rather than protein.

Giving amino acids, instead of the usual glucose, would then permit the improvement of negative nitrogen balance and by not encouraging release of high levels of insulin in the circulation would permit lipolysis (Blackburn, et. al., 1973).

C. Nitrogen Balance:

As judged by the degree of the urinary nitrogen excretion (mean between 9 and 11 g nitrogen loss per day), the degree of trauma in the present series of surgical patients appears low, compared with, for instance, massive urinary nitrogen excretion reported by Cuthbertson (1964), from patients following fracture. However, his patients received a diet with 70 g protein/day, unlike the surgical patients who received none for the first few days following the operation. Another factor to be borne in mind is the influence of previous diet. If low in protein, this might lead to minimal increased loss of urinary nitrogen following trauma (Munro, 1964). Conclusions drawn from urinary nitrogen figures alone are dangerous. A proper nitrogen balance must be estimated.

CHAPTER IV
STUDY IN OBESITY AND ELDERLY SUBJECTS

A. Impaired Glucose Tolerance

The obese and elderly subjects showed a chronic or permanent form of insulin resistance.

Both groups showed impaired glucose tolerance, shown by the significantly higher mean values for average blood glucose concentrations during oral GTT (Table 28), higher and delayed peak of glucose levels during oral GTT (Fig. 18A and Fig. 27A), and significantly lower values of K_G during iv GTT (Table 29), compared with the mean values in the normal subjects.

The impaired glucose tolerance in the obese subjects was related to higher mean levels of average plasma insulin concentrations and peak insulin response during oral GTT (Tables 28 and 30), and to significantly higher mean peak plasma insulin concentration (Table 30) during iv GTT, than in the normal control subjects.

In contrast to the obese, impaired glucose tolerance in the elderly was associated with normal or marginally diminished insulin levels during oral GTT and iv GTT (Tables 28 and 30).

B. Possible Role of the Liver:

The main feature of the oral GTT in the elderly was the delayed and exaggerated peak glucose values. The main cause of which probably the impaired ability of the liver (in the elderly) to switch off glucose production in response to endogenous insulin. This impairment resulted in prolonged hyperglycaemia. But one must also

Table 28

The mean values for fasting blood glucose and plasma insulin, average concentrations of blood glucose and plasma insulin, and insulin:glucose molar ratio during oral GTT in control, obese and geriatric subjects (± SEM). Number of observations in parenthesis.

	Mean fasting concentrations		*Mean of the Average Value for 150 min.			10^6 x mean insulin: glucose molar ratio
	Blood glucose (m mol/l)	Plasma insulin (μ unit/ml)	Blood glucose (m mol/l)	Plasma Insulin (μ unit/ml)	Plasma Insulin (10^6 x m mol/l)	
I Controls (13)	4.62 ± 0.16	12.8 ± 1.31	5.71 ± 0.18	33.5 ± 3.51	240.4 ± 25.2	42.2 ± 4.50
I x II		*	**	**	**	*
I x III			**			**
II Obese (16	4.89 ± 2.93	21.4 ± 2.88	6.73 ± 0.28	69.1 ± 10.61	495.8 ± 76.4	73.5 ± 11.89
II x III		***	*	***	***	***
III Geriatric (23)	4.92 ± 0.11	12.3 ± 0.54	7.72 ± 0.28	31.6 ± 2.52	227.0 ± 18.1	29.8 ± 2.27

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 29

The mean values for K_G, K_{G+1} and K_F in the control, obese and geriatric subjects (± SEM). Number of observations in parenthesis.

		Mean Values		
		K_G (%/min)	K_{G+1} (%/min)	K_F (%/min)
I	Control	1.81 ± 0.28 (10)	4.55 ± 0.51 (10)	3.10 ± 0.73 (6)
	I x II	*	*	ns
	I x III	***	***	ns
II	Obese	1.11 ± 0.18 (16)	2.81 ± 0.35 (16)	2.86 ± 0.90 (4)
	II x III	ns	ns	ns
III	Geriatric	0.98 ± 0.86 (23)	2.29 ± 0.16 (23)	2.21 ± 0.34 (10)

* $p < 0.05$; *** $p < 0.001$; ns = not significant

acknowledge that the insulin response to oral glucose administration was sluggish in the elderly as shown by the delayed peak insulin level during this test.

It is unlikely that the delayed peak insulin level was the result of delayed absorption rate of glucose, as suggested by Smith and Hall (1973), but proof is lacking. The evidence which is against this argument is a. the increase in blood glucose level was equal in the elderly and in young people during the first 30'; b. after 30' the elderly still increased their blood glucose level nearly as much again as they did in the first 30 minutes. The peak level of blood glucose corresponded with the peak insulin level at 90'; and c. the simple iv alanine tolerance test showed that the elderly has increased levels of blood glucose which were not suppressed by their endogenous insulin secretion.

The impairment of iv GTT was also probably due to the decreased ability of endogenous insulin to suppress gluconeogenesis. The mean K_G value was significantly lower in the elderly (Table 29), while the mean peak insulin level, although lower, was not significantly so (Table 30).

The logarithm values of K_G were significantly correlated with 120' blood glucose values during oral GTT in the combined group of the obese, elderly and control subjects ($p < 0.001$; Fig. 32). This suggests that iv GTT would be just as good (or as bad) as the oral GTT in detecting impairment in glucose tolerance.

C. Insulin Sensitivity:

1. Blood glucose and NEFA levels:

While interpretation of the iv GTT might be ambiguous, the iv GITT seems

Figure 32. Correlation between K_G value (%/min.) during iv GTT and 120' value of blood glucose concentration during oral GTT, in the obese, geriatric and control subjects.

(●) obese patients. (○) overt diabetic obese patient.

(▲) geriatric patients. (△) overt diabetic geriatric patient.

(■) normal control subjects.

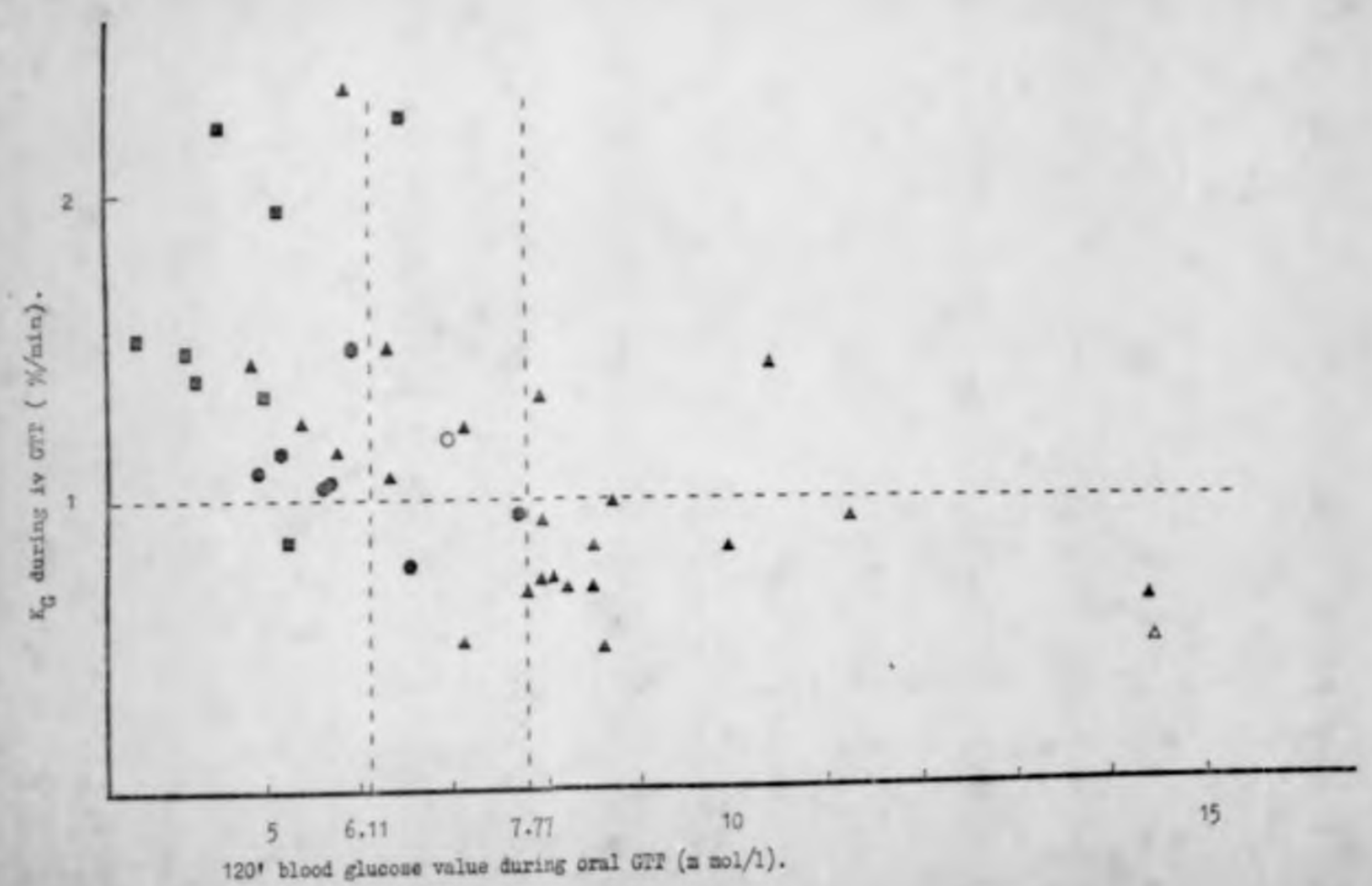

K_G during iv GTT (%/min).
2
1
5
6.11
7.77
10
15
120' blood glucose value during oral GTT (m mol/l).

Table 30

The mean peak insulin concentrations during oral GTT and iv GTT (± SEM), in control, obese and geriatric subjects. Number of observations in parenthesis.

		Mean Peak Insulin Concentrations (μ unit/ml)			
		Oral GTT		Iv GTT	Ratio of oral: iv peak insulin
I	Controls (9)	72.0 ± 7.26	ns	54.0 ± 13.60	1.33
	I x II	**		*	
	I x III	*		ns	
II	Obese patients (5)	160.6 ± 24.98	ns	138.4 ± 11.17	1.16
	I x II	***		***	
III	Geriatric patients	48.0 ± 5.34		41.6 ± 5.13	1.15

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns = not significant

quite clearly to measure peripheral tissue responsiveness. It is very unlikely that liver gluconeogenesis was not suppressed (even in the obese and the elderly) by the relatively high concentration of exogenous insulin which was given. The circulating levels 10 minutes after injection of insulin was found to lie in the range of 150 μ unit/ml to 400 μ unit/ml, i.e. about 5 to 10 times higher than peak levels of endogenous insulin measured during iv GTT. Both the obese and the elderly showed significantly decreased mean K_{G+I} values (Table 29), indicating that peripheral tissue response to insulin (as judged by glucose uptake) is reduced in these obese and elderly people.

In the elderly, it has been argued that this impaired peripheral tissue sensitivity extends also to suppression of hepatic glucose output (see Chapter II B. Oral GTT). There seems no good reason why these arguments should not also apply, though perhaps to a lesser degree, to the interpretation of the oral GTT of the obese.

Both in the study in obesity and old age, the evidence showed that this decrease in insulin sensitivity in relation to carbohydrate is more pronounced than in relation to fat metabolism. This is shown by the absence of any significant difference in the NEFA curves during oral GTT (Figs. 18C and Fig. 27C) or K_F values during iv GTT (Table 29), suggesting that the lipolytic action is not yet effected by obesity or old age.

This is perhaps not surprising since insulin concentrations which maximally stimulate glucose uptake by insulin sensitive tissues are considerably higher than concentration required to inhibit lipolysis.

2. Correlation of glucose tolerance (K_G) with insulin sensitivity (K_{G+1})

This work demonstrated highly significant correlations between K_G and K_{G+1} values ($p < 0.001$, Table 27, Fig. 35). It is interesting that this significant correlation was present in even the obese group alone ($p < 0.01$) or the geriatric group alone ($p < 0.05$), and given a larger number of control subjects, they would certainly have shown the same correlation. Another interesting fact is that in each individual group, the shape of the regression line was almost identical, except in the geriatric group which was slightly lower (Table 27). Perhaps this is a sign of some degree of β cell failure.

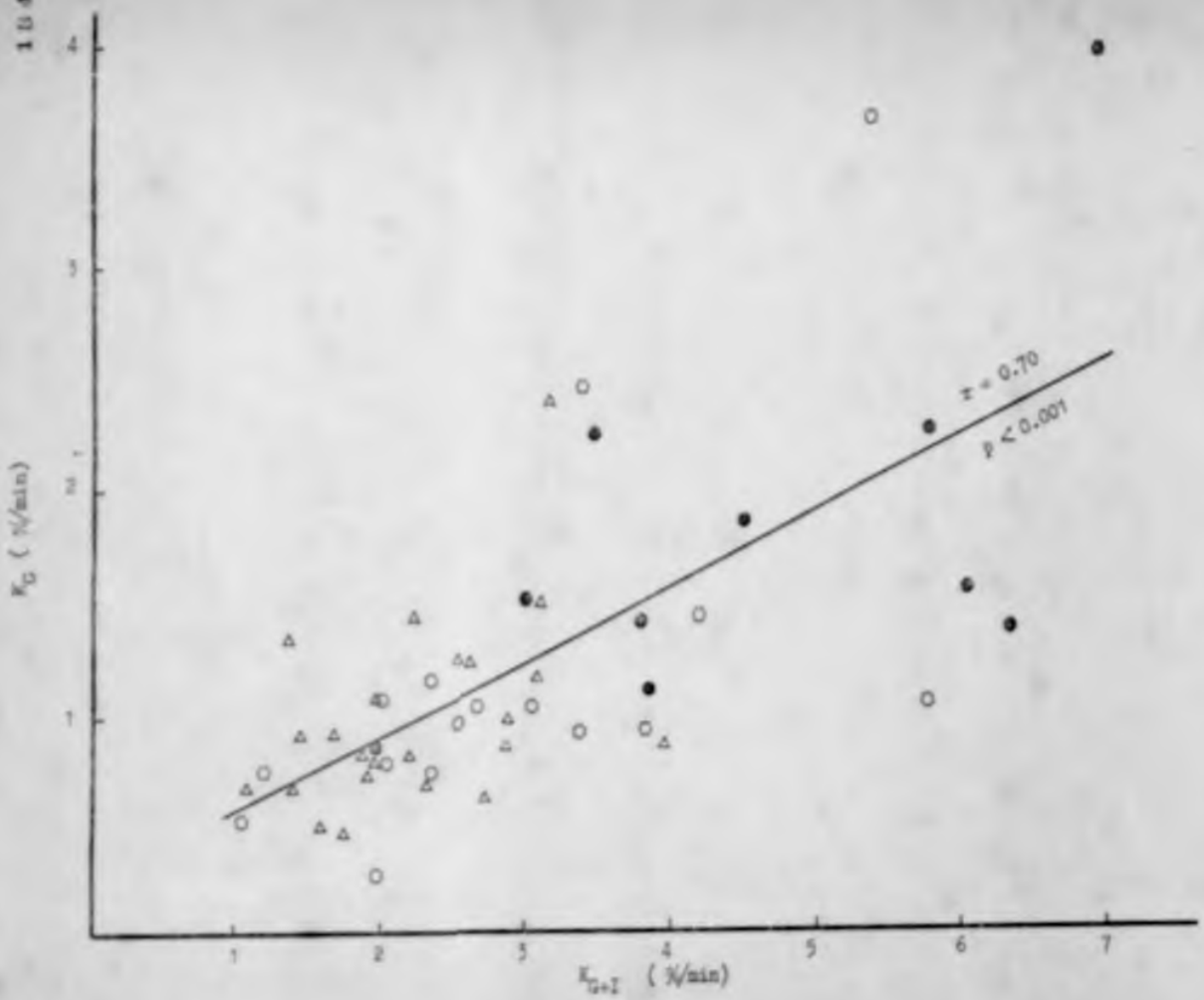
3. Hypersensitivity to insulin in 'normal' subjects:

Some apparently normal subjects showed very high values for K_{G+1} and therefore, also for K_G. It has been suggested in Part III, Chapter IV, that these subjects may be in an early phase of 'active' obesity. A longitudinal study of such people may or may not reveal that this speculation is true.

This speculation is based on the similar pattern of changes in K_G and K_{G+1} found in experimental animals. These animals were fed on diets marginally deficient in protein (Heard and Turner, 1967; Heard and Stewart, 1971). In all cases, hypersensivity to insulin was the earliest phase and led on to impaired glucose tolerance and diminished insulin sensitivity.

It would remain also to attempt to distinguish genetic from dietary influences in the development of obesity.

Figure 33. Regression line between the values of K_G and K_{G+I} in the combined group of obese (○), geriatric (△) and normal control subjects (●).


r = 0.70
p < 0.001
K_G (%/min)
K_{G+I} (%/min)
1
2
3
4
5
6
7

The phrases 'active' and 'passive' in regard to obesity, may have been independently used by other authors in a different context; e.g. Craddock (1973) used these terms for the ability or inability of obese subjects to gain more weight. While this work uses these phrases in relation specifically to insulin sensitivity (in respect to carbohydrate metabolism). (See Part III, Chapter V).

As it was shown (Part III, Chapter III), that treatment of obesity is very difficult and unreliable, a longitudinal study of insulin sensitivitity might detect subjects in the 'active' pre-obese phase or those who are prone to obesity.

Prevention of obesity rather than treatment is likely to offer greater success.

4. What determines insulin sensitivity?:

Two main groups of factors must have been likely to be important. They are: a. anti-insulin hormones, insulin antagonist, etc. (see Part II, Chapter I), and b. cellular factors including availability of receptor sites. Early enthusiasm for the concept that insulin sensitivity and receptor availability would always correlate exactly, has been rather dampened (Cuatrecasas, 1974; Keen, 1975) and more work on this aspect is still needed.

CHAPTER V
DIAGNOSTIC SIGNIFICANCE OF THE TESTS

Obviously a combination of tests would be ideal in determining an impairment in glucose tolerance. For practical purposes, however, using only one particular test, and even in extreme cases, only looking at fasting blood glucose values, would probably be sufficient; although, a few borderline cases may escape from detection.

Arbitrary elimination of subjects who had fasting blood glucose concentrations higher than 6.11 m mol/l (thus all having normal fasting blood glucose), 120' value for blood glucose during oral GTT revealed that some of the obese (> 6.11 m mol/l) and some of the geriatric subjects (> 7.77 m mol/l) were 'diabetic' (Fig. 32). Furthermore, although it was shown earlier that the logarithm values for K_G during Iv GTT were significantly correlated with 120' values of blood glucose during oral GTT, there were some subjects who would be normal by one test but 'diabetic' by the other. If one's aim to to get early detection of a disease, or an impairment, then a combination of tests is very necessary. (Fig. 32).

Iv GITT is best carried out immediately after Iv GTT, because it could serve as a measure of peripheral tissue responsiveness to insulin, since the effect of liver gluconeogenesis has been eliminated.

CHAPTER VI
CONCLUSIONS AND FUTURE WORKS

A. Surgical Patients:

A nitrogen balance study showed a negative balance amounting to 50 g protein loss per day for a period of 5 to 6 days after the operation. This period of negative nitrogen balance coincided with elevated fasting levels of plasma glucagon, NEFA, branched-chain amino acids, urinary free cortisol, urinary 17-OH-corticosteroids and with a decrease of total amino acids. Fasting levels of plasma insulin and plasma cortisol were only significantly elevated on day 1 post operatively.

A two hour infusion of glucose (0.35 g kg^{-1} h^{-1}) on day 1, resulted in hyperglycaemia and hyperinsulinaemia, suggesting a 'temporary' insulin resistance. Glucose also suppressed glucagon secretion to the same extent on day 1, and on 'recovery'. It also decreased plasma NEFA, total amino acids and plasma cortisol in both infusions. But, the fall in plasma cortisol was probably due to the effect of resting rather than the actual effect of glucose.

Several questions remained unanswered:

1. Insulin resistance seems to protect the subjects from the less desirable features of elevated insulin levels. Then perhaps amino acid administration rather than glucose would be more beneficial, since it improves the nitrogen balance without having to increase plasma insulin levels and permits mobilization of fat (Blackburn, et. al. 1973). More work, however, is still needed to establish the best post-operative regime for patients undergoing surgery.

11. It is known that protein synthesis is decreased following surgery (O'Keefe, et. al. 1974; Crane, et. al. 1976). Whether insulin resistance is also responsible in causing the decrease of protein synthesis should also be investigated.

B. Obese Subjects:

This work demonstrates that obese subjects showed impaired response to oral, and iv GTT. The impairment was associated with hyperinsulinaemia. The results showed that K_G or K_{G+1} values were not statistically correlated with fasting or peak insulin levels during iv GTT. However, higher values for K_G or K_{G+1} were usually associated with lower values of fasting or peak insulin. There was a significant correlation between K_G and K_{G+1} values in combined groups of patients and control subjects.

The logarithm values for fasting insulin were correlated with % ideal body weight ($p < 0.001$). K_G or K_{G+1} were not statistically correlated with % ideal body weight however, lower values for K_G or K_{G+1} were usually associated with higher % ideal body weight.

Unlike that of carbohydrate metabolism, this present work was unable to show any significant impairment of insulin sensitivity in regard to fat metabolism (judged by the difference in NEFA curves during oral GTT; K_F during iv GTT).

Oral GTT, iv GTT and iv GITT did not give any prediction of the possible success or failure of any dietary treatment of an obese patient.

This work speculates that there are two types of obesity (based on their insulin sensitivity): a. 'active' obesity, judged by the relatively low % ideal body weight (<160%) low values for K_G, K_{G+1} and low fasting insulin; b. 'passive' obesity judged by high % ideal body weight, low values for K_G, K_{G+1} and high fasting insulin; and c. apparently normal subjects who had very high values of K_G and K_{G+1} are probably in an early stage of 'active' obesity (see Chapter IV, C).

There are a few aspects of this study which need further investigation:

i. more 'normal' subjects with high K_G and K_{G+1} values to be investigated. Longitudinal study of such people may or may not reveal that the above speculation is true.

ii. More obese patients are needed, so that they can be classified by the combination of age and % ideal body weight. This is hoped to throw some light on the development of obesity.

C. Elderly Subjects:

This work demonstrates the familiar impairments in oral GTT and iv GTT in the elderly. Although the impairment of glucose tolerance was associated with hypoinsulinaemia and decreased peripheral tissues insulin sensitivity, the decreased ability of endogenous insulin to switch off liver gluconeogenesis seemed to be the major cause of hyperglycaemia. After intravenous injection of l-alanine, the elderly showed increased levels of blood glucose which were not suppressed by their endogenous insulin secretion.

As in the study on obesity, this work confirms earlier findings in animal experiments (Turner, 1966; Heard and Turner, 1967; Heard and Henry, 1969 a), about the significance of insulin sensitivity (K_{G+I}) in determining glucose tolerance rather than the actual levels of circulating plasma insulin. This was shown by the significant correlation between the K_G and K_{G+I} values in combined groups of elderly patients and control subjects, and also in the combined group of obese, elderly and normal control subjects.

Also, as in the obese, this work was unable to show any significant impairment of insulin sensitivity in regard to fat metabolism (NEFA curves during oral GTT; K_F values during iv GTT).

Few aspects in the study in the elderly need further investigation:

I. As old age is not merely the passing of years, but a generalized picture of many malfunctions added together, the question now arises, whether the ageing phenomenon should be prevented. And if it should, in what way?

II. As skinfold measurements are unreliable (see Chapter II, C) and % ideal body weight is unable to be measured, what is a good, simple criterion in judging the nutritional status of the very old?

III. A possible role of the liver in producing hyperglycaemia needs further investigation. By including a tracer dose of labelled glucose in the ordinary glucose load, and measuring the plasma glucose concentrations and the specific activity of labelled glucose at various times during the test, one would be able to get more information about the impaired suppressibility of liver gluconeogenesis, in the elderly (perhaps also in the obese).

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Abstract No. 50

METABOLIC AND HORMONAL CHANGES AFTER SURGERY: HYPERINSULINAEMIA DURING GLUCOSE INFUSION.

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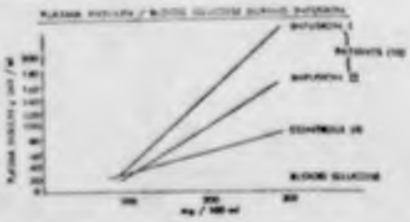
Eleven patients were studied, 3 with chest and 8 with abdominal surgery. Measurement of N balance showed the equivalent of about 50 g protein loss per day for 5-8 days post operative. This period coincided with elevated levels of fasting plasma glucagon, NEFA, branched-chain amino acids, urinary free cortisol, urinary 17-OH-corticosteroids and with a decrease of total plasma amino acids. Fasting blood glucose, plasma insulin and plasma cortisol were elevated only on day 1 after operation.

A 2-hour glucose infusion (0.5 g glucose/Kg/hr) was carried out on day 1 after operation and on "recovery" (day 9-21). Mean blood glucose levels during infusion were not significantly different on the 2 occasions, but there was a trend to a higher 2-hour level on day 1 (220 mg/100ml on day 1 and 186 mg/100ml on "recovery"). Both curves were significantly higher than in normal subjects in whom blood glucose values reached a plateau at 110 mg/100 ml.



Infusion of glucose caused a fall in plasma glucagon, NEFA and amino acids concentrations. The rise in plasma insulin relative to glucose level was much greater on day 1 than on "recovery" when the response, though approaching normal was still elevated. (See Fig.).

This work confirms that hyperglucagonaemia follows surgical operation (1) and demonstrates that this is suppressible with glucose. Although low plasma insulin:glucose values have been reported in severe burns and during operation (1, 2), after operation the insulin response is markedly exaggerated, suggesting insulin resistance.

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Glucose tolerance, plasma insulin levels and insulin sensitivity in geriatric patients. By C. R. C. Heard, W. S. Soesjodibroto and Sylvia M. France, *Clinical Nutrition and Metabolism Unit, Department of Human Nutrition, London School of Hygiene and Tropical Medicine, London WC1E 7HT*, and A. N. Exton-Smith, *Department of Geriatrics, University College Hospital, London*

Venous blood glucose values, 120 min after an oral load of 50 g glucose (G_{120}) are used to diagnose diabetes (WHO, 1965). Values $\leq 6 \cdot 11$ mmol/l are classified as normal and $> 7 \cdot 22$ mmol/l as diabetic. However, G_{120} increases steadily with age and this raises problems in the elderly both of clinical significance and of physiological interpretation.

This communication reports the results of an investigation of twenty-four geriatric patients (mean age 79 years) in whom both availability of insulin and insulin sensitivity were assessed.

Each patient received two tests on successive days: (1) oral glucose tolerance test (GTT) (50 g glucose) lasting 150 min, (2) intravenous (i.v.) GTT (0·33 g glucose/kg) lasting 60 min followed immediately by i.v. glucose + insulin (0·33 g glucose and 0·133 units insulin/kg) with blood sampling for a further 60 min (Heard & Henry, 1969). Half the patients had the oral test on the first day, half had the i.v. test. Patients were fasted from midnight. Blood was sampled and glucose and insulin injected via an indwelling butterfly needle in the arm. Glucose (glucose oxidase method) and insulin (Radiochemical Centre method) were estimated in plasma. Glucose disappearance rates (k; % per min) in the i.v. tests were calculated to give k_G for glucose alone and k_{G+I} for glucose + insulin. Normal values are about 2 and 5% per min respectively (Franckson, Malaise, Arnould, Rasio, Balasse, Conrad & Bastenie, 1966).

Although only one patient was overtly diabetic (fasting glucose 8·10 mmol/l), sixteen of the twenty-four had G_{120} values $> 7 \cdot 77$ mmol/l. Of these, fourteen also had severely impaired i.v. glucose tolerance ($k_G < 1 \cdot 0$% per min). Only two other patients had $k_G < 1 \cdot 0$% per min. Clear signs of glucose malabsorption occurred in three patients, who therefore showed very low plasma insulin levels during the oral GTT compared with values during the i.v. GTT. Another five patients without malabsorption also showed low plasma insulin levels during the oral GTT suggesting impairment of insulinogenic gut factors. Although plasma insulin levels during both tests were lower than those reported for young normal subjects, insulin sensitivity (k_{G+I}) was also low in all the patients (mean 2·4% per min). The shape of the oral GTT curves suggested that failure to suppress hepatic glucose release was a feature of this insulin insensitivity.

The extent to which these changes are typical of old age per se and whether deterioration in nutritional status contributes to the effects remain to be established.

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