THE EFFECT OF DIETARY EGG ON HUMAN PLASMA CHOLESTEROL AND TRIGLYCERIDE LEVELS

by

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INTRODUCTION

"Physiologists and Biologists have been fascinated with cholesterol because of its essential functions in membranes of animal cells, where it modulates fluidity and maintains the barrier between cell and environment, and because it is the raw material for the manufacture of steroid hormones and bile acids." (Brown and Goldstein, 1986).

Furthermore, Brown and Goldstein (1986) aptly describe cholesterol as a "Janus faced molecule" since : its insolubility in aqueous media can either be useful, as in cell membranes; or it can be lethal, as in the formation of atherosclerotic plaques in arteries.

The body cells require a constant supply of cholesterol in order to grow, reproduce and function normally i.e. cholesterol is vital for survival. Yet, instead of being regarded as an essential substance it is regarded with an element of fear. This is because elevated levels of serum cholesterol is an important risk factor in atherosclerosis (Brisson, 1981).

Most studies involving the possible influence of dietary cholesterol on serum cholesterol levels have been done in the belief that elevated blood cholesterol levels are closely linked to the accelerated development of atherosclerosis, hence increasing CHD risk (Brisson, 1981).

According to Berger and Marais (1987) coronary heart disease has a multifactorial aetiology, but there is little doubt that serum total cholesterol and serum total saturated fatty acids in triglycerides are two of the most important contributory factors.

The effect of dietary fat on blood fat levels has been extensively studied over the past 30 years. This relationship has been examined in cross-sectional population surveys, in outpatients consuming various experimental diets, and in studies of patients confined to metabolic wards. These show different results (Flynn et al., 1986). Furthermore, the blood levels of cholesterol and triglycerides were not reported in the period after ingestion up to say 6 hours after the meal (post-ingestive state).

The aim of this study is to determine the change in plasma cholesterol and triglyceride levels over a 6 hour period after a meal rich in cholesterol and triglyceride.
Twenty four free-living volunteers were selected for this study. They were separated into two groups on the basis of their blood cholesterol and triglyceride levels in order to compare their respective responses to dietary egg.

I. LITERATURE SURVEY

Cholesterol, a lipid which occurs in all cells of the human body and is essential to life, was discovered by Michel Eugene Chevreul of France.

Work on the elucidation of the structure of cholesterol began in 1903 by Alolf Windaus of Freiburg, with 1932 seeing the confirmation of the cholesterol structure (McGill, 1979).

"Cholesterol is the most highly decorated small molecule in biology." To date, thirteen Nobel prizes have been awarded to scientists who have worked on cholesterol (Brown and Goldstein, 1986).

Cholesterol is the raw material of every steroid manufactured by the body and is furthermore an essential constituent of membranes, modulating their fluidity. Although its insolubility in water causes difficulty in its handling, (eg. it requires a special transport mechanism and has a tendency to form deposits as lipid plaques in arteries), it is the foundation of the various and essential functions of cholesterol (Marx, 1976; Brisson, 1981).

Another major plasma lipid is triglyceride. Triglycerides are fatty acid esters of glycerol, usually consisting of a mixture of 2 or 3 different fatty acids (Thompson, 1989). Triglycerides can be stored in the tissues or used as fuel (Troxler and Schwertner, 1985).

(A) CHEMISTRY AND SOURCES OF CHOLESTEROL

According to Brown and Goldstein (1986), cholesterol was first isolated from gallstones in 1784. The cholesterol molecule consists of carbon and hydrogen atoms, and an hydroxyl group. The carbon atoms are linked to form a complex four-ring structure with the hydroxyl group on carbon 3 (Fig. 1).

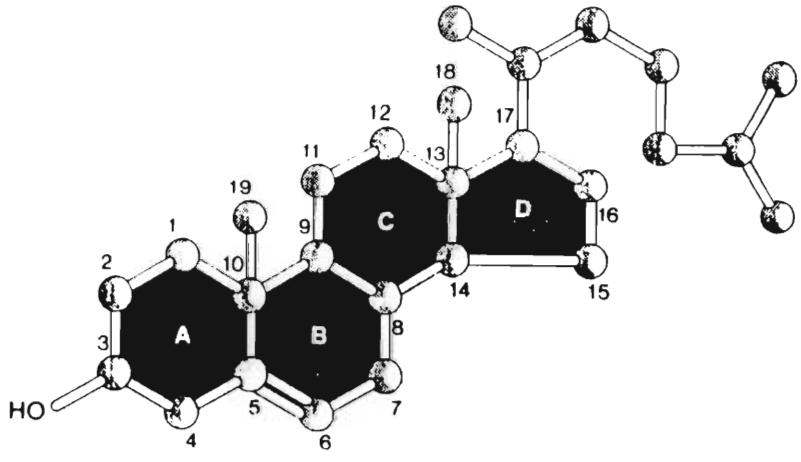

Fig. 1. Structure of Cholesterol

This is the basic structure of the steroids. Cholesteryl esters are formed when the hydroxyl group reacts with fatty acids. Thus cholesterol occurs as free cholesterol, cholesteryl esters or lipoprotein complexes (Brisson, 1981).

(B) PHYSIOLOGICAL ROLE OF CHOLESTEROL

Brisson (1981) emphasizes that cholesterol is the parent compound of numerous substances vital to life, eg. bile acids, vitamin D, male and female steroid hormones and the adrenocorticoid steroid hormones. Bile acids are synthesized in the liver and participate in the digestion of lipids. The role of the various steroids (physiological and metabolic) are essential for life. Moreover, cholesterol is necessary for cell membrane formation and stabilization. The body cells require a constant supply of cholesterol in order to grow, reproduce and function normally. This cholesterol is obtained from two sources. Cholesterol from the diet is referred to as the exogenous cholesterol whilst that synthesized in the hepatocytes is the endogenous cholesterol.

(C) BIOSYNTHESIS OF CHOLESTEROL

Burley and Turner (1983) claim that since the body synthesizes all the cholesterol it requires, any cholesterol from the diet is surplus.

The hepatocytes, are the most active, being responsible for about 85% of the total cholesterol synthesis (Troxler and Schwertner, 1985).

1. BIOSYNTHETIC PATHWAY

Marx (1976) asserts that virtually all cells of the body can synthesize cholesterol. In individuals consuming a "typical Western diet", 66% of the total body cholesterol is produced endogenously according to Tobert (1987). This endogenous cholesterol synthesis occurs via numerous steps with all the carbon atoms being derived from acetyl-coenzyme A which is a 2-carbon substrate. Brown and Goldstein (1986) go on to inform that at least 30 enzymes are required in this synthesis.

In the first step there is condensation of two molecules of acetyl-CoA to form acetoacetyl-CoA. Acetoacetyl-CoA then reacts with another acetyl-CoA molecule and 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) is formed.
The reduction of HMG-CoA to mevalonate is catalyzed by HMG-CoA reductase (Fig. 2) in what is regarded to be an

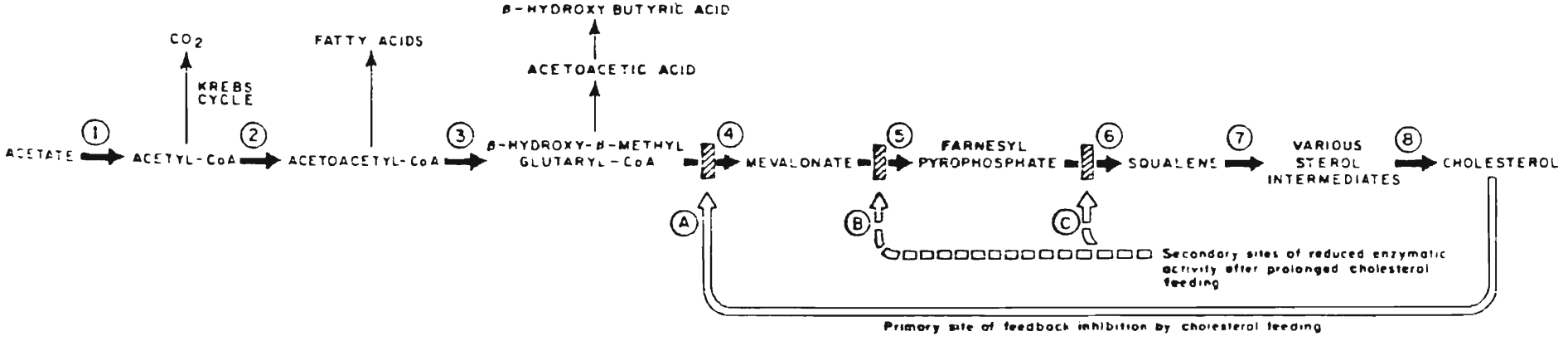

FIGURE 2. *Diagrammatic Representation of the Major Steps in the Synthesis of Cholesterol and the Biochemical Steps where Metabolic Control Takes Place.*

This simplified scheme shows only certain key steps in the biochemical sequence. The specific conversions indicated by numbers may represent a single enzymatic step (for example, Step 4 — the reduction of β-hydroxy-β-methylglutaryl-CoA to mevalonate by the enzyme β-hydroxy-β-methylglutaryl-CoA reductase) or a sequence of enzymatic steps, such as Step 6, the conversion of farnesyl pyrophosphate to squalene. The three sites at which feedback control is thought to be mediated are shown by the hatched blocks — Step 4, the primary site of feedback inhibition, and Steps 5 and 6, the sites of secondary control.

(Dietschy and Wilson, 1970).

important rate limiting step. Olson (1987) states that this is the first committed step in cholesterol synthesis.

After a series of reactions squalene, a C_{30} compound is formed. Ring closure of squalene - 2,3 - oxide produces lanosterol which undergoes inter alia, oxidative demethylation and decarboxylation to eventually produce cholesterol. (Tobert 1987; Murray et al., 1988)

2. REGULATION OF BIOSYNTHESIS

It is reported in Grundy et al. (1988) that the regulation of endogenous cholesterol synthesis is affected by a number of key enzymes.

For plasma cholesterol to remain unaltered in response to dietary cholesterol, the feedback inhibition on endogenous synthesis must equal the percentage absorbed - thus ensuring that there is a nett change of zero in the cholesterol pools. Individuals whose plasma cholesterol increases in response to an intake of dietary cholesterol have a feedback system that is not perfectly regulated i.e. they have an "insensitive feedback mechanism." (Grundy et al., 1988).

In 1976 Marx claimed that an increased intake of dietary cholesterol inhibits the synthesis of endogenous cholesterol by the hepatocytes.

According to Ganong (1983) the cholesterol in the cell provides feedback of its concentration by the following reactions :

(i) It inhibits HMG-CoA reductase (Fig. 2) and thus intracellular cholesterol synthesis,
(ii) Esterification of excess cholesterol released is stimulated, and
(iii) Synthesis of new LDL receptors is inhibited.

In support of LDL receptor inhibition Goldstein and Brown (1985a; 1987) assert that when cells have an excess of cholesterol the synthesis of new LDL receptors is shut-off. It is noteworthy that when cholesterol is needed by the liver or extrahepatic tissues for synthesis of steroid hormones, for example, these tissues synthesize LDL receptors which take up LDL and in effect cholesterol by receptor - mediated endocytosis.

(D) LIPID METABOLISM

1. LIPID DIGESTION

Pancreatic esterase hydrolyses the dietary cholesteryl esters in the intestinal lumen while pancreatic lipase acts on dietary triglyceride in the duodenum to liberate fatty acids, glycerol and monoglycerides. Micelles, which solubilize lipids, are formed when lipids (such as fatty acids, monoglycerides, and cholesterol) interact with bile salts.

These micelles diffuse through the unstirred water layer in the lumen to reach the brush border of the mucosal cells. The lipids then diffuse out of the micelles so that a saturated solution of lipids is kept in contact with the mucosal cells. The lipids enter the cells by passive diffusion and undergo esterification. This ensures a concentration gradient from the intestinal lumen into the cells (Ganong 1983).

2. LIPID ABSORPTION

Castelli (1986) claims that once within the intestinal cells, the fate of the fatty acids is dependent on their size. Those with a maximum of ten carbon atoms (short-chain fatty acids) are absorbed as free fatty acids and enter the portal circulation, via the intestinal cell, and

are conveyed directly to the liver. On the other hand, the long-chain fatty acids immediately undergo re-esterification into triglycerides. Some of the cholesterol is also esterified.

The triglyceride and cholesterol esters are coated with a layer of protein, cholesterol and phospholipids, thus forming very large lipoprotein complexes called chylomicrons which are released by exocytosis to enter the lymph. It is to be noted that almost all the absorbed cholesterol is incorporated into chylomicrons (Brisson, 1981; Ganong, 1983). Furthermore, 84% of the chylomicron is triglyceride or exogenous fat (Troxler and Schwertner, 1985).

3. LIPID TRANSPORT

According to Brisson (1981) cholesterol and triglycerides are practically insoluble in water. Therefore special mechanisms are required for their transport in plasma from the sites of absorption and synthesis to the sites of utilization. They combine with proteins and phospholipids to form large aggregates called lipoproteins which are water soluble.

There are six classes of lipoproteins. These lipoprotein particles are large and globular and consist of a nonpolar lipid core with a coat of phospholipid, free cholesterol

and apoproteins. The core consists of cholesteryl esters and/or triglycerides.

There are two components to this lipoprotein transport pathway in blood (Fig. 3) as follows :

(a) EXOGENOUS PATHWAY - for the transport of lipids that enter from the gut.

(b) ENDOGENOUS PATHWAY - for the transport of lipids released from the liver and all other tissues with the exception of the intestine (Brown and Goldstein, 1985).

(a) EXOGENOUS PATHWAY

The first step here is the formation of chylomicrons from dietary cholesterol and triglyceride in the intestine. These large lipoprotein complexes enter the circulatory system via the lymphatic system. (Brown and Goldstein, 1985).

The APO CII component of the chylomicron activates lipoprotein lipase, an enzyme bound to the surface of the endothelial cells of the capillaries of muscle and adipose tissue, which clears the circulation of chylomicrons by catalyzing the breakdown of the triglyceride to free fatty acids and glycerol (Ganong, 1983; Castelli, 1986).

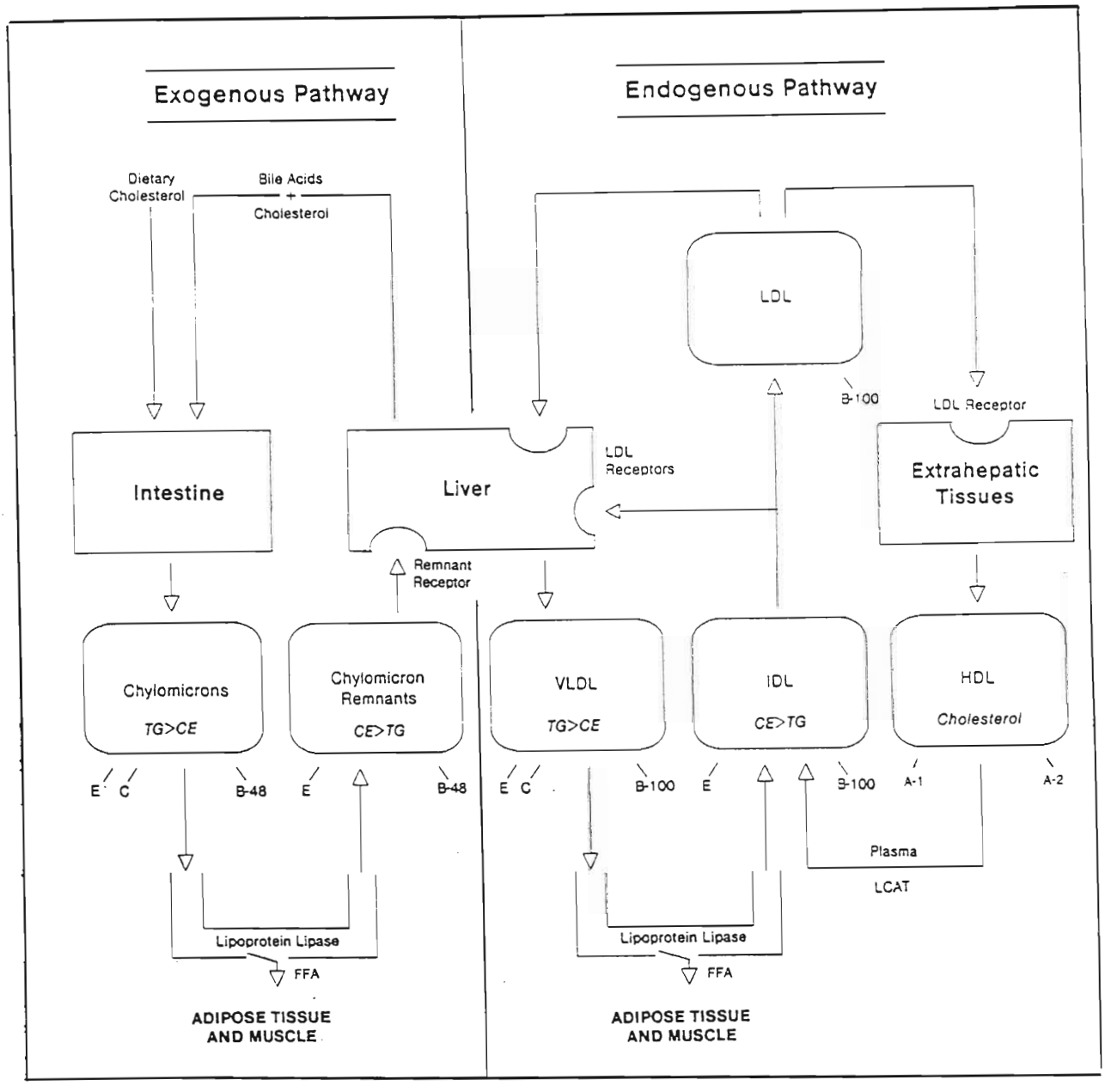

Fig. 3 Model for the metabolism of plasma lipoproteins, showing the separate pathways for transport of endogenous and exogenous lipids

CE	:	Cholesteryl esters
FFA	:	Free fatty acids
TG	:	Triglycerides
HDL	:	High density lipoprotein
IDL	:	Intermediate density lipoprotein
LCAT	:	Lecithin:cholesterol acyltransferase
LDL	:	Low density lipoprotein
VLDL	:	Very low density lipoprotein

A-1, A-2, B-48, B-100, C'S and E represent the apoproteins associated with the indicated lipoprotein particle.

(Modified from Goldstein, Kita and Brown, 1983).

According to Troxler and Schwertner (1985) approximately two-thirds of the dietary triglyceride enters the tissues and one-third the hepatocytes.

Once most of the triglycerides have been removed, the chylomicrons re-enter the bloodstream. These chylomicrons are now smaller in size, have a decreased triglyceride content but an unchanged cholesteryl ester content. They are therefore referred to as chylomicron remnants. These cholesterol-rich chylomicron remnants then enter the liver cells by receptor-mediated endocytosis. These receptors recognise apoproteins E and B-48, two protein components of the chylomicron remnant. The chylomicron remnants are degraded in the lysosomes of the liver cells. During this process the cholesteryl esters undergo cleavage and free cholesterol is generated. One of several things can happen to this free cholesterol:

(i) it can be used in the formation of endogenous lipoproteins;
(ii) it can be stored within hepatocytes as cholesteryl esters;
(iii) it can be excreted into the bile as cholesterol or bile acids; or
(iv) it can be used for the synthesis of membranes and steroid hormones (Brown and Goldstein, 1985; Castelli, 1986).

(b) ENDOGENOUS PATHWAY

The liver is an additional source of triglyceride. After a meal rich in fat most of the free fatty acids released by the action of lipoprotein lipase are conveyed to the liver where they undergo re-esterification to triglycerides.

Furthermore excess calories from carbohydrate, protein, and alcohol are converted to fatty acids and then to triglycerides.

Sometimes, an increased intake of saturated fatty acids causes an increased production of cholesterol.

From all of these activities, the liver produces very low density lipoproteins (VLDL). The VLDL's consist mainly of 51% triglyceride and cholesterol (20% by weight) formed from chylomicron remnants and from acetyl-coenzyme A. They also contain APO B 100, as well as C, E, and A APO's. Their primary function is to convey these endogenous triglycerides to the peripheral tissues.

The first step in the endogenous pathway is the release of cholesterol and triglycerides as components of very-low-density-lipoprotein (VLDL) from the liver. The triglyceride component of the VLDL is cleaved in the capillaries by the same lipoprotein lipase enzyme that is involved in the digestion of the chylomicrons. The end-

product of this digestion is a VLDL remnant or intermediate density lipoprotein (IDL) which is fairly enriched in cholesterol ester. Once this IDL particle dissociates from the capillary endothelium and re-enters the circulation one of two things can happen to it :

(i) it can either be rapidly cleared by the liver or

(ii) it can remain in the circulation.

The IDL particles are taken up by the liver by a receptor-mediated mechanism. The low density lipoprotein (LDL) receptor is responsible for this uptake and it binds apoprotein E or B-100 containing lipoproteins.

The LDL receptor has a dual role :

(i) by enhancing the uptake of IDL it suppresses LDL synthesis and

(ii) it mediates cellular uptake of LDL thus actively promoting LDL metabolism.

Approximately 50 % of the IDL particles remain in the circulation. The major portion of the remaining triglyceride is removed resulting in the core becoming further enriched in cholesterol ester. The resultant particle is called a low density lipoprotein (LDL) and consists of 45% cholesterol by weight and 11% triglyceride.

Cholesterol is transferred to the peripheral cells where it is used in new cell formation.

The LDL has a half-life of 1.5 days in the circulation. Two-thirds are metabolized after binding to LDL receptors in liver and other body cells whilst one-third is degraded by receptor-independent means. Significantly 60 - 70 % of total plasma cholesterol is found in LDL which is the major transport lipoprotein for endogenous cholesterol.

Free cholesterol continually enters the circulation as the body cells die and the "cell membranes undergo turnover".

This free cholesterol is adsorbed onto high density lipoprotein (HDL) particles where the enzyme lecithin : cholesterol acyltransferase (LCAT) promotes its esterification with a long-chain fatty acid.

A cholesteryl ester transfer protein transfers these cholesteryl esters from HDL to VLDL or LDL particles.

Therefore (as illustrated in Fig. 3) the cycle by which cholesterol is transferred to the tissues, and is returned to new LDL particles by means of the combined actions of HDL, LCAT, and the transfer protein is complete (Goldstein and Brown, 1983; Brown and Goldstein, 1985; 1986; Troxler and Schwertner, 1985; Schaefer and Levy, 1985; Castelli, 1986).

It is thus clear that whilst the exogenous lipids are transported in chylomicrons and their remnants, the endogenous cholesterol and triglycerides are transported in VLDL, LDL, IDL and HDL.

4. LIPID EXCRETION

Approximately 1 g/day of cholesterol is eliminated from the body. About half of this is converted to bile acids and eliminated in the faeces whilst the rest is excreted as neutral steroids. A great portion of the bile salts return to the liver via the portal circulation and is re-excreted in the bile. This is termed the enterohepatic circulation (Murray et al., 1988).

(E) CHOLESTEROL HOMEOSTASIS

Oliver (1976) contends that both cholesterol synthesis and the enterohepatic circulation of bile acids form an essential homeostatic mechanism.

Some of the bile acids that are released into the duodenum are reabsorbed in the ileum and transported back to the liver. If this return is reduced, the liver increases its production of bile acids and vice versa. Hence, the enterohepatic circulation adjusts the body's response to variations in dietary cholesterol intake and possibly acts

in a "protective" way.

Furthermore, Hassan (1986) informs that it is generally accepted that the liver is the most important organ in the maintenance of cholesterol homeostasis. He adds that whilst HMG-CoA reductase is the rate limiting enzyme in the cholesterol biosynthetic pathway, the rate limiting enzyme in the biosynthesis of bile acids from cholesterol is most probably hepatic cholesterol 7 alpha-hydroxylase.

Lin and Connor (1980) observed that cholesterol homeostasis in the body is governed by an interplay of cholesterol absorption, synthesis, storage, and excretion.

They carried out a long-term (up to 25 weeks) sterol balance study. Their results showed that compensatory mechanisms to increased dietary cholesterol included "incomplete cholesterol absorption" by the intestine, "reduction in cholesterol synthesis, and increase in faecal steroid excretion of bile acids and/or neutral steroids".

In their study the suppression of cholesterol biosynthesis through the feedback inhibition of dietary cholesterol and an increase in faecal bile acid excretion was observed.

On the other hand, Quintao et al. (1971) found an increased re-excretion of cholesterol i.e. endogenous faecal neutral

steroids - this suggests that "absorbed cholesterol is re-excreted through the liver before being converted to bile acids." Nevertheless, a suppression of cholesterol biosynthesis was also observed.

Sterol balance studies show that individuals exhibit varying extents of suppression (Quintao et al., 1971; Pyorala, 1987).

The variability of individuals with respect to absorption of dietary cholesterol is emphasized by McNamara (1985) and Kesaniemi and Miettinen (1986).

McNamara (1985) reports that experiments have demonstrated that the human adult absorption range of dietary cholesterol was from as little as 20% to as much as 85% with an average of 50-60%.

Keys (1985) in his reply to McNamara emphasizes that other contributory factors are differences in the reabsorption of cholesterol in the bile, in cholesterol biosynthesis and in the direct absorption of dietary cholesterol.

(F) EFFECT OF DIETARY CHOLESTEROL ON PLASMA LIPOPROTEINS

Although it has been demonstrated that elevated plasma cholesterol levels in response to a rise in cholesterol consumption is primarily due to an increase in LDL cholesterol, controversy surrounds the mechanism by which this occurs.

Kinetic studies have shown that this occurs by an increase in the rate of LDL synthesis and a decrease in the fractional removal rate of LDL which is probably caused by suppression of LDL receptor activity.

Some researchers have reported that there is a change in the composition of LDL particles with the core becoming enriched in cholesterol esters at the expense of triglycerides (Pyorala, 1987; Grundy et al., 1988). Yet other researchers found no changes in either the physical properties or the composition of LDL and concluded that an increase in the quantity of LDL was responsible for the increased plasma LDL cholesterol (Pyorala, 1987).

Increased dietary cholesterol also affects the chylomicron by increasing its cholesterol concentration. Therefore the chylomicron remnant would also be cholesterol-enriched. It is also suggested that an increased intake of dietary cholesterol affects triglyceride-rich endogenous lipoproteins (Grundy et al., 1988).

(G) DIFFERENCES BETWEEN INDIVIDUALS IN PLASMA CHOLESTEROL RESPONSE TO CHANGES IN DIETARY CHOLESTEROL

Research has shown that individuals exhibit pronounced differences in plasma cholesterol response with respect to identical changes in dietary cholesterol. This range in response is from little to moderate to marked.

Beynen and Katan (1985a) conducted this experiment in order to determine whether free-living individuals show "consistent differences" in plasma cholesterol response to a low cholesterol diet.

Thirty four subjects that had participated in an experiment in 1976 and who habitually ingested a minimum of 1 egg per day were used. It was found that the large differences between subjects that was observed in the 1976 experiment with respect to plasma cholesterol response on cessation of egg consumption was still partly reproducible in 1982-83.

Beynen and Katan (1985b) performed two identical experiments to investigate the reproducibility of plasma cholesterol response to dietary cholesterol. In these experiments 6 healthy volunteers consumed a low cholesterol diet for 10 days and then added 6 egg yolks per day, for a further 10 days, to their diet. This increased the cholesterol intake by 1600 mg/day. It was found that the mean serum total cholesterol level increased by 13% with

the major portion being resident in LDL cholesterol which rose by 21%.

It was suggested that the rise in serum total cholesterol was due to the cholesterol in the egg yolk as opposed to the monounsaturated fat.
It was observed that the response for a selected individual was not reproducible. This was explained by " 'spontaneous' intra-individual fluctuations" in the level of serum cholesterol.

Katan et al. (1986) reports on 3 controlled trials (using healthy volunteers) performed to assess the effect of dietary cholesterol provided by egg yolk on serum cholesterol levels. The aim of these experiments was to ascertain whether individuals respond consistently to a change in dietary cholesterol. Consequently, those with a high response were categorized as hyperresponders and those with a low response as hyporesponders.

In the first trial these "putative hyper - and hyporesponders" were identified whilst the second and third served to assess the reproducibility of these subjects serum cholesterol response to dietary cholesterol.

It was found that the individual response was only partly reproducible. However, the selected hyperresponders exhibited much higher plasma cholesterol responses than the hyporesponders.

It was also found that subjects that were hyperresponsive in one trial were hyporesponsive in another. It was concluded that this was due to the "diet-independent within-person variability of serum cholesterol".
These studies show "that on average, dietary cholesterol from egg yolk does raise serum cholesterol".

The study by Oh and Miller (1985) also demonstrates the variability of plasma cholesterol response among free-living individuals. They found "2-stage thresholds of response" to cholesterol consumption.

According to Beynen and Katan (1986) the above-mentioned study "nicely illustrates the impact of within-person fluctuations of serum cholesterol on the classification of subjects as hypo- or hyperresponsive on the basis of only one cholesterol determination on a low and high cholesterol diet. Such a selection procedure will cause the inclusion in the hyperresponder group of some subjects whose serum cholesterol through chance fluctuations happened to be depressed at the beginning or elevated at the end of the high cholesterol diet period".

Flynn et al. (1986) reports on a 22 week cross-over study in which 2 groups of free-living normolipemic healthy subjects ate 3 eggs/day for 10-12 weeks and no eggs for 10-12 weeks. Group A subjects on the 3 egg diet for 10 weeks showed statistically significant increases in average plasma cholesterol level with a significant decrease within two weeks of crossing over to the egg-free diet.
In Group B a significant decrease in plasma cholesterol was seen following their initial egg-free period of 12 weeks. However, no longitudinally significant increase was observed when they then consumed 3 eggs/day for 10 weeks.

Thus the variability of plasma cholesterol response to dietary cholesterol consumed by free-living individuals is shown in this investigation, too. No significant change was observed in the average plasma triglyceride levels. Porter et al. (1977) and Flynn et al. (1979) performed similar trials and made similar observations.

Grossman (1988) examined intra-individual variations of plasma cholesterol levels in office patients. This retrospective study confirmed that plasma cholesterol levels may be variable in individuals.

Ahrens (1984) claims that he has, in his studies over the years, measured the heterogeneity of response to diet. He asserts that it is difficult to differentiate between responders and non-responders.

(H) ATHEROSCLEROSIS AND CORONARY HEART DISEASE (CHD)

1. CHOLESTEROL

Alolf Windaus, while working on the structure of cholesterol, discovered "that the atheromatous aorta contained up to 20 times as much cholesterol and cholesterol esters as the normal aorta".
James Herrick thereafter identified myocardial infarction and associated it with coronary atherosclerosis. None of these researchers related dietary cholesterol to atherosclerosis (McGill, 1979).

According to Lee (1988), although CHD has a multifactorial aetiology, there is little doubt that one of the most predictive factors is serum total cholesterol.

Oh and Miller (1985) contend that the widely accepted idea is that the cholesterol component of the egg elevates the blood cholesterol level, resulting in cholesterol being deposited in the coronary arteries and hence enhancing atherosclerotic heart disease.

The role of cholesterol in the aetiology and development of atherosclerosis has been extensively researched (Ganong, 1983). According to Zilversmit (1979), "most scientists working in the atherosclerotic area see cholesterol as a pivotal element in the aetiology of cardiovascular disease".

According to the Consensus Conference on Lowering Blood Cholesterol to Prevent Heart Disease (1985), there is much evidence (eg. genetic and epidemiological) that shows a cause-and-effect relationship between hypercholesterolaemia and CHD.

Burley and Turner (1983) state that every change of 100 mg/dl in dietary cholesterol alters the serum cholesterol by 6 mg/dl and this can significantly affect CHD risk.

According to Troxler and Schwertner (1985) "atherosclerosis results when the influx of cholesterol in the arterial wall exceeds the egress from the tissues".

Thompson (1989) reports that the fibrous plaque is the characteristic lesion of atherosclerosis. It consists of an outer cap of smooth muscle cells and collagen and an inner core of foam cells and cholesterol crystals. Lipid (mainly cholesterol) contributes up to 45% of the lesion. This cholesterol is mainly from the blood. Under conditions of diet-induced hypercholesterolaemia monocytes attach to the endothelium of arteries. They penetrate the endothelium, accumulate lipid and become foam cells or macrophages that have accumulations of cholesterol esters.

Following years of hypercholesterolaemia, there is retraction of the endothelium covering clusters foam cells (fatty streaks) which may expose the macrophages to the circulation. This leads to adherence of platelets, with thrombus formation in the arterial wall. This results in proliferation of smooth muscle cells and eventually a proliferative lesion.

According to Guyton (1986), with time fibroblasts cause sclerosis of the arteries. Thereafter calcium is deposited to form calcified plaques. By this time the arteries are hardened and the disease is referred to as arteriosclerosis.

The Lipid Research Clinics Programme (1984) reported a 2% decrease in the number of deaths due to CHD for every 1% fall in serum cholesterol initiated by cholestyramine. Indications were that LDL-cholesterol was the most important contributory factor because the greater decrease in CHD risk was found in the group with the greater reduction in LDL-cholesterol. Troxler and Schwertner (1985) claim that this was also supported by other researchers. Thus it is apparent that decreasing serum cholesterol decreases the number of deaths due to CHD. However, Troxler and Schwertner (1985) also state that "the assumption that this can be accomplished by merely lowering dietary cholesterol is both unwarranted and unscientific". Moreover, since cholesterol is also manufactured by the

body (about 85% by the liver) more attention should be focussed on those factors responsible for the regulation of endogenous cholesterol synthesis under varying levels of dietary cholesterol.

Plasma LDL and HDL levels appear to be involved in atherosclerosis. Furthermore it seems that there is an inverse relationship between HDL levels and the disease (Walker, 1980; Levy, 1986). Some investigators believe that the total cholesterol to HDL-cholesterol ratio is most predictive of CHD. This is explained by taking into account the fact that total cholesterol moves to the tissues whilst HDL removes deposited cholesterol away from the tissues. It has also been demonstrated that the seriousness of atherosclerosis is directly related to the total-cholesterol to HDL-cholesterol ratio (Ganong, 1983; Troxler and Schwertner, 1985; Castelli and Anderson, 1986; Murray et al., 1988).

Grundy et al. (1988) reports that a review of "four investigations found a positive association between the cholesterol intake and subsequent rates of CHD, and in some studies, this association appeared to be independent of plasma cholesterol concentration".

According to Grundy et al. (1988) it has been proposed that "postprandial lipoproteins containing dietary cholesterol are atherogenic".

As a result of animal work and in vitro studies that suggest "that remnants of triglyceride-rich lipoproteins could be atherogenic, several investigators have continued to speculate that induction of cholesterol-rich chylomicron remnants by dietary cholesterol may be an atherogenic factor that is independent of fasting cholesterol levels".

It is also noted that investigations have shown that increased dietary cholesterol consumption increases the cholesterol content of chylomicrons and their remnants which should enhance their atherogenicity.

Similarly, dietary cholesterol could either increase the cholesterol content of VLDL remnants or inhibit their hepatic uptake - both of which may enhance their atherogenicity (Grundy et al., 1988).

2. TRIGLYCERIDE

Hulley et al. (1980) reports that there is no strong support for a causal relationship between triglycerides rich in polyunsaturated fatty acids and CHD.

However, according to Carlson and Bottiger (1981), a 14.5 year follow-up of 3486 men showed that plasma triglyceride was an independent risk factor for ischaemic heart disease.

(I) MOTIVATION

Egg is a convenient source of dietary cholesterol and is commonly consumed in excess. It therefore accounts for a large percentage of hypercholesterolaemia with the cholesterol being deposited in the coronary arteries thus promoting atherosclerosis (Oh and Miller, 1985).

Therefore, eggs were used in the present study to determine the effect of dietary cholesterol and triglyceride on blood cholesterol and triglyceride levels.

In all the studies mentioned above, the experimental periods were several weeks or longer. No study was found reporting the blood levels of cholesterol and triglyceride after the ingestion of dietary egg up to say 6 hours after the meal (post-ingestive state). This forms the basis of the present study.

The purpose of this study is to determine the effect of dietary egg on human plasma cholesterol and triglyceride after ingestion of freshly boiled egg up to the post-ingestive period of 6 hours.

A preliminary study done in 1988 used too few subjects (three). Therefore, in the present study the number

of subjects used was increased.

A further consideration was whether individuals with elevated plasma cholesterol and/or triglyceride levels handled these nutrients any differently than individuals with normal plasma cholesterol and triglyceride levels. Thus in this investigation 2 groups of individuals were selected for study. Group 1 consisted of individuals with random normal baseline plasma cholesterol and triglyceride levels and group 2 consisted of individuals with random elevated baseline plasma cholesterol and triglyceride levels.

II. MATERIALS AND METHODS

In this chapter the various materials used, and the principles and procedures of the adopted methods, are outlined.

(A) SUBJECT SELECTION

In accordance with the protocol of this investigation twelve healthy, non-smoking Asian male volunteers with normal random blood cholesterol and triglyceride levels (group 1) and twelve healthy, Asian male volunteers with elevated baseline blood cholesterol and/or triglyceride levels (group 2) served as subjects.

The search for such subjects, especially group 2, was difficult and was accomplished only after approaches were made to local hospitals, medical practitioners and the general community.

The pre-requisite for the group 2 subjects was a cholesterol concentration of greater than 6.5 mmol/l. The 24 volunteers between the ages of 18 and 51 years were finally selected after about six months of searching for, and screening of numerous potential subjects.

1. HEALTH SCREENING

All subjects were examined medically to determine their suitability for participation in the study. This examination was conducted by a medical doctor and included the following :

(i) Medical history and physical examination.

(ii) Height and mass measurements.

(iii) Vital signs : Measurement of respiratory rate, radial pulse rate, blood pressure and oral temperature.

(iv) Haematological studies : Determination of haemoglobin, packed cell volume, red blood cell count, white blood cell count, differential count, platelet count and mean corpuscular haemoglobin.

(v) Urinalysis : Determination of pH and the presence or absence of protein, glucose, ketones, bilirubin and blood in the urine.

(vi) Measurement of blood glucose concentration after 12 hours of fasting.

2. ETHICAL CONSIDERATIONS

Preceding the trial, the nature and purpose of the research to be undertaken including the risks involved were explicitly explained to all the subjects.

The voluntary nature of the study was emphasized and they were informed that they could withdraw from the study at any time. They were also requested to sign the "Drug Studies Unit Informed Consent Form" (Appendix 1) in the presence of a witness.

This study was carried out in conformance with the local legal requirements as well as the recommendations for clinical trials in man, as set out in the "Declaration of Helsinki" (Venice Amendment, 1983).

Furthermore, the study was approved by the Ethics Committee of the University of Durban-Westville.

(B) HAEMATOLOGICAL STUDIES

The Haematological analyses were performed at the Pathology Laboratory of Ferguson, Roux and Partners in Durban.
The "Technicon H*1" system was used (Simson, Long Island Jewish Medical Centre, New York).

1. PRINCIPLE OF THE "TECHNICON H*1" SYSTEM

The principle is that of flow cytometry. It involves the following steps :

(i) preparation of the blood by cyto-chemical reactions.

(ii) measurement of specific cell properties by a cytometer (that is based on differential light scattering).

(iii) the conversion of these values into readings for cell count, for example, by algorithms.

The four channels making up this system are the haemoglobin, peroxidase, red cell/platelet and the basophil/lobularity (nuclear) channels.

(a) Principles of the Four Channels

Haemoglobin : The red blood cells are broken down by a surfactant which releases the haemoglobin. The haem portion combines with cyanide and this mixture is then read in a colorimeter at 546 nm.

Peroxidase : This channel is used for the white blood cell count and the differential count (except basophils).

The white blood cells are fixed and stained using a chromogen and hydrogen peroxide. Two detectors in an optical channel detect the light scattered and the intensity of staining thus characterizing the cells.

Basophil/lobularity (nuclear) channel : Under the action of a surfactant at low pH only the basophils remain intact enabling them to be counted (by a nuclear channel cytometer which can distinguish differences in nuclear shape).

RBC/Platelets : The optical assembly is the same as that for the nuclear channel. Cellular volume (size) and optical density (haemoglobin concentration) are measured by the light scattered at low and high angles. A computer converts these signal pairs into a cytogram, one histogram for red cell volume and another for haemoglobin concentration. The packed cell volume and mean corpuscular haemoglobin are calculated from the measured haemoglobin, red blood cell count and the mean corpuscular volume. The high angle detector measurements are used to produce a platelet histogram.

(C) URINALYSIS

1. PRINCIPLES OF THE VARIOUS TESTS

(i) Blood - The peroxidase-like activity of haemoglobin catalyzes the reaction of cumene hydroperoxide and 3,3',5,5'-tetra-methylbenzidine. The colour changes from orange through green to dark green.

Reagents: Cumene hydroperoxide, 3,3',5,5'-tetra-methylbenzidine, buffer and nonreactive ingredients.

(ii) Protein - This test is based on the error-of-indicators principle. If protein is present a green colour develops.

Reagents: tetrabromphenol blue, buffer and nonreactive ingredients.

(iii) pH - This test is based on the double indicator principle. It has a colour range from orange through yellow and green to blue; and a pH range from 5 to 8,5.

Reagents: methyl red, bromothymol blue and nonreactive reagents.

(iv) Glucose - This test involves a double sequential reaction:-

(a) Glucose is converted to gluconic acid and hydrogen peroxide by the action of the enzyme glucose oxidase.

(b) Peroxidase catalyzes the reaction of hydrogen peroxide with a potassium iodide chromogen resulting in a green to brown colour.

Reagents: Glucose oxidase, peroxidase, potassium iodide, buffer and nonreactive reagents.

(v) Bilirubin -Bilirubin is coupled with diazotized dichloroaniline in a strongly acidic medium. The colour change is from tan to purple.

Reagents: 2,4-dichloroaniline diazonium salt, buffer and nonreactive reagents.

(vi) Ketones - acetoacetic acid reacts with nitroprusside. The colour ranges from buff pink to maroon as positivity increases.

Reagents: Sodium nitroprusside and buffer.

2. PROCEDURE

Each subject's freshly voided urine sample was analyzed immediately using the Ames Bili-labstix reagent strips. These firm, plastic reagent strips have separate reagent areas for the determination of blood, bilirubin, ketones, glucose, protein and pH. This allows the status of carbohydrate metabolism, kidney and liver functions and acid based balance to be assessed.

A reagent strip was dipped into the urine sample and the test areas were matched with the corresponding colour charts on the bottle label.

Reading times were specified for each test, for example whilst the glucose readings were to be taken at 10 seconds, bilirubin values were read at 20 seconds.

(D) BLOOD GLUCOSE CONCENTRATION

1. PRINCIPLE AND PROCEDURE

Blood glucose concentration was measured by using the Ames Glucometer II. The reagent strip used in this method has the enzyme glucose oxidase as well as a colour reagent. A drop of blood obtained by finger prick, is placed on the

reagent strip. The glucose fraction is converted to gluconate and H_2O_2 by the action of glucose oxidase. The H_2O_2 reacts with the colour reagent to form a coloured complex. The glucometer reads the absorbance of this coloured complex and converts it into a glucose concentration reading.

(E) DIETARY RECORDS

The volunteers were also requested to record their daily food intake for seven days starting a week prior to conducting the study so that their normal diets could be assessed.

Their average daily intake of the various nutrients was then calculated with the use of the NRIND Food Quantities Manual (Langenhoven et al., 1986) and NRIND Food Composition Tables, (Gouws and Langenhoven, 1986).
These tables give the nutrient distribution per 100g of a particular food type. Therefore, if a volunteer consumed more or less than 100g of that food type, the nutrient distribution had to be calculated proportionately.

Since volunteers had widely varying diets the calculation of these values was a long and ardous task.

1. PROCEDURE FOR CALCULATION OF DAILY NUTRIENT INTAKE

The 7 day dietary records of each subject was used to convert food intake into nutrient intake. Hence the totals of each nutrient contained in all the food consumed over the 7 days were calculated. These values were then divided by 7 to get the average daily intake of each nutrient.

The individual nutrient values were calculated as follows:

Subject 1 of group 1 had consumed 40 different food items over the 7 days. One such item is "60g of peanuts (salted)".

Now, the Food Composition Manual (1986) give the quantities of each of 35 nutrients per 100g of food item. Thus in 100g of salted peanuts for example, there is 10.2g of total carbohydrate (TOTCHO) (See NRIND, Food Composition Tables, 2nd edition, (1986), page 59, code 6007).

Hence calculating proportionately, in the 60g of salted peanuts there is :

(60 x 10.2)/100 = **6.12g** of total carbohydrate.

Likewise, for each of the 35 nutrients listed in the Food Composition Tables, the quantities of each nutrient consumed per 60g of peanuts was calculated.

This entire set of 35 calculations (once for each nutrient) was repeated for all 40 different food items consumed by Subject 1 of group 1 as listed in Appendix 2.

Similarly, tables were drawn up for each of the 24 subjects.

Approximately 26 680 calculations had to be done to draw up these tables, i.e.

$$\pm 32(\text{food items}) \times 35(\text{nutrients}) \times 24(\text{subjects})$$

$$= \pm 26\ 680 \text{ calculations !}$$

(F) STUDY PROCEDURE AND COLLECTION OF BLOOD SAMPLES

The experimental procedure was carried out in the Department of Physiology, University of Durban-Westville under the supervision of a medical doctor.

The volunteers were given detailed instructions concerning the trial performance. It was emphasized that they present themselves on the trial days following an overnight fast of at least twelve hours.

The sequence of the trial days was such that :

The group 1 subjects received egg yolk (100 g) on their first trial day and whole egg (100g of egg yolk plus 200g of egg white) on their second trial day. The group 2 subjects had just one trial day on which they received whole egg.

With the exception of the type of egg ingested the sequence of events followed on the various days of the trials was identical - the details of which are outlined below.

Each subject was initially required to deliver a urine sample which was analyzed for the above-mentioned parameters.

An indwelling cannula (18 gauge; 44.5 mm long) was then inserted into a major upper limb vein in each subject (Figs. 4 and 5). The 18 gauge cannula was chosen in order to prevent haemolysis.

A baseline blood sample was drawn immediately, with a second baseline sample being drawn fifteen minutes later.

In the intervals between the drawing of samples the cannula was kept patent by a slow infusion of normal saline. Approximately 500 ml of saline was infused into the bloodstream over the 6 hour study period. Blood was drawn using a three way stopcock that was fitted onto the modified saline drip. The initial 3 - 5 mls drawn consisted of a mixture of blood and saline and was therefore discarded together with the syringe.

A new syringe was used to draw the next 5 mls of blood which was collected into a 5 ml heparinized vacutest tube. Thus the used syringe was used to draw the "sample" to be discarded whilst a new syringe was used to draw the sample to be analyzed.

After the baseline samples were drawn, the subjects consumed freshly hard-boiled egg yolk (100g) or whole egg (egg yolk 100g plus egg white 200g), depending on the trial being undertaken, and then 200 mls of water.

Fig. 4. The attachment of the drip to the subject's arm

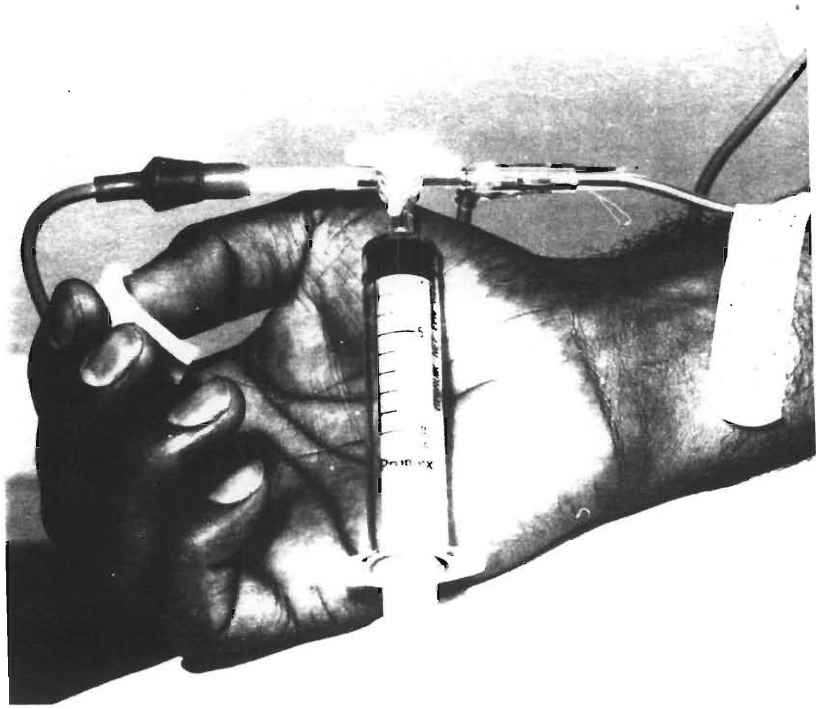

Fig. 5. Close-up of the attachment of the drip to the subject's arm. Blood was withdrawn at regular intervals, using a syringe, by turning the stopcock 90 degrees to the left

Further blood samples were then drawn at the following time intervals : 1 hour, 2 hours, 2.5 hours, 3 hours, 3.5 hours, 4 hours, 4.5 hours, 5 hours, 6 hours.

These time intervals were chosen after an analysis of the results obtained from a pilot study (1988) in which blood samples were drawn at 15 minute intervals. It was found that the triglyceride concentration peaked between 2.5 and 3.5 hours and on this basis it was decided that the above-mentioned time intervals were appropriate.

The subjects were also allowed to drink 200 mls of water at the end of each 2 hour period. During the course of the experimental procedure they were allowed to sit and read or watch a film.

On the day of each test 11 blood samples (i.e. a maximum of 110 mls of blood) were drawn from each subject. These blood samples were centrifuged immediately (at maximum speed in the Rotor Unit II for ten minutes). Two aliquots of plasma was then transferred from each sample into labelled sample tubes which were stored frozen until assayed.

The plasma cholesterol and triglyceride concentrations were determined by means of the TD_x System (Abbott Laboratories Diagnostic Division, System Assays II, 1985) in the Drug Studies Unit at the University of Durban-Westville.

(G) CHOLESTEROL DETERMINATION

1. PRINCIPLE

The TD_x REA cholesterol assay is a reagent system for the quantitative measurement of plasma cholesterol (TD_x System, Systems Assay II, 1985). This assay utilizes a technique known as Radioactive Energy Attenuation (REA).

In this assay the coupled enzymatic reactions of a lipolytic enzyme, cholesterol oxidase and horseradish peroxidase generate the chromophore. The amount of chromophore produced is directly proportional to the cholesterol level of the specimen. (The fluorescent compound used in this assay is chemically non-reactive.)

2. REACTIONS

Cholesterol esters $\xrightarrow[\text{enzyme}]{\text{cholesterol esterase}}$ free fatty acids + cholesterol.

Cholesterol $\xrightarrow[\text{oxidase}]{\text{cholesterol}}$ cholest-4-en-3-one + H_2O_2.

H_2O_2 + 4AAP + DHBS $\xrightarrow[\text{peroxidase}]{\text{horseradish}}$ quinoneimine dye.

Note : DHBS = 3.5 - dichloro-2-hydroxybenzenesulphuric acid

4 AAP = 4-aminoantipyrine.

A standard curve is generated to establish the precise relationship between the concentration of cholesterol and the measured fluorescence intensity. This is achieved by running cholesterol calibrators and measuring the attenuated fluorescence signal. Thereafter, when a sample of unknown cholesterol concentration is run, its concentration is calculated from the stored calibration curve.

3. REAGENTS

The TD_x REA cholesterol reagent pack that was used contained the following reagents:

(i) P Substrate Fluorescein Solution

DHBS, 4 AAP, fluorescein and surfactant in Solvent (3 ml).

Preservative : 0.1% sodium azide.

(ii) S Enzyme Solution

Lipolytic enzyme, cholesterol oxidase and horseradish peroxidase (3 ml).

Preservative : 0.1% benzoic acid.

(iii) T Buffered Solution

Potassium ferrocyanide in sodium phosphate buffer (3 ml).

Preservative : 1.1% sodium azide

The TD_x cholesterol calibrators consisting of 6 vials with accurately measured amounts of free cholesterol in solution at the following concentrations (Table 1) were used to calibrate the Autoanalyser.

Table 1. Concentration of cholesterol calibrators

VIAL	CHOLESTEROL CONCENTRATION (mmol/l)
A	0.00
B	1.30
C	2.59
D	5.18
E	7.77
F	10.36

The TD_x REA cholesterol controls consisted of 3 vials of free cholesterol in solution that read within the following ranges (Table 2) and were accepted.

TABLE 2. CONCENTRATION OF CHOLESTEROL CONTROLS

VIAL	CHOLESTEROL CONCENTRATION (mmol/l)
LOW	1.68 - 2.20
MEDIUM	5.82 - 7.12
HIGH	8.15 - 9.97

Note : At the beginning of each run and when a new kit was used, the controls were tested to ensure that the system was still standardized.

4. PROCEDURE

The volume of sample used in the sample cup was 50 microlitres.

Since cholesterol calibrators and controls contain a volatile solvent they were capped securely to prevent evaporation.

Cholesterol calibrators and controls tend to foam when pipetted, therefore droppers which cause less foaming than micropipettes were used in the transfer of these solutions.

All bubbles were removed from the sample cartridge before the assay was run.

In order to obtain an acceptable REA cholesterol calibration curve it was ensured that the Percent Fluorescence Intensity Error (ERR) was less than $\pm$ 0.8 for all the calibrators; and that the Root Mean Squared Error (RMSE) was less than 0.5 and that the Low, Medium and High controls were all within the acceptable ranges.

5. SPECIFIC PERFORMANCE CHARACTERISTICS

(i) Sensitivity : The lowest measurable level, defined as that concentration of cholesterol distinguishable from zero with 95%

confidence, was 0.16 mmol/l. The concentration ranges of the TD_x REA cholesterol calibrators (0 - 10.36 mmol/l) is adequate to accurately determine the cholesterol concentration in most samples, however, when concentrations exceed 10.36 mmol/l, 'HI' is printed out instead of a result.

(ii) Specificity : The REA cholesterol assay measures total cholesterol in serum or plasma and is specific for cholesterol.

(iii) Precision : Reproducibility studies yielded coefficients of variation of less than 5%.

(iv) Accuracy by correlation with reference assays : Comparison with established methods yielded correlation coefficients ranging between 0.990 and 0.998.

(H) TRIGLYCERIDE DETERMINATION

The TD_x REA triglyceride assay is a reagent system for quantitative measurement of plasma triglycerides (TD_x System, Systems Assays II, 1985).

1. PRINCIPLE

This assay is similar to that for cholesterol except that here the combined catalytic activities of lipase, glycerol kinase, glycerol phosphate dehydrogenase and diaphorase generate the chromophore.

2. REACTIONS

Triglycerides $\xrightarrow{\text{Lipase}}$ glycerol + free fatty acids.

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

NAD^+ + glycerol-3-phosphate $\xrightarrow{\text{G-3-PDH}}$ dihydroxyacetone phosphate + NAD + H^+

NADH + H^+ + MTT $\xrightarrow{\text{DIA}}$ NAD^+ + formazan - MTT

NOTE :
- ATP = Adenosine triphosphate
- ADP = Adenosine diphosphate
- NAD(H) = Nicotinamide adenine dinucleotide (reduced)
- G-3-PDH = Glycerol-3-phosphate dehydrogenase
- MTT = Monotetrazolium dye
- DIA = Diaphorase

The triglyceride concentration of a sample of unknown triglyceride concentration is determined in the same way as in the cholesterol concentration determination.

3. Reagents

The TD_x REA triglyceride reagent packs contained the following:

(i) P Indicating solution
Flourescein and monotetrazolium dye (MTT) in solvent (3 ml).

(ii) S Substrate solution
Nicotinamide adenine dinucleotide, adenosine 5 -triphosphate and magnesium chloride in buffer containing stabilizers (3 ml).
Preservative : 0.05% sodium azide.

(iii) T Enzyme solution
Lipase, glycerol kinase, glycerol phosphate dehydrogenase and diaphorase in buffer and protein stabilizers (3 ml).
Preservative : 0.02% sodium azide.

The TD_x REA triglyceride calibrators consisting of 6 vials with accurately measured amounts of glycerol in serum equivalent to the following triglyceride concentrations (Table 3) were used to calibrate the Autoanalyzer.

TABLE 3. CONCENTRATION OF TRIGLYCERIDE CALIBRATORS

VIAL	TRIGLYCERIDE CONCENTRATION (mmol/l)
A	0.00
B	1.13
C	2.26
D	3.39
E	4.52
F	5.65

Preservative: 0.1% sodium azide.

The TD_x REA triglyceride controls consisted of 3 vials of triglycerides in serum that read within the following ranges (Table 4) and were accepted.

TABLE 4. CONCENTRATION OF TRIGLYCERIDE CONTROLS

VIAL	TRIGLYCERIDE CONCENTRATION (mmol/l)
LOW	1.20 - 1.63
MEDIUM	2.54 - 3.11
HIGH	3.56 - 4.35

Preservative: 0.1% sodium azide.

Note : At the beginning of each run and when a new kit was used, the controls were tested to ensure that the system was still standardized.

4. PROCEDURE

The volume of sample used in the sample cup was 100 microlitres.

After each triglyceride carousel the probe was cleaned.

In order to obtain an REA triglyceride calibration curve it was ensured that the Percent Fluorescence Intensity Error (ERR) was less than $\pm$ 1.50 for all the calibrators; the Root Mean Squared Error (RMSE) was less than 0.65 and that the Low, Medium and High controls were all within the acceptable ranges.

5. SPECIFIC PERFORMANCE CHARACTERISTICS

(i) Sensitivity : The lowest measurable level, defined as that concentration of triglycerides distinguishable from zero with 95% confidence, was 0.11 mmol/l. The concentration range of the TD_x REA triglyceride calibrators (0 - 5.65 mmol/l) is adequate to accurately determine the triglyceride concentrations in most samples, however, when concentrations exceed 5.65 mmol/l 'HI' is printed out instead of a result.

(ii) Specificity : The enzymes (lipase and glycerol kinase) and the indicator system (glycerol-3-phosphate dehydrogenase, diaphorase and monotetrazolium dye) is highly specific for triglycerides and glycerol.

(iii) Precision : Reproducibility studies yielded coefficients of variation of less than 5%.

(iv) Accuracy by correlation with reference assays : Comparison with established methods yielded correlation coefficients ranging between 0.993 and 0.998.

(I) LIPOPROTEIN ELECTROPHORESIS

Serum from the subjects with elevated cholesterol and/or triglyceride levels (group 2) was, in addition to the above-mentioned tests, subjected to an electrophoretic separation of the lipoproteins. This was done in an attempt to determine the type of hyperlipidaemia prevalent in the various subjects.

1. PRINCIPLE

When lipoproteins are placed in a buffer of pH 8.6, they acquire an electric charge and therefore migrate towards one of the electrodes when placed in an electric field.

Lipoprotein particles which consist essentially of lipid and protein components in varying proportions have varying physical properties. Whilst the lipids are essentially neutral the proteins usually acquire a charge depending on the difference between their isoelectric point (pI) and the pH of the medium. Proteins with a pI less than the pH of the medium become negatively charged (by donating a proton) and where the pI is greater than the pH of the buffer they become positively charged (by accepting a proton).

It is thus clear that the different lipoprotein particles will acquire different charges and therefore migrate at varying velocities when subjected to electrophoresis.

The velocity of this migration depends on the strength of the electric field, the nett electrical charge on the protein and the frictional resistance. The electrophoretic mobility of these particles increases with increasing electric field strength.

With the buffered agarose gel electrophoresis technique the plasma lipoproteins are separated into four major groups i.e. chylomicrons, beta or low density lipoproteins (LDL), pre-beta or very low density lipoproteins (VLDL), and alpha or high density lipoproteins (HDL).

HDL has the smallest diameter and hence experiences the least frictional resistance to its movement. As HDL has the highest percentage of protein, it is the most negatively charged particle. It therefore migrates the furthest and is found closest to the anode. Chylomicrons on the other hand, are the largest particles and experience the greatest resistance to movement. They have the highest percentage of fat and the lowest percentage of protein making them the least charged particles. They are therefore found closest to the origin.

Based on this, the order of these groups on electrophoresis is chylomicrons, LDL or beta-lipoprotein, VLDL or pre-beta-lipoprotein and HDL or alpha-lipoprotein, from cathode to anode.

The Beckman Paragon Electrophoresis System was utilized for this purpose.

2. LIMITATIONS

As the electrophoretic mobility of the lipoproteins could be altered by drugs, it was ascertained that none of the subjects of group 2 had taken any drugs over the last 2 weeks.

3. PROCEDURE

(i) Blood samples from subjects (who fasted for at least 12 hours prior to the test) were freshly drawn from an upper limb vein into 5 ml vacutest tubes containing EDTA as the anticoagulant. The blood and anticoagulant were mixed by gently inverting the vacutest tube 3 - 5 times. This sample was centrifuged at 3000 rpm immediately for ten minutes. One ml of the serum was then removed with a disposable pasteur pipette and placed into a plastic sample tube.

(ii) The lipo gel was removed from its foil package, placed on a paper towel on a flat surface, and blotted gently with a gel blotter.

(iii) A gel template was placed on the gel aligned with the "c" position marks on the gel.

(iv) A fingertip was gently run across the templates to ensure the seal.

(v) Using a five microlitre pipette, 3-5 microlitres of each sample was applied across each template slot corresponding to gel tracks 1-8. Thereafter the samples were allowed to diffuse into the gel for five minutes.

(vi) The template was then blotted gently with a template blotter to remove excess serum.

(vii) The gel was placed onto the gel bridge assembly such that its positive (+) and negative (-) sides were aligned with those marked on the gel bridge assembly.

This assembly (Fig. 6) was then placed into the Paragon electrophoresis cell and covered. 45 mls of the B-2 barbital buffer solution, pH 8.6 was poured into each reservoir of the Paragon Electrophoresis Cell. This solution was prepared just before use by dissolving the contents of the buffer bottle in 1500 mls of deionized water.

(viii) The Paragon Electrophoresis Cell was connected to the power supply. The voltage was set to 100 volts, and the electrophoresis process was allowed to progress for thirty minutes.

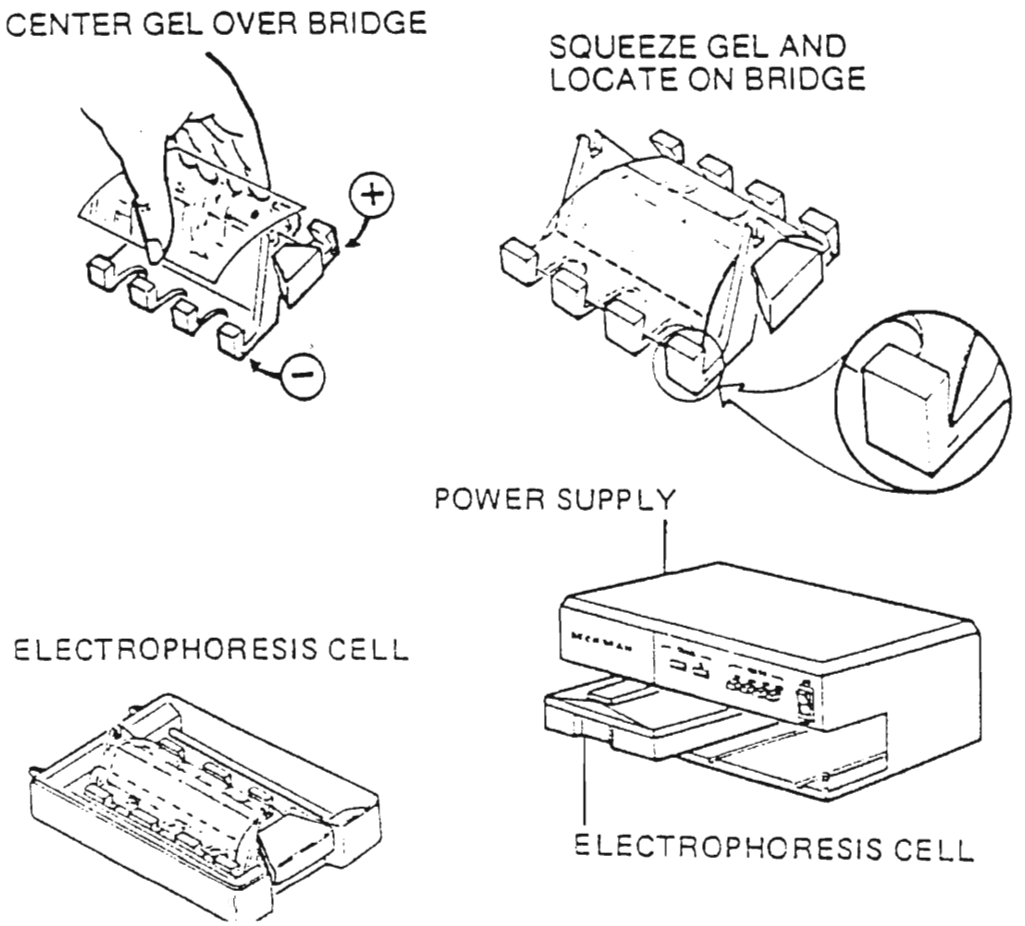

Fig. 6. Apparatus for Lipoprotein Electrophoresis

(ix) The gel was removed and placed in a gel frame which was then placed in the frame holder.

The containers of the Paragon Wet Processor were prepared by filling them with 300 mls of each of the following solutions :

(a) Fixative Solution - The fixative solution was prepared by adding 90 mls of deionized water and 30 mls of glacial acetic acid to 180 mls of reagent alcohol and then mixing thoroughly.

(b) Lipoprotein Working Stain Solution - The lipoprotein working stain solution (0.07%) was prepared by adding 3 mls of Paragon Lipostain (Sudan Black B Stain) to 165 mls of reagent alcohol (95% ethanol denatured with methanol). 135 mls of deionized water was added to this with stirring and then mixed thoroughly for ten minutes.

(c) Destaining Solutions I/II/III - The destaining solutions were prepared by mixing 550 mls of deionized water thoroughly with 450 mls of reagent alcohol.

(x) The frameholder was placed in the fixative solution for 5 minutes to immobilize the lipoproteins in the gel.

(xi) The gel was then placed flat in an oven until it was dried.

(xii) The dried gel was then placed in working stain solution for 5 minutes and then destained as detailed below :

(a)	Destain Solution I	-	three dips;
(b)	Destain Solution II	-	three dips;
(c)	Destain Solution III	-	five minutes.

(xiii) The gel was subsequently placed in an oven until completely dry.

(xiv) It was then ready for visual inspection and interpretation (Table 5).

(xv) One millilitre of the remaining serum was observed after 12 hours of standing for any change in appearance (Table 5).

Table 5. Interpretation of changes

TYPE OF HYPER-LIPIDAEMIA	LIPOPROTEIN ELECTROPHORESIS	SERUM CHOLESTEROL CONCENTRATION	SERUM TRIGLYCERIDE CONCENTRATION	APPEARANCE OF SERUM AFTER 12 HOURS
Normal	No chylomicron band, prominent beta band, faint pre-beta band.	Normal	Normal	Clear
TYPE 1	Intense chylomicron band.	Normal, slightly increased.	Increased	Creamy supernatant, clear infranatant.
TYPE 2(a)	Intense beta band.	Increased		Clear
TYPE 2(b)	Intense beta and pre-beta bands.	Increased	Increased	Moderately cloudy.
TYPE 3	Broad beta band.	Increased	Increased	Turbid
TYPE 4	Intense pre-beta band.	Slightly increased	Increased	Turbid
TYPE 5	Chylomicron and intense pre-beta band.	Increased	Increased	Creamy supernatant, cloudy infranatant.

(J) STATISTICAL ANALYSIS

The "SAS" statistical software program was applied for the statistical analysis of all the data obtained in the cholesterol and triglyceride assays of all subjects.

The extent and rate of triglyceride absorption were assessed by maximum concentration (Cmax), time to maximum concentration (tmax), and area under the concentration vs time curve (AUC). AUC was determined by the linear trapezoidal method. The mean residence time (MRT) was obtained by dividing the area-under-the-(first)-moment vs time curve (AUMC) by the AUC, i.e. MRT = AUMC / AUC.

The "Lotus 123" software program was used to manipulate the raw data as well as to draw the graphs.

III. RESULTS

In this chapter the results obtained from the various procedures are presented together with the analyses carried out.

(A) HEALTH SCREENING

1. MEDICAL HISTORY AND PHYSICAL EXAMINATION

All the volunteers were examined by a medical doctor and it was observed that all were healthy.

However, subject number 12 (group 2) reported having nine bouts of pancreatitis between 1971 and 1981. Chronic pancreatitis affects pancreatic enzyme release. As pancreatic lipase is involved in digestion of lipids it was noted that this subject might exhibit an abnormality in his metabolism of lipids i.e. a decrease both in lipid digestion and absorption.

2. HEIGHT AND MASS MEASUREMENTS

Body mass was no more than ten percent (10 %) above or below the normal mass for height and age according to the 1983 Metropolitan height and mass tables (Geigy Scientific Tables, 1984, Vol.3, p.325-6) as is reflected in Tables 6 and 7.

Table 6. Age, height and mass of group 1 subjects

SUBJECT	AGE (yrs)	HEIGHT (m)	ACTUAL MASS (kg)	IDEAL MASS (kg)
1	27	1.73	67.7	73.0
2	21	1.72	68.0	70.9
3	32	1.59	58.9	63.7
4	22	1.72	64.3	70.9
5	25	1.69	63.0	69.7
6	21	1.65	58.5	64.9
7	36	1.55	55.0	60.3
8	26	1.68	71.9	68.5
9	19	1.64	53.0	58.5
10	22	1.72	64.0	70.9
11	22	1.66	59.5	65.7
12	18	1.74	60.0	65.8

Table 7. Age, height and mass of group 2 subjects

SUBJECT	AGE (yrs)	HEIGHT (m)	ACTUAL MASS (kg)	IDEAL MASS (kg)
1	33	1.66	66.7	69.1
2	45	1.73	82.5	75.7
3	50	1.67	66.3	71.5
4	33	1.80	76.0	81.2
5	34	1.79	73.0	80.0
6	41	1.75	70.08	77.6
7	26	1.69	75.8	69.7
8	26	1.67	72.0	68.0
9	38	1.73	82.3	74.8
10	51	1.70	68.0	73.9
11	25	1.76	72.98	75.8
12	41	1.66	77.0	70.2

3. VITAL SIGNS

It is evident from Tables 8 and 9 that all subjects had normal blood pressure, oral temperature, respiratory and pulse rates.

Table 8. Vital signs (resting) of group 1 subjects

SUBJECT	RESPIRATORY RATE (per min.)	RADIAL PULSE RATE (per min.)	BLOOD PRESSURE (mmHg)	ORAL TEMPERATURE (F)
1	14	72	115/70	98.5
2	15	70	130/75	99.5
3	20	92	105/70	98.0
4	12	60	105/65	98.5
5	14	60	110/70	98.5
6	18	86	105/60	98.0
7	16	66	105/70	99.0
8	13	48	110/75	98.0
9	19	83	120/70	98.5
10	15	66	115/75	100.0
11	16	70	105/65	98.5
12	15	60	105/70	98.5

Table 9. Vital signs (resting) of group 2 subjects

SUBJECT	RESPIRATORY RATE (per min.)	RADIAL PULSE RATE (per min.)	BLOOD PRESSURE (mmHg)	ORAL TEMPERATURE (F)
1	14	64	125/70	98.0
2	14	72	120/70	98.5
3	13	71	105/65	98.5
4	15	80	110/70	100.0
5	16	88	105/75	98.5
6	12	64	115/70	99.0
7	13	70	105/65	99.5
8	12	65	110/70	98.5
9	13	63	105/70	98.5
10	19	87	105/60	101.0
11	15	60	105/70	98.0
12	14	60	110/65	99.0

(B) HAEMATOLOGICAL STUDIES

As can be seen in Tables 10 and 11 all subjects exhibited values for red blood cell count, haemoglobin, mean corpuscular haemoglobin, packed cell volume and white blood cell count that were either within, or deviated slightly from the normal range (Dacie and Lewis, 1975). This indicated that these subjects have normal blood profiles.

Table 10. Blood Profiles of group 1 subjects

SUBJECT	Hb (g/dl)	PCV (l/l)	RBC (10 /l)	WBC (10 /l)	PLATELET (10 /l)	MCH (pg)	GLUCOSE (mmol/l)
1	14.8	0.443	5.13	7.10	273	28.8	4.9
2	15.9	0.469	5.21	7.97	377	30.6	4.6
3	14.7	0.446	5.34	8.08	328	27.4	5.0
4	14.5	0.431	4.73	6.29	272	30.7	3.4
5	14.1	0.420	4.68	7.52	311	30.0	5.1
6	16.2	0.483	5.53	6.65	301	29.3	5.0
7	14.2	0.422	4.90	8.16	244	29.0	4.5
8	14.2	0.428	5.00	4.44	190	28.4	4.4
9	14.6	0.433	4.93	5.24	241	29.6	4.1
10	15.9	0.476	5.49	5.28	208	29.0	3.5
11	14.7	0.431	4.59	*3.43	310	32.0	4.5
12	16.1	0.480	5.34	6.97	205	30.1	3.6
NORMAL RANGE	13 - 18	0.40 - 0.54	4.5 - 6.1	4 - 11	150 - 440	26 - 34	

KEY : Hb - Haemoglobin; PCV - Packed Cell Volume; RBC - Red Blood Cell Count; WBC - White Blood Cell Count; MCH - Mean Corpuscular Haemoglobin; Platelet - Platelet Count.

Table 11. Blood Profiles of group 2 subjects

SUBJECT	Hb (g/dl)	PCV (l/l)	RBC (10 /l)	WBC (10 /l)	PLATELET (10 /l)	MCH (pg)	GLUCOSE (mmol/l)
1	15.5	0.466	5.19	7.00	234	29.9	4
2	14.8	0.445	5.18	5.13	267	28.6	5.3
3	15.9	0.490	5.75	10.80	280	27.6	3.6
4	*12.0	*0.384	*6.18	8.40	226	*19.4	3.6
5	14.8	0.447	4.92	4.50	184	30.2	4.5
6	15.1	0.460	5.15	5.10	336	29.3	3.3
7	16.2	0.481	5.66	7.50	288	28.6	4.2
8	17.2	0.514	6.02	5.60	189	28.6	3.4
9	15.7	0.464	5.16	8.10	212	30.5	4.3
10	*12.2	*0.378	*4.24	5.50	267	28.8	3.3
11	16.4	0.495	5.32	7.20	281	30.9	3.6
12	14.1	0.425	4.96	7.60	246	28.5	5.5
NORMAL RANGE	13 - 18	0.40 - 0.54	4.5 - 6.1	4 - 11	150 - 440	26 - 34	

KEY : Hb - Haemoglobin; PCV - Packed Cell Volume; RBC - Red Blood Cell Count; WBC - White Blood Cell Count; MCH - Mean Corpuscular Haemoglobin; Platelet - Platelet Count.

(C) URINALYSIS

On all occasions protein, glucose, ketone and bilirubin tested negatively for all subjects whilst the pH ranged between 5.5 and 7.5.

These factors led to the conclusion that carbohydrate metabolism, kidney and liver functions, as well as acid balance were normal in all subjects.

(D) BLOOD GLUCOSE CONCENTRATION

A study of Tables 10 and 11 reveals that the blood glucose levels (after a 12 hour fast) was normal for all subjects.

(E) DIETARY RECORDS

Tables 12 and 13 shows the daily nutrient intake for all the subjects.
As far as the dietary cholesterol is concerned, the intake that was recommended by the NIH Consensus Development Statement (1985) is 250 - 300 mg/day.

Whilst only four of the group 1 subjects have cholesterol intakes of less than 300 mg/day, eight of the group 2 subjects have intakes in this range.

Table 12. Daily nutrient intake for group 1 subjects

NUTRIENT	SUBJECT 1	2	3	4	5	6	7	8	9	10	11	12	AVERAGE
TOTCHO-G	270.1	146.7	77.9	208.6	267.0	235.5	125.3	557.5	257.7	251.3	221.6	204.0	235.3
VITA-RE	1058.7	195.6	662.8	681.5	494.5	756.1	255.6	2262.2	941.3	1276.3	477.1	794.3	821.3
MOIST-%	1700.3	977.2	458.8	948.2	1209.8	1191.3	639.9	2617.7	915.2	1465.5	1214.3	869.4	1184.0
FIBRE-G	19.8	2.0	5.1	7.1	14.9	12.3	9.3	57.9	10.1	30.9	10.4	11.5	15.9
THIA-MG	1.5	0.3	0.4	0.7	1.3	0.6	0.6	4.4	1.3	2.6	0.9	0.7	1.3
KILOCAL	2794.7	1330.1	747.1	1676.4	2677.0	2038.4	1157.5	6024.8	2665.8	3002.1	2154.1	1713.8	2331.8
SUGAR-G	79.5	66.3	25.7	91.2	63.0	44.0	16.3	73.7	39.5	11.8	44.4	52.2	50.6
RIBOF-MG	1.5	0.5	0.4	0.7	1.4	1.2	0.4	6.8	1.4	4.5	1.4	0.7	1.7
KILOJOUL	11734.9	5572.6	3133.4	7042.3	11216.8	8563.5	4844.9	25254.9	11179.2	12588.3	9017.8	7192.5	9778.4
CA-MG	710.6	166.4	158.7	366.8	523.4	764.4	224.1	2600.1	410.7	1776.8	624.8	302.2	719.1
NICOT-MG	31.3	6.4	6.5	17.1	20.7	7.6	6.6	61.8	21.0	34.8	13.3	11.5	19.9
PROT-G	167.9	46.2	25.5	71.2	116.1	61.4	40.0	255.5	90.7	138.4	75.0	55.9	95.3
FE-MG	11.8	3.6	3.3	6.8	11.5	6.6	5.3	43.0	11.9	23.8	9.3	6.5	12.0
VITB6-MG	2.5	0.5	0.4	0.9	1.2	0.9	0.3	4.6	1.1	2.8	0.8	0.8	1.4
PLPROT	26.3	9.8	7.7	18.1	33.1	1099.3	19.7	83.9	27.4	39.3	21.5	19.5	117.1
MG-MG	295.3	74.0	82.1	153.9	250.4	161.3	110.6	783.1	188.4	434.3	190.9	138.9	238.6
FOL-UG	273.0	53.5	32.0	103.9	166.9	157.6	63.6	802.9	137.9	481.5	128.9	90.4	207.7
ANPROT	141.6	33.0	17.8	53.1	69.3	42.3	20.3	171.6	63.3	99.0	53.5	36.4	66.8
P-MG	1065.8	466.5	388.4	817.5	1178.2	1074.9	466.8	3708.2	990.1	2159.8	1069.2	715.6	1175.1
B12-UG	11.9	2.4	1.3	2.6	4.9	3.9	1.8	12.3	5.0	7.4	5.2	2.2	5.1
T/FAT-G	119.2	64.9	36.7	61.4	130.7	97.0	53.8	288.0	138.7	149.1	104.3	72.6	109.7
K-MG	4115.2	832.0	858.2	1264.0	2238.8	1690.5	871.8	5960.8	1925.3	3381.2	1767.0	1313.0	2184.8
PAMT-MG	4.2	1.8	1.1	2.6	4.0	3.9	1.3	19.4	3.5	12.1	4.1	2.4	5.0
SATFAT-G	45.4	16.5	9.3	16.8	32.9	28.4	12.7	86.0	41.0	48.6	30.0	19.4	32.3
NA-MG	3012.8	586.8	459.2	1497.6	2126.3	1786.9	1373.7	5598.5	2318.2	2934.4	1904.4	1645.9	2103.7
BIOT-UG	32.8	15.5	4.1	8.5	21.1	25.5	8.1	73.6	12.7	40.8	31.1	8.9	23.6
MUFAT-G	32.2	14.6	9.0	21.1	35.3	26.7	12.5	81.4	49.4	40.2	28.5	21.9	31.1
ZN-MG	13.5	4.2	3.6	7.5	13.7	8.4	4.5	32.4	15.1	16.9	9.8	8.0	11.5
ASCRB-MG	302.8	2.6	9.2	22.0	25.6	70.3	5.6	342.0	41.6	208.0	23.0	82.7	94.6
PUFAT-G	25.3	28.8	15.2	19.1	54.5	35.2	24.8	84.2	40.6	40.3	36.0	26.5	35.9
CU-MG	2.5	0.5	0.5	0.9	1.4	0.9	0.6	4.0	1.2	2.0	0.9	0.9	1.4
VITD-UG	0.7	0.9	0.1	1.3	1.2	1.3	1.3	4.6	1.6	2.3	1.7	0.6	1.5
CHOL-MG	596.1	363.7	68.3	223.6	493.0	409.1	211.5	1245.1	315.6	658.8	565.1	201.9	446.0
VITA-IU	7546.7	1261.5	6416.0	5261.0	3522.6	5481.9	2352.8	20975.9	6294.6	12929.8	2549.8	7080.3	6806.1
VITE-MG	20.3	20.6	16.7	14.0	39.3	34.9	15.5	82.2	22.4	37.9	26.2	23.1	29.4

KEY : TOTCHO - TOTAL CARBOHYDRATE;
VITA - VITAMIN A;
MOIST -MOISTURE;
THIA - THIAMINE;
KILOCAL - KILOCALORIE;
RIBOF - RIBOFLAVIN;
KILOJOUL - KILOJOULE;
CA - CALCIUM;
NICOT - NICOTINIC ACID;
PROT - PROTEIN;
FE - IRON;
VITB6 - VITAMIN B6;
PLPROT - PLANT PROTEIN;
MG - MAGNESIUM;
FOL - FOLIC ACID;
ANPROT - ANIMAL PROTEIN;
P - PHOSPHORUS;
B12 - VITAMIN B12;
T/FAT - TOTAL FAT;
K - POTASSIUM;
PANT - PANTOTHENIC ACID;
SATFAT - SATURATED FAT;
NA - SODIUM;
BIOT - BIOTIN;
MUFAT - MONOUNSATURATED FAT;
ZN - ZINC;
ASCRB - ASCORBATE;
PUFAT - POLYUNSATURATED FAT;
CU - COPPER;
VITD - VITAMIN D;
CHOL - CHOLESTEROL;
VITE - VITAMIN E;
G - GRAM;
RE - RETINOL EQUIVALENTS;
MG - MILLIGRAM;
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Table 13. Daily nutrient intake for group 2 subjects

NUTRIENT	SUBJECT												AVERAGE
	1	2	3	4	5	6	7	8	9	10	11	12	
TOTCHO-G	226.6	113.0	193.5	245.1	228.4	335.6	87.9	234.4	302.1	134.8	275.1	233.1	217.5
VITA-RE	502.3	716.3	780.0	277.7	698.0	1735.6	625.5	285.9	1642.4	401.2	914.4	556.0	761.3
MOIST-%	1157.5	709.1	673.0	1377.1	1320.8	1394.1	534.9	860.1	1395.0	1002.3	893.8	1422.0	1061.6
FIBRE-G	12.9	12.6	21.5	12.0	9.7	27.9	6.8	8.3	15.4	11.3	16.4	11.3	13.9
THIA-MG	1.0	0.7	1.3	0.9	0.7	1.7	0.4	0.7	1.3	1.0	1.0	1.1	1.0
KILOCAL	2061.0	1282.8	1456.8	2034.5	2166.0	3051.5	782.6	1943.1	2571.1	1765.1	2806.7	2164.7	2007.2
SUGAR-G	55.8	15.8	15.8	69.6	61.3	85.0	32.5	77.5	115.7	31.6	66.0	65.1	57.6
RIBOF-MG	1.2	0.6	0.6	0.9	0.9	1.2	0.4	0.7	1.9	0.7	1.2	1.2	0.9
KILOJOUL	8648.2	5363.8	6093.0	8529.9	9080.2	12772.4	3288.4	8132.6	10778.5	7388.1	11768.0	9071.4	8409.5
CA-MG	642.2	237.0	246.3	488.0	445.1	491.0	206.6	343.8	867.3	237.7	751.8	341.2	441.5
NICOT-MG	14.5	11.9	13.4	14.7	10.6	25.7	10.7	10.0	18.6	12.9	21.3	22.9	15.6
PROT-G	77.0	50.9	49.8	66.5	62.7	118.4	43.6	54.3	69.9	62.7	118.1	96.9	72.6
FE-MG	8.4	6.7	10.3	6.9	7.5	15.6	4.3	6.3	10.1	7.2	12.4	10.2	8.8
VITB6-MG	1.2	0.6	0.8	1.0	0.7	1.5	0.7	0.5	1.1	0.6	1.1	1.2	0.9
PLPROT	23.8	18.5	33.0	25.4	24.0	44.4	8.3	23.5	17.7	21.5	134.4	26.7	33.4
MG-MG	213.5	156.9	243.0	179.3	152.4	336.0	85.9	147.8	220.5	151.2	260.5	212.2	196.6
FOL-UG	113.9	82.8	167.2	109.5	97.2	196.4	58.7	86.7	134.3	70.9	147.9	122.8	115.7
ANPROT	53.1	32.4	16.8	41.1	38.7	74.0	35.3	30.8	52.2	41.2	91.7	70.2	48.1
P-MG	1068.5	637.1	718.0	927.9	851.1	1450.8	526.4	638.9	1277.8	645.0	1538.3	1084.9	947.1
B12-UG	3.8	1.8	1.5	1.9	3.0	6.2	4.4	2.1	1.8	2.4	9.3	3.7	3.5
T/FAT-G	92.9	64.9	45.0	85.6	109.1	131.8	26.9	89.9	119.3	107.1	133.0	92.1	91.5
K-MG	2143.8	1232.5	1492.7	1697.0	1382.1	2688.9	855.5	1287.7	2478.8	1273.7	2291.9	2044.8	1739.1
PAMT-MG	3.2	1.8	2.1	2.7	2.9	3.8	1.4	2.2	2.7	2.1	3.9	3.5	2.7
SATFAT-G	31.2	13.9	6.5	19.7	25.2	33.5	6.7	21.6	39.0	29.0	34.4	26.1	23.9
NA-MG	1719.0	925.0	1332.2	1628.2	1694.8	3447.7	703.4	1514.6	2453.2	1858.5	3388.7	1391.6	1838.1
BIOT-UG	12.9	7.8	16.7	8.7	13.8	21.9	2.7	8.8	11.2	6.7	15.9	12.3	11.6
MUFAT-G	26.8	16.5	8.8	22.8	29.1	32.5	8.2	23.3	36.1	36.9	41.2	28.2	25.9
ZN-MG	10.4	7.1	6.7	7.3	9.5	12.5	4.7	6.8	8.3	8.4	13.7	12.4	9.0
ASCRB-MG	83.2	11.2	28.2	14.3	7.9	41.4	8.6	8.3	90.8	16.0	30.3	33.6	31.2
PUFAT-G	29.2	25.8	18.2	38.5	47.8	51.9	8.9	39.9	36.6	33.3	48.7	31.3	34.2
CU-MG	1.2	0.9	1.2	0.9	0.9	1.9	0.6	0.8	1.4	0.8	1.9	1.2	1.1
VITD-UG	0.4	0.7	1.1	0.8	1.2	3.1	1.6	0.5	2.5	0.3	5.9	0.6	1.6
CHOL-MG	244.3	120.9	162.5	148.6	249.0	448.9	128.2	147.1	317.4	157.9	591.0	363.5	256.6
VITA-IU	4122.3	5772.1	7178.6	1044.4	4810.6	16509.8	6061.9	1831.3	12934.4	3249.3	5498.7	4527.8	6128.4
VITE-MG	26.5	27.3	19.2	41.0	48.4	31.1	2.8	34.2	42.5	30.7	45.2	24.2	31.1

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Interestingly, the highest level of intake in the group 2 subjects (590.95 mg in subject 11) is still lower than the highest level of the group 1 subjects (1245 mg in subject eight). What must be re-emphasized here, is the point that subjects were assigned to group 2 because their plasma cholesterol levels were greater than 6.5 mmol/l.

On the day of the trial, however, the baseline levels of the majority of these subjects was recorded as being less than 6.5 mmol/l (see Table 14). Attempts were made to trace the reason for this change and most of the subjects admitted that they had drastically altered their eating habits on learning of their raised cholesterol levels as they had become concerned or paranoid about having "heart attacks".

It appears that in these subjects a change in diet plan was sufficient to effect a decrease in their plasma cholesterol levels.

It is also apparent that whilst the maximum number of eggs consumed per week by the group 2 subjects was 5.5 (subject 6), five of the group 1 subjects actually consumed more than this number (two ate 7 -, two ate 8 -, and one ate 9.5 eggs per week).

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Table 14. Baseline plasma cholesterol and triglyceride concentrations of group 2 subjects

SUBJECT	PRELIMINARY TESTING: CHOLESTEROL (mmol/l)	PRELIMINARY TESTING: TRIGLYCERIDE (mmol/l)	TEST DAY: CHOLESTEROL (mmol/l)	TEST DAY: TRIGLYCERIDE (mmol/l)
1	6.54	1.50	6.54	1.50
2	6.84	1.93	5.42	1.39
3	9.93	2.21	5.55	2.28
4	6.62	2.87	5.65	2.87
5	8.70	2.15	8.11	1.83
6	8.62	4.26	6.60	2.38
7	7.21	2.54	5.85	1.58
8	6.51	2.16	6.15	1.71
9	6.65	1.73	5.60	1.52
10	7.31	1.63	6.12	1.38
11	6.64	1.98	6.19	2.45
12	6.65	4.86	4.61	4.78

Although the group 1 subjects consumed more eggs and cholesterol on the whole, their plasma cholesterol levels were normal. It is therefore evident that their endogenous synthesis of cholesterol was suppressed due to their normal negative feedback inhibition of the cholesterol biosynthetic pathway, as explained by Marx in 1976.

The group 2 subjects, on the other hand, consumed less eggs and cholesterol but showed an elevated plasma cholesterol level. Evidently their negative feedback mechanism of endogenous cholesterol synthesis is sub-optimal.

The kilocalorie value is a reflection of the energy intake with the daily requirement being age-related. (Recommended Daily Allowances, 1980).

Group 1 subjects 1, 5 and 10 and group 2 subjects 6, 9 and 11 have the recommended daily energy intake whilst group 1 subject 8 has an intake that is approximately twice that recommended for him. However, on enquiry it was found that this subject is very athletic and therefore had a much higher energy requirement. The remaining subjects' energy intakes were below the recommended allowances which is in keeping with their sedentary life-styles.

(F) CHOLESTEROL

A study of the coefficients of variation as per Table 15 shows that measurements taken at any point in time would still be within 8.66% of the mean. This implies that the coefficient of variation is very small and therefore negligible.

Table 15. Statistical analysis of the cholesterol and triglyceride data for all subjects

SUBJECT	GROUP 1 EGG YOLK				GROUP 1 WHOLE EGG				GROUP 2 WHOLE EGG			
	CHOLESTEROL		TRIGLYCERIDE		CHOLESTEROL		TRIGLYCERIDE		CHOLESTEROL		TRIGLYCERIDE	
	MEAN ± SD	C.V.	MEAN ± SD	C.V.	MEAN ± SD	C.V.	MEAN ± SD	C.V.	MEAN ± SD	C.V.	MEAN ± SD	C.V.
1	4.15 ± 0.14	3.44	1.49 ± 0.29	20.92	4.23 ± 0.23	5.84	0.89 ± 0.18	21.80	6.24 ± 0.46	7.84	2.26 ± 0.42	19.78
2	4.33 ± 0.17	4.10	1.07 ± 0.35	34.22	4.62 ± 0.26	5.89	1.30 ± 0.24	19.76	5.33 ± 0.22	4.26	1.59 ± 0.22	14.74
3	5.81 ± 0.19	3.42	1.25 ± 0.29	24.84	5.19 ± 0.14	2.85	1.31 ± 0.34	27.13	5.35 ± 0.17	3.44	3.78 ± 0.96	26.92
4	3.98 ± 0.20	5.18	0.65 ± 0.11	17.62	3.64 ± 0.11	3.08	0.77 ± 0.17	22.60	5.73 ± 0.15	2.85	4.04 ± 0.68	17.78
5	6.39 ± 0.14	2.24	2.75 ± 0.77	29.53	6.03 ± 0.28	4.81	2.51 ± 0.64	26.97	7.77 ± 0.26	3.55	2.82 ± 0.59	22.02
6	3.26 ± 0.21	6.94	1.27 ± 0.38	31.49	3.45 ± 0.16	4.96	0.81 ± 0.14	18.24	6.43 ± 0.19	3.08	3.89 ± 0.91	24.54
7	4.62 ± 0.28	6.45	1.36 ± 0.30	23.16	4.42 ± 0.10	2.46	1.78 ± 0.74	43.91	5.85 ± 0.15	2.79	2.75 ± 0.67	25.66
8	4.07 ± 0.25	6.51	1.24 ± 0.42	35.90	4.58 ± 0.27	6.30	1.10 ± 0.32	30.69	6.23 ± 0.19	3.19	2.68 ± 0.73	46.48
9	3.04 ± 0.09	3.09	0.80 ± 0.31	40.95	3.37 ± 0.18	5.78	0.86 ± 0.38	46.98	5.65 ± 0.08	1.57	2.47 ± 0.56	24.08
10	4.20 ± 0.15	3.85	0.57 ± 0.11	19.97	3.92 ± 0.32	8.66	0.43 ± 0.08	20.60	6.22 ± 0.20	3.32	2.16 ± 0.36	17.66
11	4.20 ± 0.09	2.22	0.69 ± 0.27	42.04	4.08 ± 0.18	4.69	0.62 ± 0.28	47.77	5.83 ± 0.45	8.11	3.89 ± 0.90	24.42
12	3.79 ± 0.12	3.34	0.63 ± 0.19	31.94	4.07 ± 0.22	5.67	0.97 ± 0.25	26.69	4.73 ± 0.32	7.16	6.23 ± 1.06	18.02

KEY : SD = Standard Deviation C.V. = Coefficient of variation

Since the coefficient of variation of the assay system used was approximately 5%, it appears that variations in measurements could most probably have been due to assay error.

Furthermore, the TD_x Autoanalyser's reproducibility of results was verified as follows :

> Ten identical samples run on a carousel gave an intra-coefficient of variation of 1.37%, whilst 10 identical samples run consecutively on different carousels gave an inter-coefficient of variation of 4.79% - both being less than 5%.

From these statistics it appears as though, plasma cholesterol levels do not deviate at all or the deviations are very small over the 6 hour post-ingestive period. This suggests that the absorption (if any) of the dietary cholesterol over this post-ingestive period is minimal. It therefore appears that repeat measurements taken both close to each other, and at any time for a dietary egg post-ingestive period of at least 6 hours will give similar readings.

Tables 16 and 17 show the cholesterol concentrations at baseline and after ingestion of whole egg for the group 1 and group 2 subjects respectively, whilst Table 18 shows the same data for group 1 subjects on the egg yolk diet only.

Table 16. Cholesterol concentration of group 1 subjects over a 6 hour period, after ingestion of whole egg

TIME (HOURS)	CHOLESTEROL CONCENTRATION (mmol/l)											
	SUBJ. 1	SUBJ. 2	SUBJ. 3	SUBJ. 4	SUBJ. 5	SUBJ. 6	SUBJ. 7	SUBJ. 8	SUBJ. 9	SUBJ. 10	SUBJ. 11	SUBJ. 12
0.00	4.01	4.06	5.02	3.64	5.72	3.27	4.37	4.33	3.06	4.06	4.01	3.93
1.00	4.07	5.04	5.35	3.67	5.98	3.48	4.39	4.59	3.77	4.23	4.22	4.10
2.00	4.19	4.72	4.96	3.40	5.92	3.15	4.38	4.46	3.35	4.18	4.16	3.60
2.50	4.15	4.70	5.19	3.67	6.21	3.64	4.50	4.56	3.32	3.84	4.18	4.27
3.00	3.94	4.37	5.27	3.66	5.72	3.28	4.53	4.89	3.36	3.98	4.14	4.24
3.50	4.18	4.60	5.30	3.62	5.91	3.46	4.45	4.90	3.34	3.07	4.14	3.99
4.00	4.36	4.52	5.41	3.66	5.83	3.50	4.21	4.62	3.24	3.72	4.06	4.34
4.50	4.13	4.54	5.20	3.58	6.03	3.56	4.56	3.95	3.35	4.20	3.62	4.09
5.00	4.43	4.73	5.12	3.66	6.65	3.69	4.50	4.85	3.29	3.92	3.97	3.85
6.00	4.79	4.88	5.06	3.86	6.31	3.51	4.31	4.61	3.61	3.96	4.32	4.27

Table 17. Cholesterol concentration of group 2 subjects over a 6 hour period, after ingestion of whole egg

TIME (HOURS)	CHOLESTEROL CONCENTRATION (mmol/l)											
	SUBJ. 1	SUBJ. 2	SUBJ. 3	SUBJ. 4	SUBJ. 5	SUBJ. 6	SUBJ. 7	SUBJ. 8	SUBJ. 9	SUBJ. 10	SUBJ. 11	SUBJ. 12
0.00	6.54	5.42	5.55	5.65	8.11	6.60	5.85	6.15	5.60	6.12	6.19	4.61
1.00	6.51	5.12	5.14	5.58	7.50	6.39	6.02	5.95	5.62	6.61	5.12	4.21
2.00	5.95	5.27	5.45	5.43	7.75	6.67	6.04	6.25	5.71	6.31	5.88	4.90
2.50	6.14	5.05	5.56	5.67	7.50	6.39	6.05	6.10	5.53	6.38	5.77	4.97
3.00	6.08	5.38	5.22	5.69	7.29	6.43	5.59	6.14	5.56	6.06	5.82	4.41
3.50	6.08	5.09	5.35	5.79	8.08	6.28	5.70	6.65	5.73	5.86	5.85	4.81
4.00	5.12	5.46	5.15	5.74	7.90	6.02	5.74		5.66	6.31	5.20	5.00
4.50	6.62	5.16	5.21	5.97	7.65	6.29	5.79	6.40	5.65	6.15	5.51	4.25
5.00	6.54	5.61	5.27	5.95	8.00	6.56	5.73	6.21	5.60	6.09	6.54	5.00
6.00	6.84	5.71	5.64	5.81	7.87	6.62	5.97	6.25	5.83	6.28	6.44	5.15

Table 18. Cholesterol concentration of group 1 subjects over a 6 hour period, after ingestion of egg yolk

TIME (HOURS)	CHOLESTEROL CONCENTRATION (mmol/l)											
	SUBJ. 1	SUBJ. 2	SUBJ. 3	SUBJ. 4	SUBJ. 5	SUBJ. 6	SUBJ. 7	SUBJ. 8	SUBJ. 9	SUBJ. 10	SUBJ. 11	SUBJ. 12
0.00	4.17	4.17	5.92	3.82	6.14	3.31	4.44	4.61	3.17	4.01	4.24	3.80
1.00	3.93	4.30	6.22	4.19	6.62	3.15	4.77	4.32	3.16	4.21	4.16	3.72
2.00	4.30	4.65	5.87	3.53	6.42	3.36	4.78	4.32	3.06	4.33	4.24	4.03
2.50	3.97	4.32	5.64	3.95	6.25	3.28	4.68	4.01	3.05	4.05	4.11	3.70
3.00	3.97	4.32	5.73	3.97	6.43	2.71	4.55	3.94	3.05	4.03	3.99	3.70
3.50	4.15	4.47	5.83	4.21	6.36	3.45	4.66	3.96	3.13	4.23	4.25	3.58
4.00	4.21	4.00	5.98	3.90	6.57	3.46	4.49	3.73	2.95	4.24	4.20	3.89
4.50	4.23	4.24	5.61	4.11	6.31	3.49	3.93	3.90	2.91	4.09	4.31	3.89
5.00	4.32	4.35	5.75	3.96	6.33	3.22	4.87	3.88	3.03	4.22	4.27	3.78
6.00	4.24	4.46	5.56	4.16	6.45	3.20	5.01	4.02	2.92	4.54	4.24	3.82

Fig. 7. Cholesterol concentration of group 1 subjects (1-6) over a 6 hour period, after ingestion of whole egg

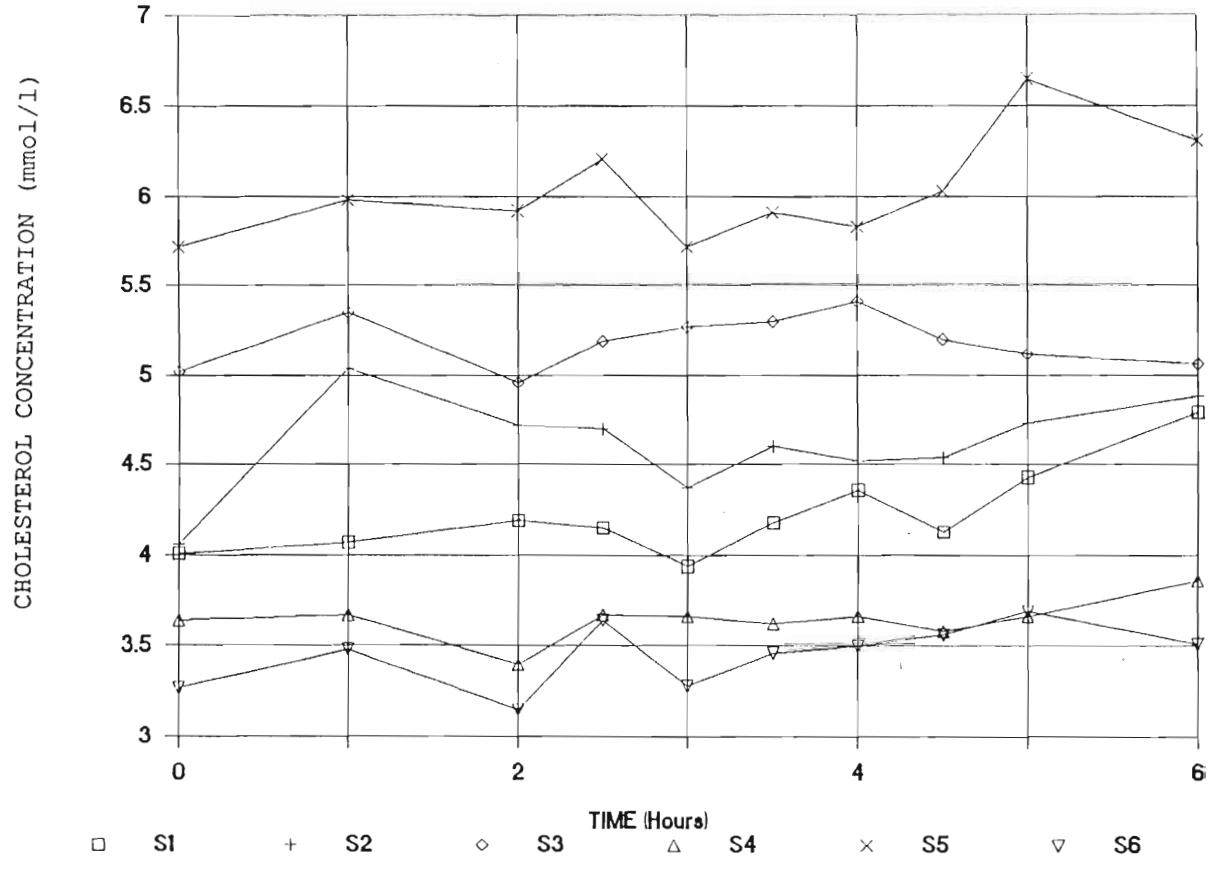


Fig. 8. Cholesterol concentration of group 1 subjects (7-12) over a 6 hour period, after ingestion of whole egg

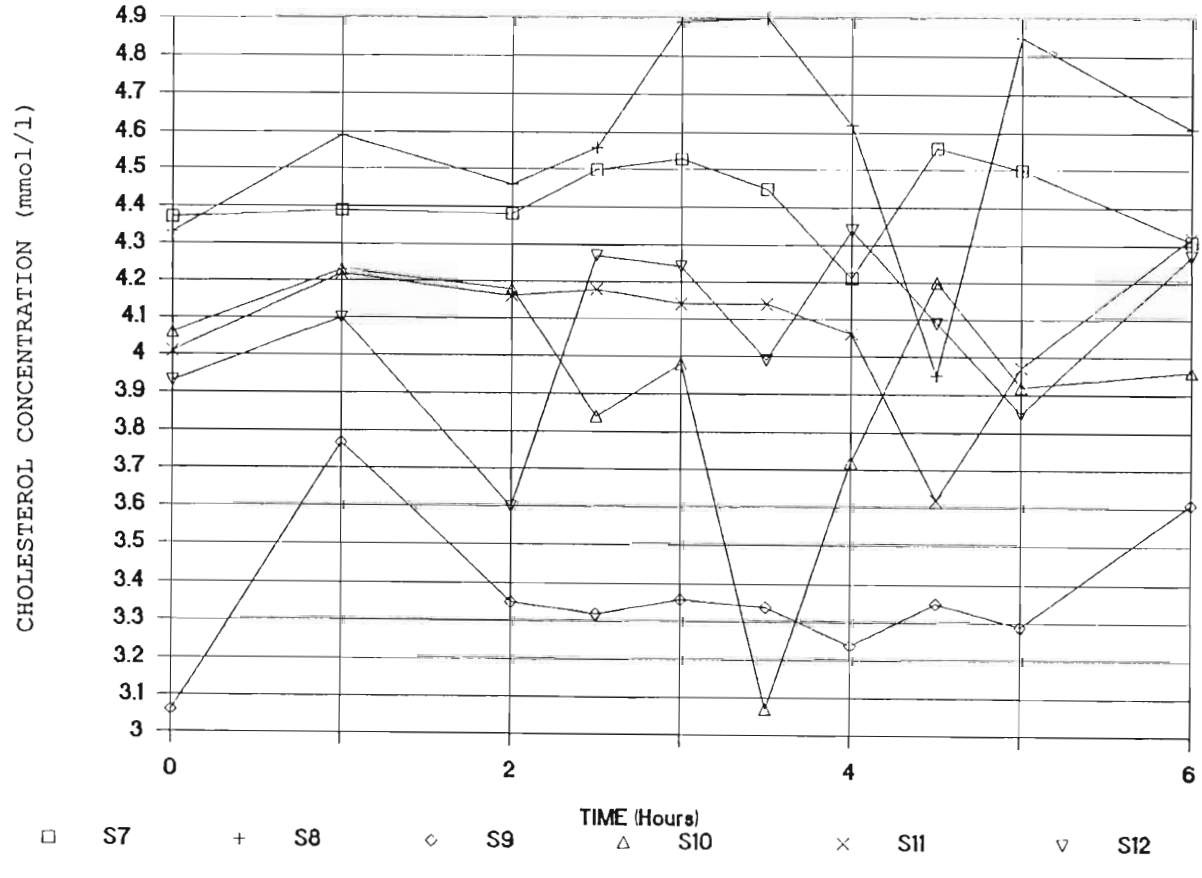


Fig. 9. Cholesterol concentration of group 2 subjects (1-6) over a 6 hour period, after ingestion of whole egg

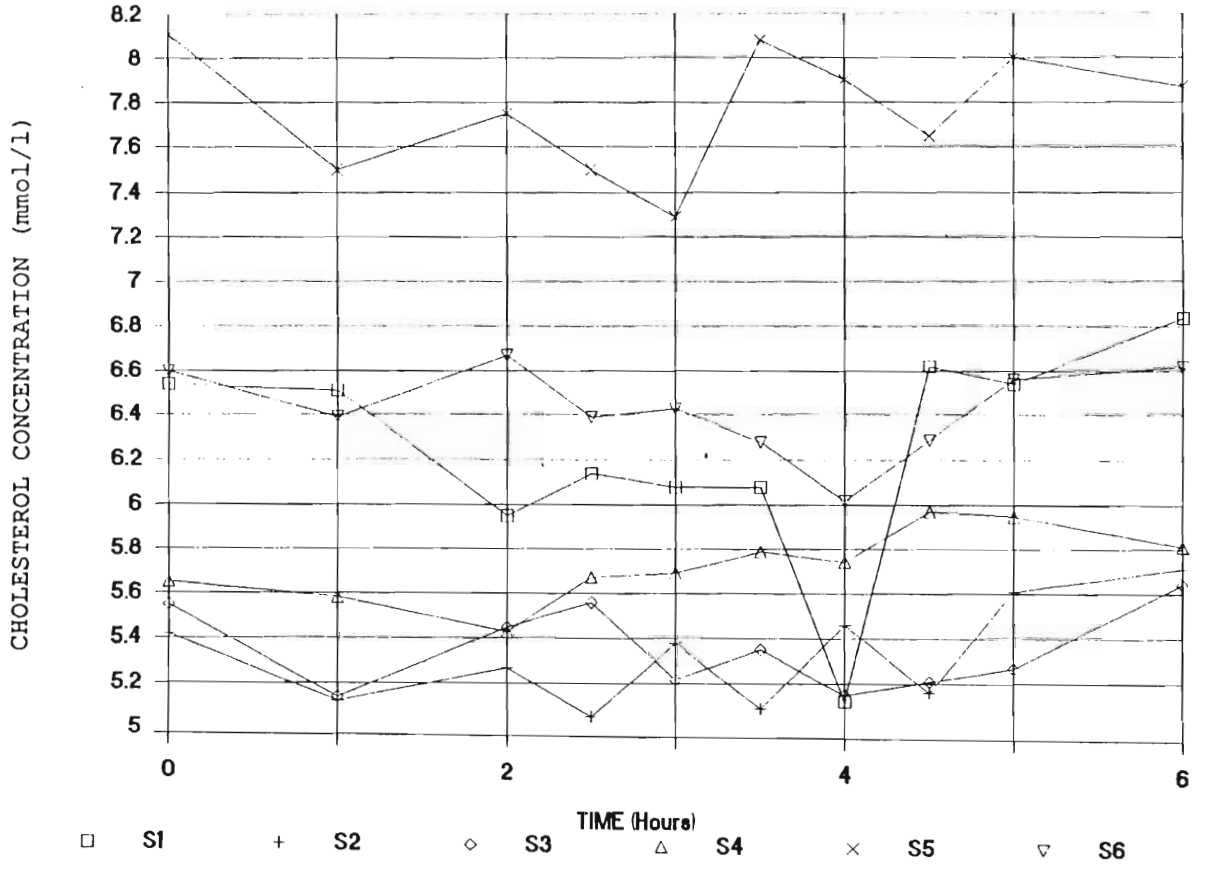


Fig. 10. Cholesterol concentration of group 2 subjects (7-12) over a 6 hour period, after ingestion of whole egg

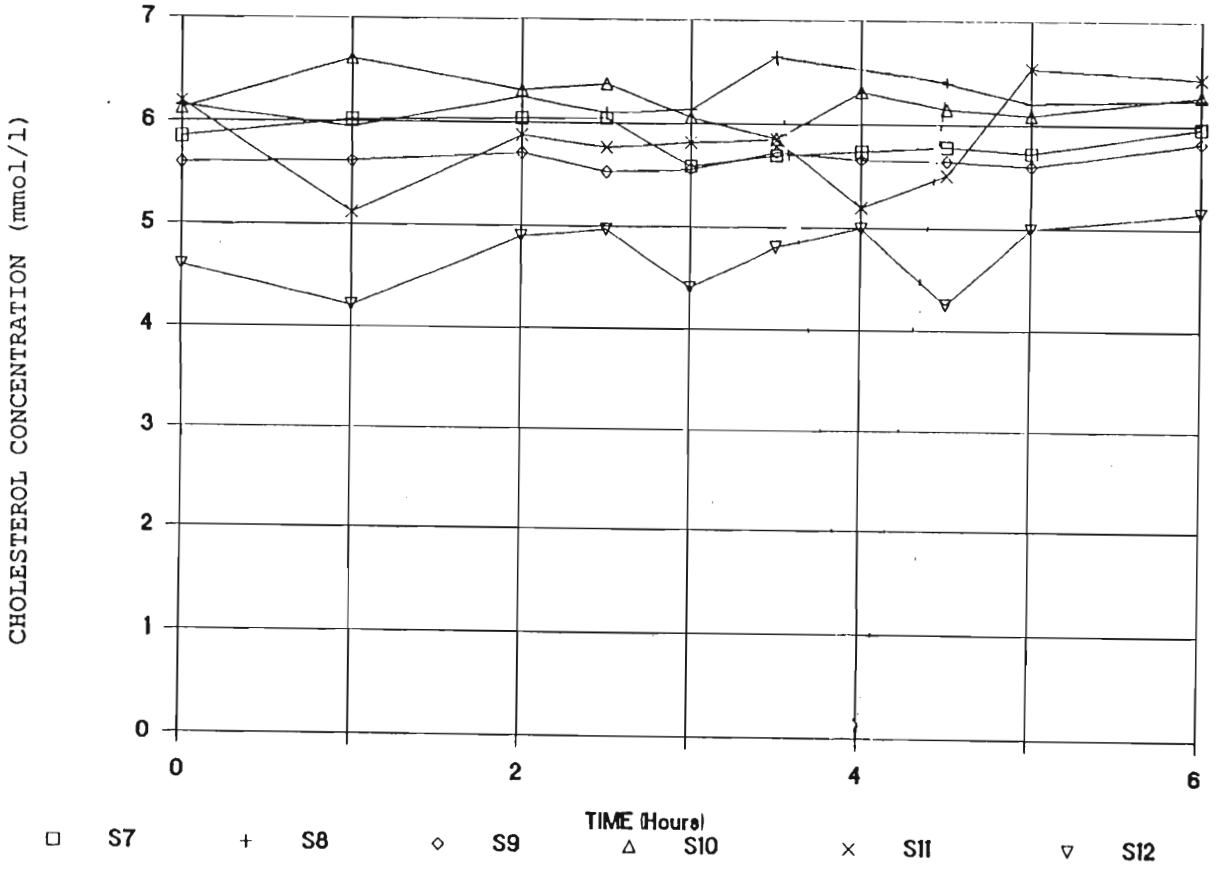


Fig. 11. Cholesterol concentration of group 1 subjects (1-6) over a 6 hour period, after ingestion of egg yolk

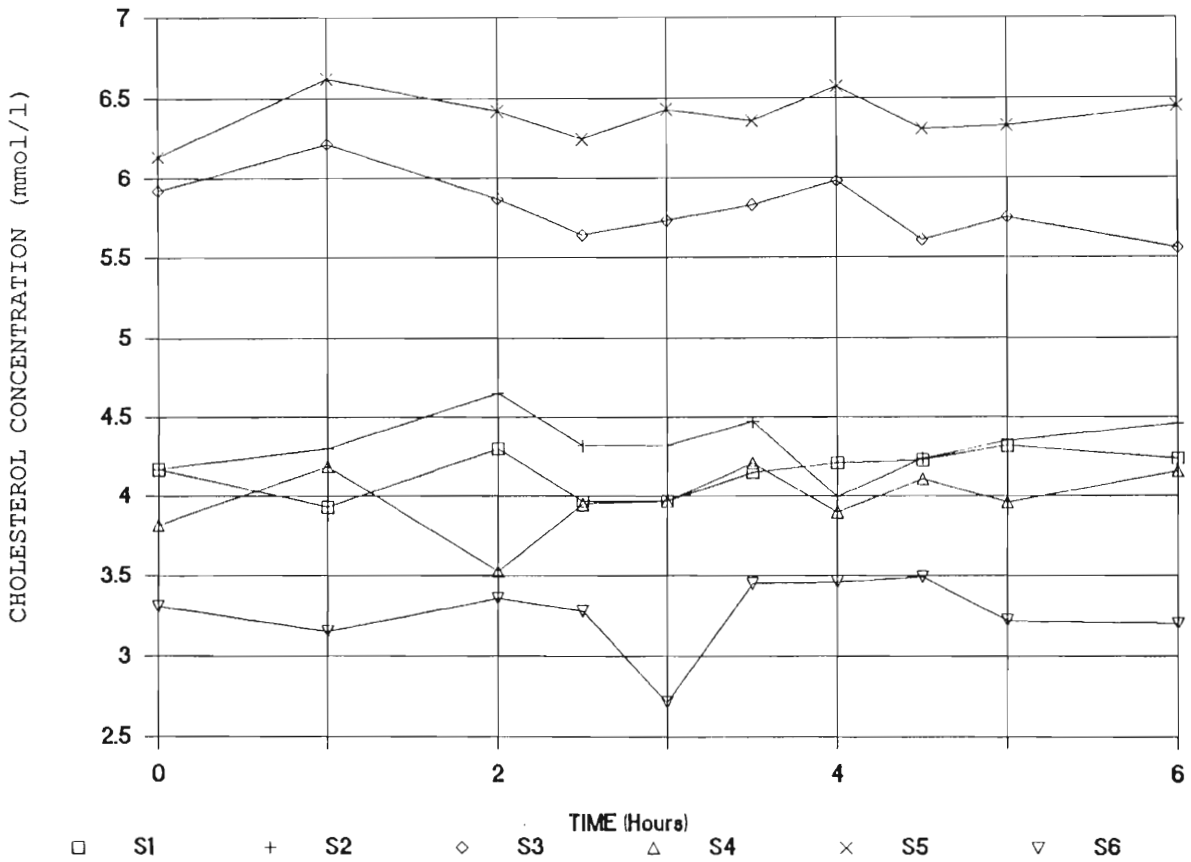
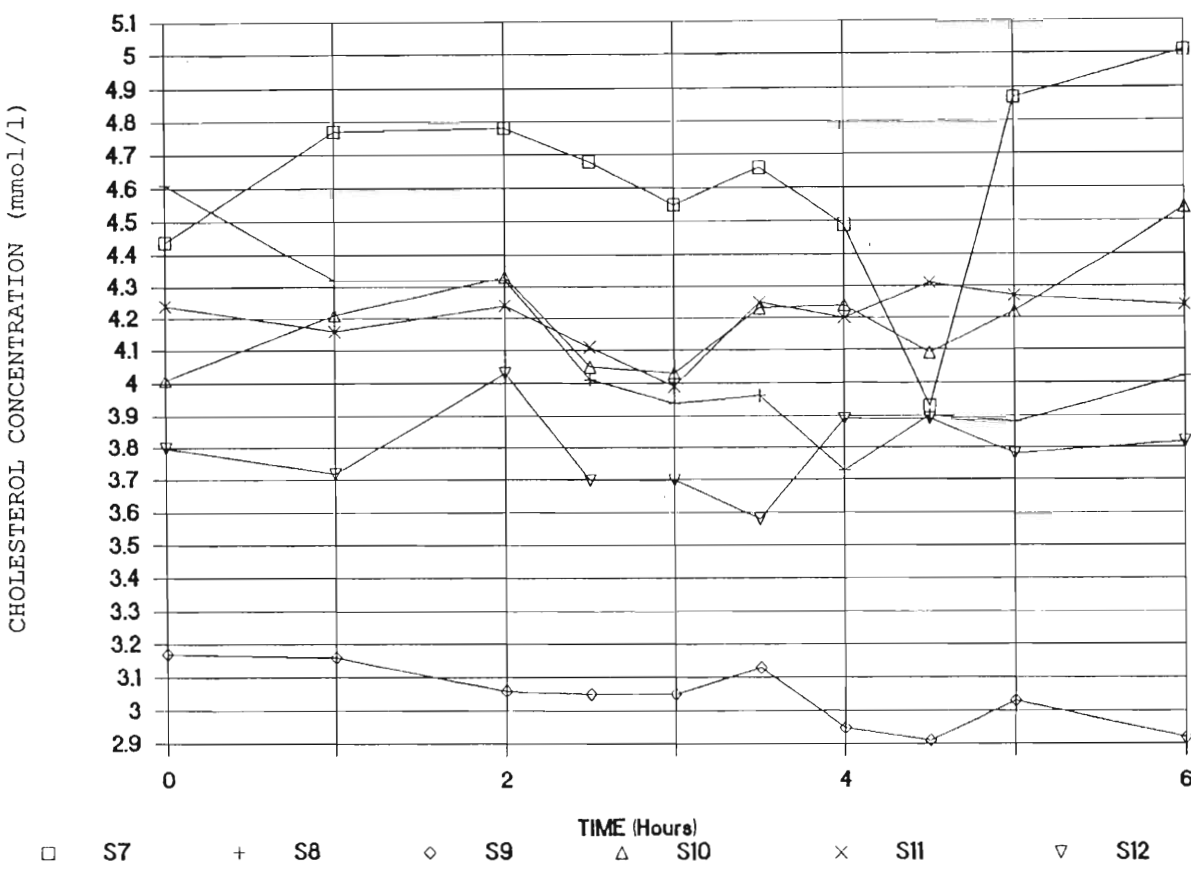


Fig. 12. Cholesterol concentration of group 1 subjects (7-12) over a 6 hour period, after ingestion of egg yolk



These cholesterol concentration values exhibit several small peaks and troughs in all subjects throughout the 6 hour post-ingestive period as shown in Figs. 7 to 12.

These trends are emphasized in Fig. 19 which shows the average cholesterol concentration of the subjects on the different diets.

It should be noted that only one blood sample was lost during the entire procedure. This was the seventh sample of subject 8 of group 2.

This subject moved suddenly causing the cannula to slip out of position.

(G) TRIGYLCERIDE

A study of the coefficients of variation of the various subjects (Table 15) reveals that those of the triglyceride assay are fairly high (14.74 - 46.48%) compared to those of the cholesterol assay. This suggests that there is a definite change in the plasma triglyceride concentrations of the various volunteers during the post-ingestive egg feeding period. For this change to occur in the plasma triglyceride levels there must have been significant absorption of dietary triglyceride.

Tables 19, 20 and 21 show the basal and post-ingestive triglyceride concentrations for group 1 and group 2 subjects. These are shown graphically in Figs. 13 to 18 where it can be seen that these concentration values appear to peak prior to returning to almost baseline concentration. This indicates that there is no nett change in plasma triglyceride concentration by the end of the 6 hour post-ingestive period. However, it was observed that although in most group 1 subjects the triglyceride concentrations returned to baseline within the 6 hour period, most of the group 2 subjects showed the tendency to return, but did not quite reach the baseline by the end of the given time period.

Table 19. Triglyceride concentration of group 1 subjects over a 6 hour period, after ingestion of whole egg

TIME (HOURS)	TRIGLYCERIDE CONCENTRATION (mmol/l)											
	SUBJ. 1	SUBJ. 2	SUBJ. 3	SUBJ. 4	SUBJ. 5	SUBJ. 6	SUBJ. 7	SUBJ. 8	SUBJ. 9	SUBJ. 10	SUBJ. 11	SUBJ. 12
0.00	0.62	0.80	0.75	0.56	1.42	0.45	0.51	0.69	0.46	0.30	0.43	0.94
1.00	0.97	1.60	1.00	0.80	1.68	0.72	0.77	1.22	0.94	0.50	0.99	1.20
2.00	1.09	1.38	1.15	0.78	2.04	0.82	1.76	1.21	1.05	0.51	1.04	1.22
2.50	1.02	1.44	1.47	0.93	2.43	0.81	1.88	1.26	1.39	0.48	0.98	1.32
3.00	1.03	1.39	1.63	1.08	2.49	0.80	2.47	1.49	1.56	0.54	0.78	1.18
3.50	1.06	1.51	1.75	0.95	2.96	0.90	2.69	1.59	1.05	0.45	0.54	0.99
4.00	0.93	1.32	1.71	0.76	3.25	0.95	2.55	1.20	0.67	0.41	0.42	0.81
4.50	0.81	1.44	1.55	0.68	3.32	0.98	2.32	1.00	0.53	0.44	0.38	0.82
5.00	0.81	1.17	1.17	0.62	3.23	0.85	1.83	0.87	0.53	0.35	0.30	0.65
6.00	0.52	0.93	0.93	0.56	2.23	0.86	0.98	0.51	0.41	0.29	0.34	0.56

Table 20. Triglyceride concentration of group 2 subjects over a 6 hour period, after ingestion of whole egg

TIME (HOURS)	TRIGLYCERIDE CONCENTRATION (mmol/l)											
	SUBJ. 1	SUBJ. 2	SUBJ. 3	SUBJ. 4	SUBJ. 5	SUBJ. 6	SUBJ. 7	SUBJ. 8	SUBJ. 9	SUBJ. 10	SUBJ. 11	SUBJ. 12
0.00	1.50	1.39	2.28	2.87	1.83	2.38	1.58	1.71	1.52	1.38	2.45	4.78
1.00	1.79	1.28	2.23	2.98	2.02	2.52	1.95	2.20	1.68	1.74	2.62	4.47
2.00	2.11	1.70	2.93	3.48	2.39	3.05	2.85	2.70	2.59	2.06	3.90	5.77
2.50	2.45	1.79	3.34	3.99	2.52	3.74	3.21	3.03	2.80	2.10	4.34	6.34
3.00	2.83	1.85	4.16	4.52	3.17	4.51	3.52	3.57	2.97	2.26	5.19	6.82
3.50	2.85	1.83	4.75	4.63	3.41	4.78	3.56	3.81	3.23	2.39	3.12	5.26
4.00	2.16	1.79	4.78	4.63	3.30	4.75	3.24		2.83	2.68	4.58	7.41
4.50	2.65	1.51	4.86	4.75	3.54	4.88	3.10	3.17	2.74	2.47	4.52	7.57
5.00	2.31	1.58	4.44	4.65	3.39	4.53	2.55	2.26	2.53	2.45	4.78	7.41
6.00	1.94	1.22	3.99	3.88	2.64	3.77	1.97	1.65	1.77	2.11	3.37	6.44

Table 21. Triglyceride concentration of group 1 subjects over a 6 hour period, after ingestion of egg yolk

TIME (HOURS)	TRIGLYCERIDE CONCENTRATION (mmol/l)											
	SUBJ. 1	SUBJ. 2	SUBJ. 3	SUBJ. 4	SUBJ. 5	SUBJ. 6	SUBJ. 7	SUBJ. 8	SUBJ. 9	SUBJ. 10	SUBJ. 11	SUBJ. 12
0.00	0.93	0.89	0.67	0.51	1.61	0.67	0.85	0.96	0.52	0.45	0.49	0.76
1.00	1.10	0.99	0.92	0.58	2.04	0.69	0.86	1.66	1.01	0.51	0.80	0.79
2.00	1.51	1.26	1.10	0.67	2.20	0.99	1.26	1.82	1.02	0.58	1.06	1.03
2.50	1.59	1.33	1.21	0.77	2.47	1.32	1.49	1.76	1.34	0.67	1.08	0.74
3.00	1.71	1.46	1.48	0.71	3.44	1.50	1.71	1.65	1.22	0.76	0.91	0.64
3.50	1.84	1.41	1.55	0.85	3.71	1.70	1.79	1.30	0.84	0.66	0.87	0.58
4.00	1.81	1.03	1.70	0.68	3.67	1.75	1.36	0.98	0.63	0.65	0.53	0.50
4.50	1.68	1.29	1.40	0.61	3.69	1.61	1.37	0.83	0.50	0.57	0.45	0.40
5.00	1.49	0.28	1.34	0.60	2.76	1.43	1.57	0.73	0.51	0.51	0.36	0.47
6.00	1.20	0.73	1.11	0.48	1.95	1.04	1.32	0.73	0.44	0.38	0.32	0.40

Fig. 13. Triglyceride concentration of group 1 subjects (1-6) over a 6 hour period, after ingestion of whole egg

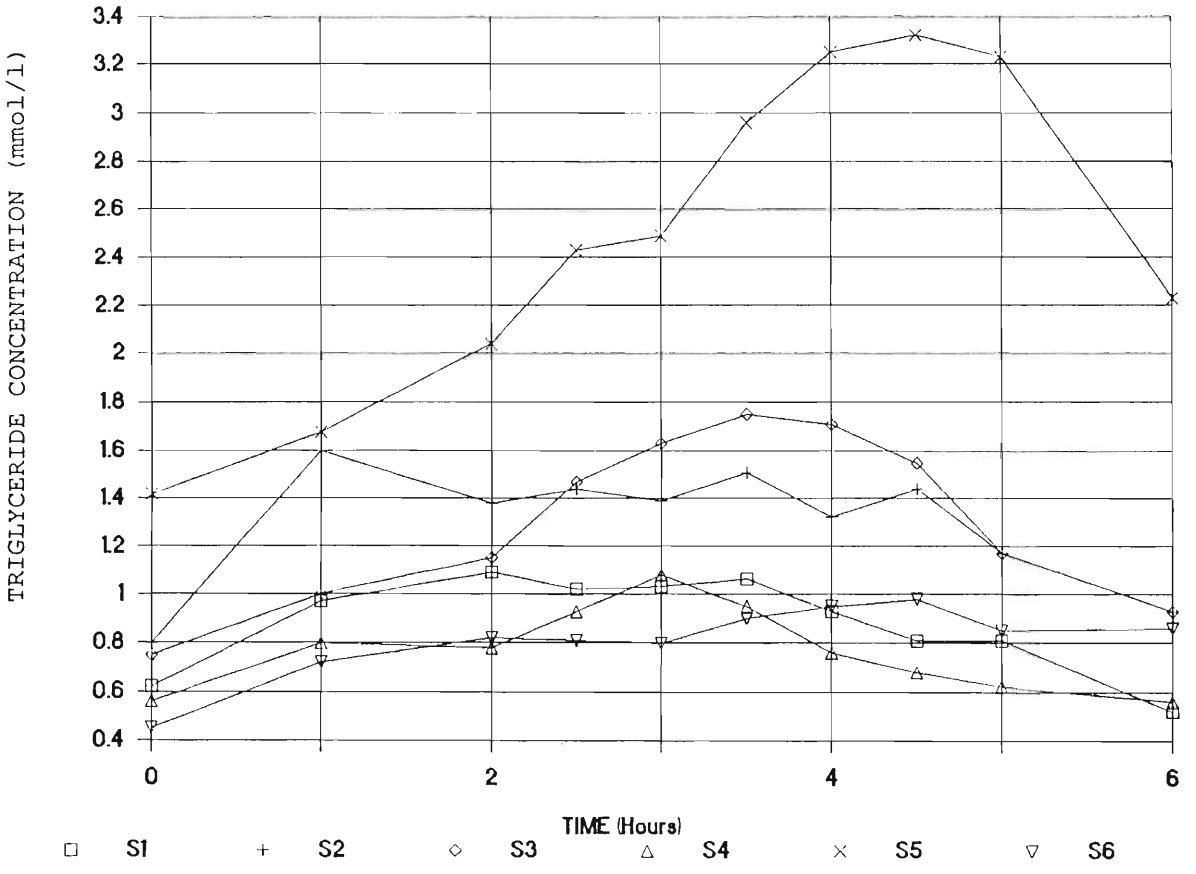


Fig. 14. Triglyceride concentration of group 1 subjects (7-12) over a 6 hour period, after ingestion of whole egg

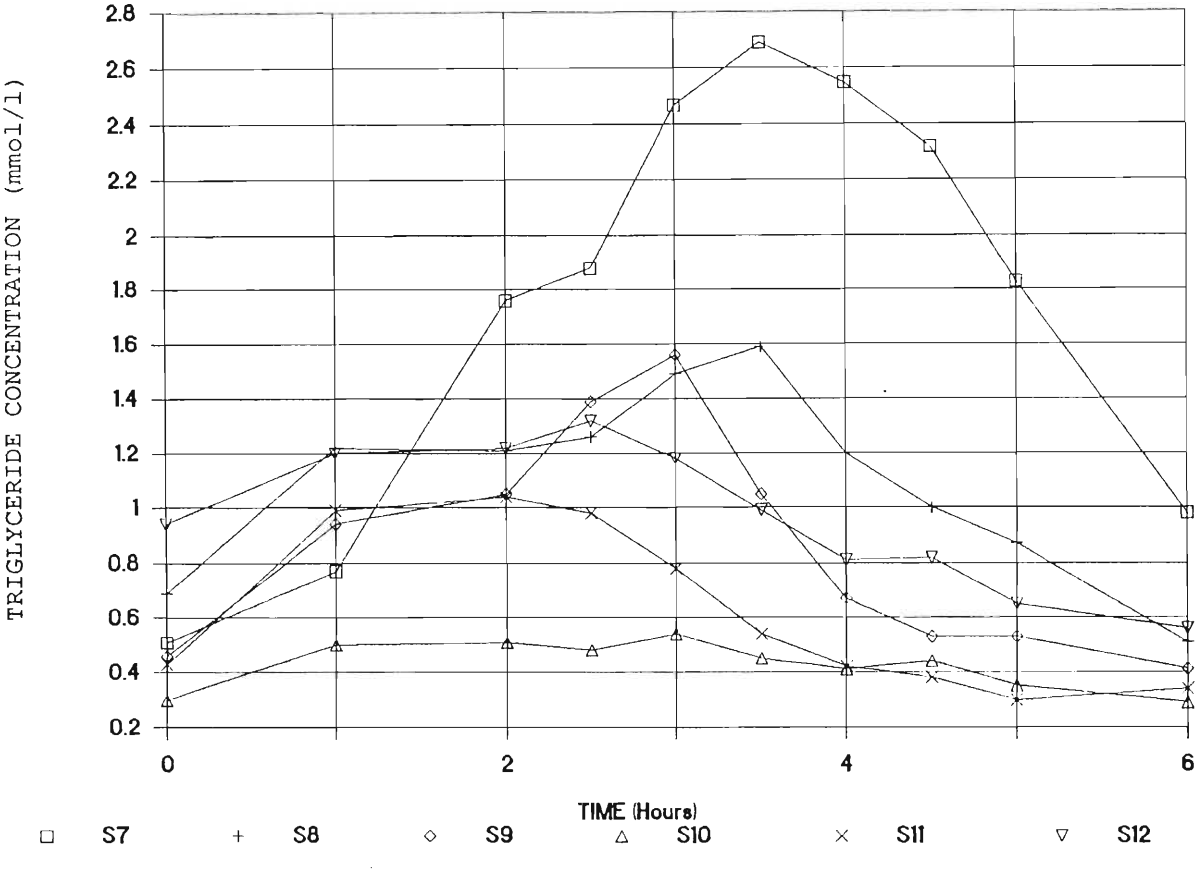


Fig. 15. Triglyceride concentration of group 2 subjects (1-6) over a 6 hour period, after ingestion of whole egg

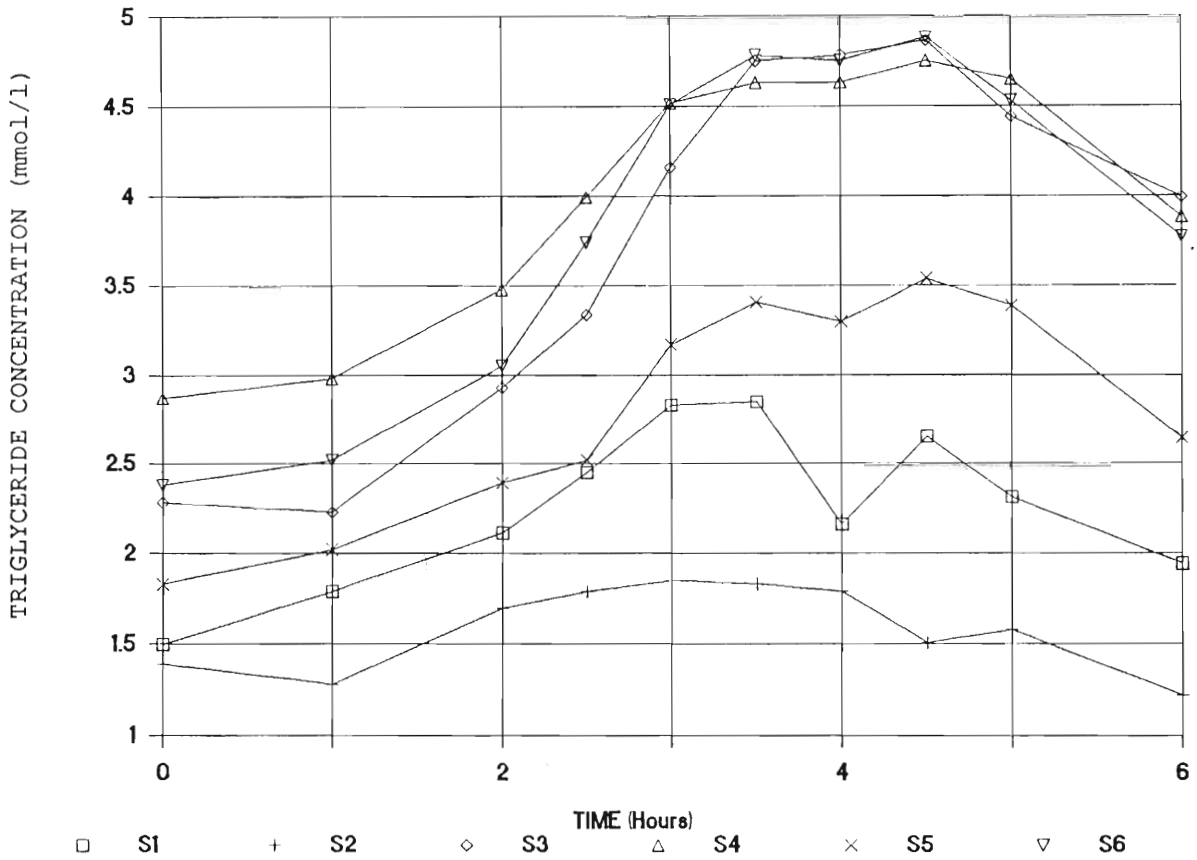


Fig. 16. Triglyceride concentration of group 2 subjects (7-12) over a 6 hour period, after ingestion of whole egg

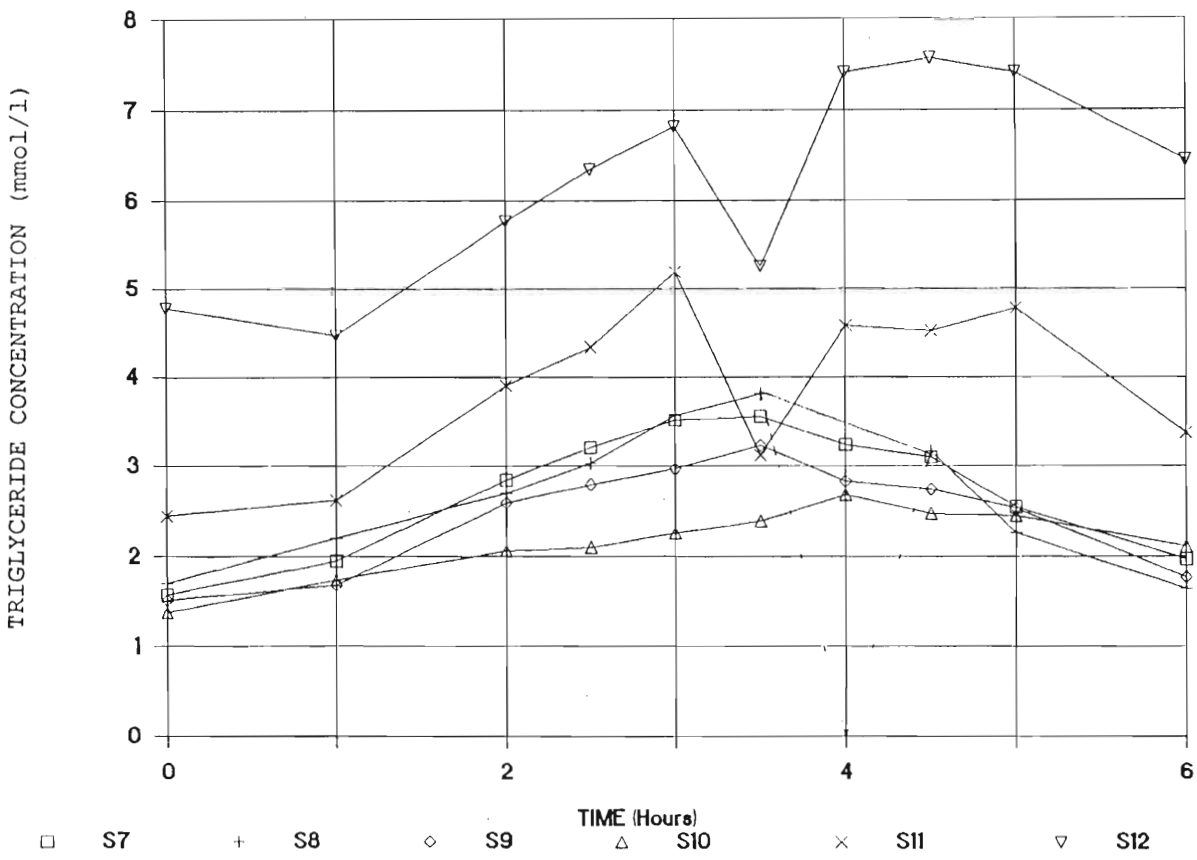


Fig. 17. Triglyceride concentration of group 1 subjects (1-6) over a 6 hour period, after ingestion of egg yolk

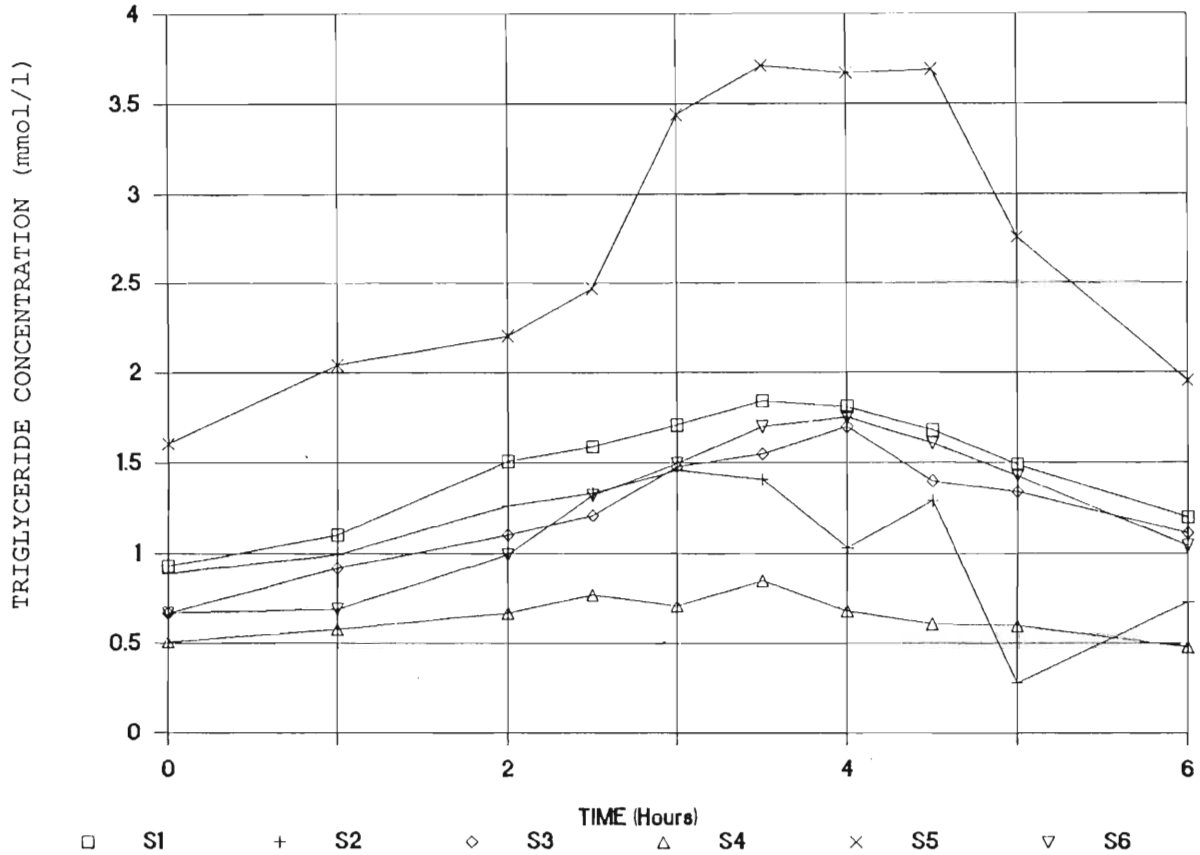


Fig. 18. Triglyceride concentration of group 1 subjects (7-12) over a 6 hour period, after ingestion of egg yolk

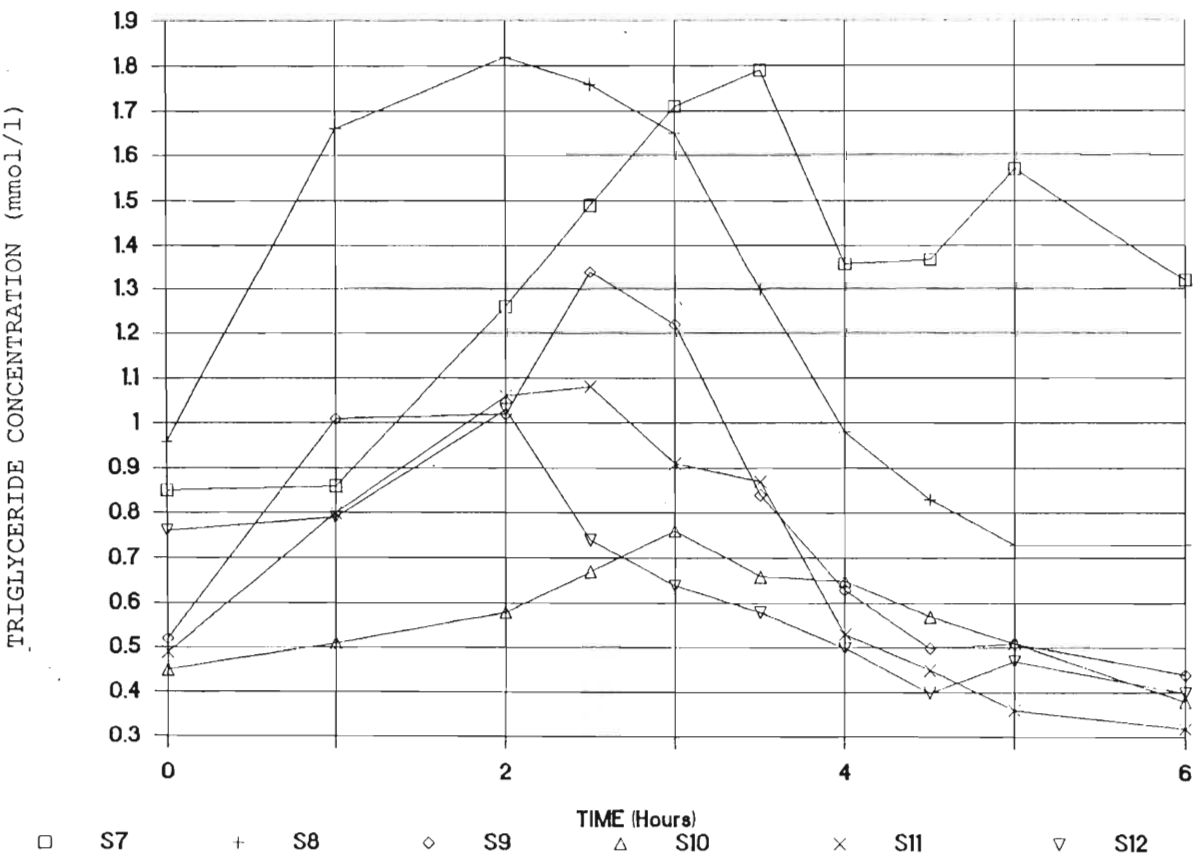


Fig. 19. Average cholesterol concentration of all subjects on the various diets, over a 6 hour post-ingestive period

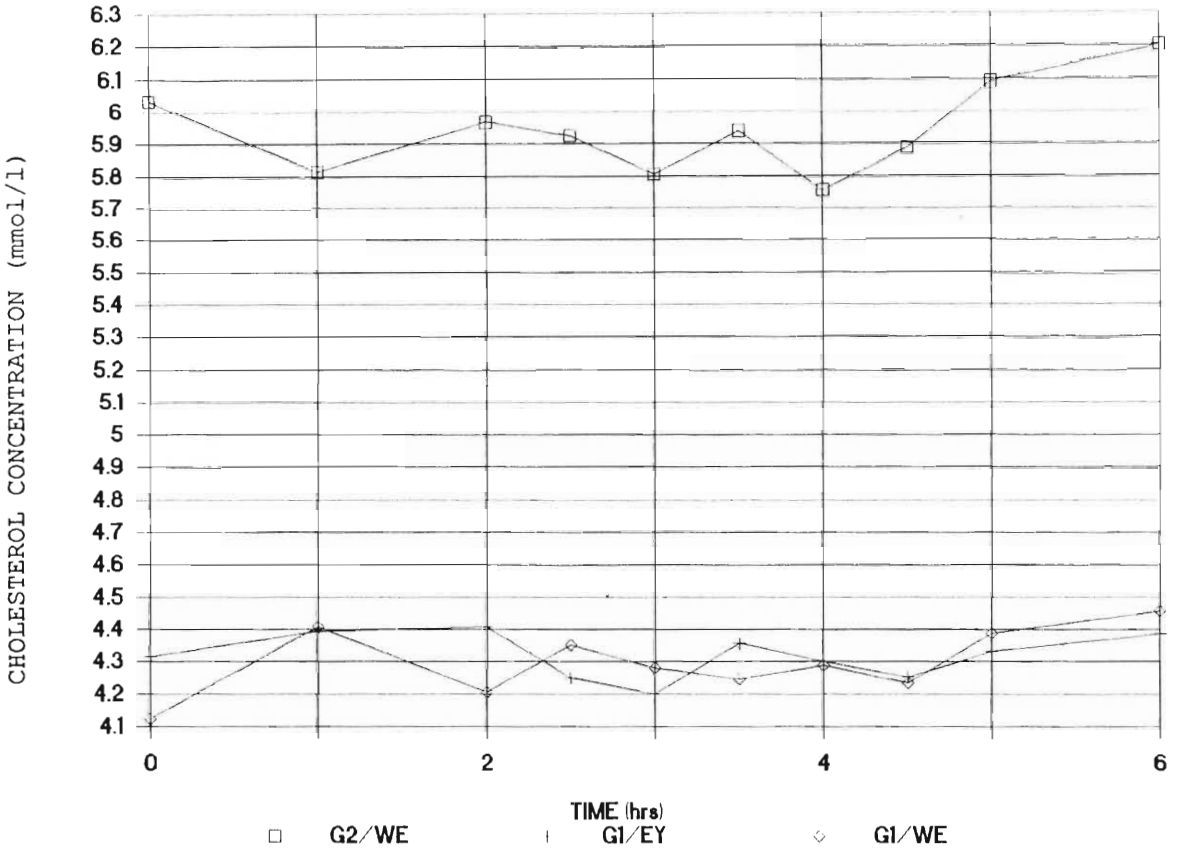


Fig. 20. Average triglyceride concentration of all subjects on the various diets, over a 6 hour post-ingestive period

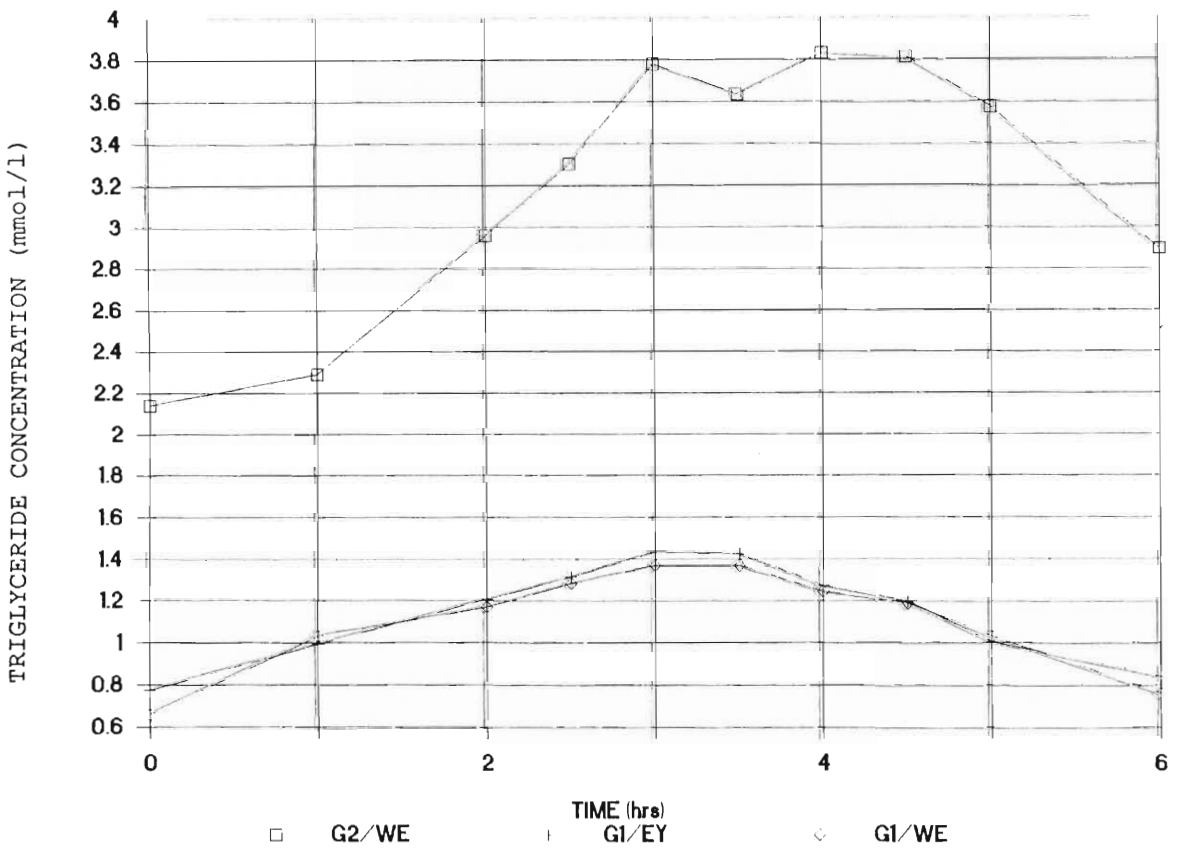


Fig. 20, which shows the average triglyceride concentrations of the subjects on the different diets, emphasizes all of the above trends.

Concentration levels before the peak refer to the absorption of triglyceride from the gastrointestinal tract into the bloodstream whilst those after the peak refer to the clearance of triglyceride from the bloodstream.

1. GROUP 2 SUBJECTS ON WHOLE EGG

Figs. 15 and 16 illustrate that group 2 subjects 3, 4, 5, 6 and 12 have peak triglyceride concentrations at 4.5 hours after the ingestion of whole egg.

The range in these subjects' baseline values is between 1.83 and 4.78 mmol/l as shown in Table 20. The remainder of the subjects show peaks from 3 to 4 hours. Their range in baseline levels is between 1.38 and 1.71 mmol/l with the exception of subject 11 whose level was 2.45 mmol/l.

The general trend here, then, appears to be that a higher baseline level results in a longer time to peak whilst a lower initial level results in a shorter time to peak.

Since the time to peak is an indication of the rate of absorption of triglycerides it is apparent then that subject 11 has a "super-efficient" absorption mechanism.

Subject 12 on the other hand is the volunteer with previous pancreatitis. His late time to peak is in keeping with his medical condition. (Note that pancreatitis causes slower digestion and absorption due to the defect/abnormality in the enzyme system especially pancreatic lipase.)

2. GROUP 1 SUBJECTS ON WHOLE EGG AND EGG YOLK

The obvious trend here too is that the triglyceride concentration reaches a peak before decreasing back to an almost baseline level.

Sixty seven percent of the subjects on the egg yolk diet attain their peak levels within an half hour period of the time it takes them to reach their peak levels on the whole egg diet.

The remaining subjects have a time difference of up to two hours, for example subject 2 exhibits a time to peak of one hour on the whole egg diet and of three hours on the egg yolk diet.

3. PEAK CHANGE IN TRIGLYCERIDE CONCENTRATION

An examination of the peak change in triglyceride concentrations (Appendix 3) reveals that whilst the range of this change is between 0.24 and 1.10 mmol/l in the

group 1 subjects on the whole egg diet, this range is between 1.30 and 2.79 mmol/l in the group 2 subjects (Appendix 4), with the following exceptions :

subjects 5 and 7 of group 1 (1.90 and 2.18 mmol/l respectively), and
subject 2 of group 2 (0.46 mmol/l).

These results indicate that the group 2 subjects attain much higher changes in triglyceride levels than the group 1 subjects. This shows that although these subjects have relatively high baseline triglyceride levels their absorption of dietary triglyceride is still considerably increased.

The group 1 subjects on the egg yolk diet display a range in their peak change in triglyceride concentration (Appendix 5) between 0.27 and 1.08 mmol/l (except for subject 5 who has a peak change of 2.10 mmol/l). It is apparent that these group 1 subjects have similar ranges in their maximum triglyceride levels, regardless of whether they ingest whole egg or egg yolk.

4. AREA UNDER THE CONCENTRATION VERSUS TIME CURVE (AUC)

The AUC's of the triglyceride concentration curves quantitatively reflects the extent of absorption of the triglycerides. After examining these AUC's (Table 22) it is inferred that the group 2 subjects have considerably increased AUC's compared to the group 1 subjects on the whole egg diet, except for group 1 subjects 5 and 7 who have high AUC's and group 2 subject 2 who had a low AUC.

This speaks of a defect in the functioning of the triglyceride transport and/or absorption mechanism in the group 2 subjects because both groups received/ingested the same quantity of dietary egg. Four group 1 subjects (1, 3, 5 and 6) show increased AUC's for the egg yolk diet compared to the whole egg diet.

The Mean Residence Time (MRT) for the triglyceride concentrations of all subjects is shown in Table 22. According to Rowland et al. (1989), "Following extravascular administration, the observed MRT is the sum of the mean residence time in the body and the mean absorption time."

TABLE 22. AUC, AUMC AND MRT FOR THE TRIGLYCERIDE LEVELS OF ALL SUBJECTS

SUBJECT	AUC (mmol.hour/l)			AUMC (mmol.hour/l)			MRT = AUMC/AUC (hour)		
	GROUP 1		GROUP 2	GROUP 1		GROUP 2	GROUP 1		GROUP 2
	EGG YOLK	WHOLE EGG	WHOLE EGG	EGG YOLK	WHOLE EGG	WHOLE EGG	EGG YOLK	WHOLE EGG	WHOLE EGG
1	3.15	1.72	4.30	10.99	4.70	15.02	3.49	2.73	3.49
2	1.36	3.13	1.29	4.10	8.86	4.21	3.01	2.83	3.26
3	3.33	3.14	8.21	11.96	10.66	33.48	3.59	3.39	4.08
4	0.79	1.25	6.50	2.43	3.39	25.47	3.08	2.71	3.92
5	6.37	6.16	5.58	22.94	23.91	21.74	3.60	3.88	3.90
6	3.28	2.15	8.33	12.56	7.51	32.84	3.83	3.49	3.94
7	2.83	7.10	6.61	10.66	25.12	22.24	3.77	3.54	3.36
8	2.27	2.60	5.91	4.66	7.19	18.92	2.05	2.77	3.20
9	1.84	2.43	5.33	4.10	6.05	18.44	2.23	2.49	3.46
10	0.73	0.81	4.54	2.26	2.10	16.62	3.10	2.59	3.66
11	1.45	1.52	8.22	3.28	2.88	30.41	2.26	1.89	3.70
12	0.23	0.81	8.30	0.44	1.60	33.72	1.91	1.98	4.06

KEY : AUC = AREA UNDER THE CONCENTRATION vs TIME CURVE
--- AUMC = AREA UNDER THE (first) MOMENT vs TIME CURVE
MRT = MEAN RESIDENCE TIME

(H) LIPOPROTEIN ELECTROPHORESIS

The following results were obtained. Samples 1 and 2 on lipogram 1 and samples 1 and 7 on lipogram 2 were obtained from individuals with normal cholesterol levels to serve as controls for the process.

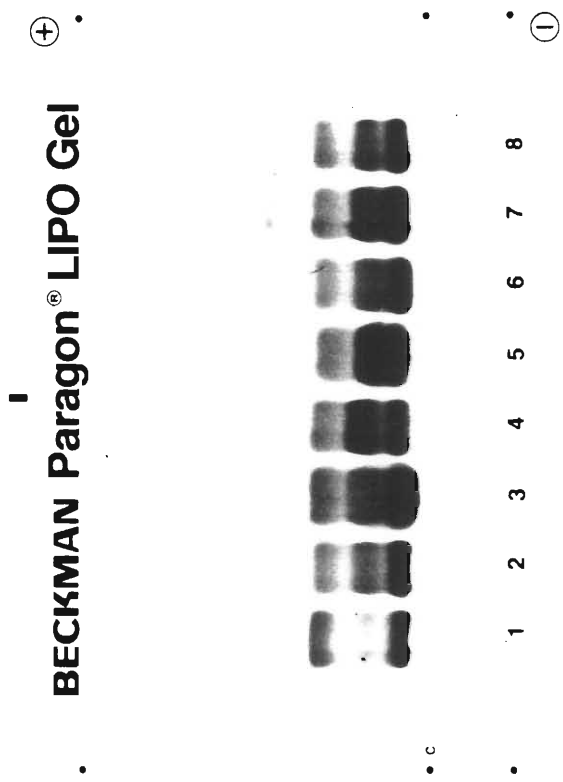


Fig. 21.

LIPOGRAM 1
KEY

SAMPLE NO	SUBJECT NO
01	CONTROL
02	CONTROL
03	06
04	03
05	12
06	07
07	05
08	09

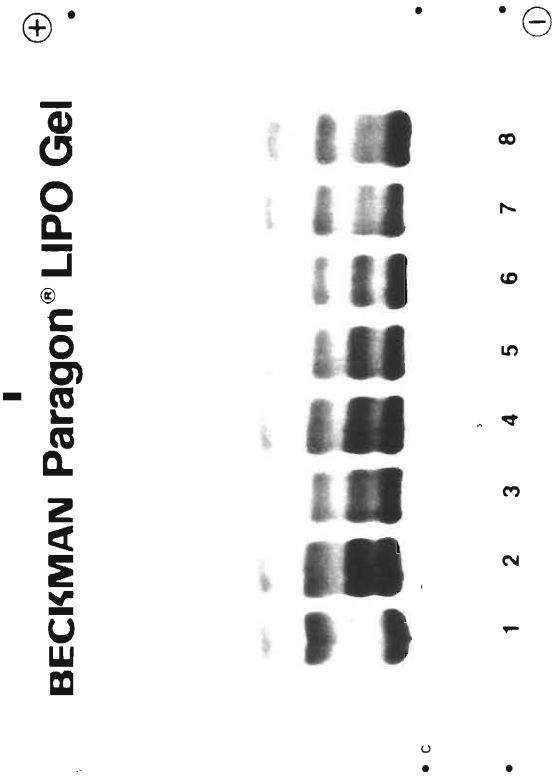


Fig. 22.

LIPOGRAM 2
KEY

SAMPLE NO	SUBJECT NO
01	CONTROL
02	08
03	10
04	01
05	04
06	11
07	CONTROL
08	02

These results were assessed qualitatively i.e. by visual evaluation of the observed pattern (Figs. 21 and 22) as outlined in the "Guide to Genetic Disorders" by Genetic Services, Department of Health, Welfare and Pensions.

Table 23. Interpretation of results

SUBJECTS	LIPOPROTEIN ELECTROPHORESIS	APPEARANCE OF SERUM AFTER 12 HOURS.
1	Slightly intense beta band.	Clear serum.
2	Intense chylomicron band.	Creamy supernatant; clear infranatant.
3	Intense beta band.	Clear serum.
4	Normal pattern.	Clear serum.
5	Fairly intense chylomicron and beta bands.	Clear serum.
6	Intense chylomicron band.	Creamy supernatant; clear infranatant.
7	Fairly intense beta band.	Clear serum.
8	Normal pattern.	Clear serum.
9	Normal pattern.	Clear serum.
10	Normal pattern.	Clear serum.
11	Normal pattern.	Clear serum.
12	Broad beta band.	Turbid serum.

Subjects 4, 10 and 11 exhibited normal lipoprotein electrophoresis patterns and were classed as normal with respect to lipoprotein profile.

Subjects 1, 5 and 7 exhibited fairly intense beta bands with subject 5 having additionally a fairly intense chylomicron band.

They were thus considered as mild to moderate heterozygous type 2(a) hyperlipidaemia patients. In this common type of hyperlipidaemia too much cholesterol is synthesized because the negative feedback mechanism that controls endogenous cholesterol synthesis has a reduced efficiency.

Furthermore, on examination of Fig. 9 it is apparent that subject 5 has an increased cholesterol level which remains elevated due most probably to an abnormality in the negative feedback control of endogenous cholesterol synthesis.

It is also possible that the duration of the experiment was too short to pick up any changes in cholesterol levels.

Subjects 3 and 8 showed intense beta bands and were classed as patients with moderate to severe homozygous type 2(a) hyperlipidaemia.

This condition correlates with problems in the handling of cholesterol and triglyceride.

Subject 9 was considered to be a patient with mild homozygous type 2(a) hyperlipidaemia compared to subject 3.

Subjects 2 and 6 exhibited intense chylomicron bands and were categorized as patients with type I hyperlipidaemia.

In this particular condition the chylomicrons are not cleared from the circulation due to an abnormality in the activity of the lipoprotein lipase enzyme. According to Ganong (1983) lipoprotein lipase clears the circulation of chylomicrons by catalyzing the breakdown of their triglycerides to free fatty acids and glycerol. Thus if the LPL enzyme is not functional then the chylomicrons cannot be converted to LDL.

Subject 12 exhibited a broad or abnormal beta band with a normal pre - beta band and was categorized as a patient with type 3 hyperlipidaemia.

This condition is similar to type I. Here VLDL cannot be converted to LDL - and an intermediary particle called IDL is formed instead.

On comparison with Fig. 16 an increased triglyceride level is seen which reflects an increased chylomicron level.

These serum samples were observed after standing for 12 hours for the appearance of the milky white supernatant and/or an increase in turbidity which is seen in serum samples that have a high triglyceride or chylomicron or VLDL concentration.

This change occurs in serum samples obtained from individuals that have type 1 or 5 hyperlipidaemia. Results of these observations compared favourably with the types of hyperlipidaemia as classified by the lipograms.

Note that the lipoprotein electrophoresis was a retrospective analysis performed two months after the test, in an attempt to determine the particular lipid abnormalities of the group 2 subjects.

Ideally, this analysis should have been performed on the day of the test so that their total status in terms of lipid profiles could have been ascertained and the particular abnormality defined.

Furthermore, a change in, for example, diet or lifestyle in the two months between the test day and the day on which the electrophoresis run was done could have altered or affected their particular lipid profiles subsequently.

Since the electrophoresis was not performed on the day of the test, the chylomicron and triglyceride levels should have been checked on the day the lipoprotein electrophoresis was run in order to confirm the abnormality.

IV. DISCUSSION

In this chapter the above results are discussed giving possible explanations for the observed trends and findings.

This particular project was embarked upon with the view of ascertaining what effect, if any, the ingestion of dietary cholesterol and triglyceride, in the form of egg, has on the plasma levels of cholesterol and triglyceride in the Asian male adult. To this end, 12 volunteers with normal baseline blood cholesterol and triglyceride concentrations (group 1) as well as 12 volunteers with raised blood cholesterol and/or triglyceride concentrations (group 2) were chosen to participate in this study.

These free-living volunteers were subjected to the following dietary regimens :

Group 1 was required to ingest egg yolk on trial day one and whole egg on trial day two, whilst group 2 had only one trial day on which they were required to ingest whole egg. The egg was freshly boiled in each instance. Connor (1982) reports that there is 1400 mg of cholesterol per 100g of egg yolk.

These volunteers were also requested to record their normal daily dietary intake for a week prior to the test being carried out. These dietary records were then used to calculate (as previously described) the average daily

intake of various nutrients in the hope that this would assist in explaining and/or correlating the various trends observed in the trials.

The lipoprotein electrophoresis procedure was followed for the group 2 volunteers in an attempt to categorize them into the various hyperlipidaemia classes.

As far as the cholesterol absorption patterns are concerned, it is apparent that regardless of the group or diet under study the cholesterol concentration over the six hour period is relatively stable. All subjects exhibit minor fluctuations that do not differ much from the baseline level. Indeed in many subjects it appears to drop marginally to below baseline levels.

The possibility exists that the time period of assessing the change in plasma cholesterol concentration (i.e. the post-ingestive period of six hours) was too short. Perhaps cholesterol from the diet takes much longer to be absorbed into the circulation.

It is also possible that the feedback inhibition mechanism for the control of endogenous cholesterol synthesis has come into operation to cut-off this predominant source of cholesterol hence preventing an increase in plasma cholesterol with the ingestion of the dietary dose of cholesterol (Marx, 1976).

Walker (1980) contends that cholesterol is not a nutritional need as the human body is capable of synthesizing all the cholesterol it needs. Evidently the diet "contributes" only 25 - 30% cholesterol to the serum cholesterol concentration.

Support for this contention comes from Tobert (1987) who reports that approximately 33% of the total body cholesterol is contributed by the diet in individuals consuming a "typical Western diet".

This assertion could, to some extent, explain the findings in the present study i.e. there was no significant change in plasma cholesterol levels on consuming 1400 mg of cholesterol.

Another possible reason for the lack of any significant change in plasma cholesterol concentration is that this dosage of 1400 mg of cholesterol ingested might have been insufficient to effect a response.

The outstanding feature of the change in the plasma triglyceride levels is that the triglyceride level rises from the baseline level to reach a maximum value before returning towards the baseline level. This indicates that plasma triglyceride responds to dietary triglyceride as an increase in dietary triglyceride results in an increased plasma triglyceride.

This phenomenon is supported in Guyton (1986) where it is stated that the chylomicron concentration rises after a meal rich in triglyceride. What is interesting though, is the fact that although in most of the group 1 subjects baseline levels are reached during the recorded post-ingestive period of six hours, the group 2 subjects levels, although displaying a distinct tendency to return to baseline levels, do not quite do so within the 6 hour period.

It is also observed that the group 2 subjects exhibit the greatest change in the triglyceride levels with respect to the baseline levels when compared to the group 1 subjects (Fig. 20).

It is also noted that most group 2 subjects have elevated initial triglyceride levels.

The trend thus seems to be that a higher initial triglyceride value leads to an increased absorption of exogenous triglyceride which results in a longer time period being necessary for a return to the initial triglyceride value.

A study of the AUC's (which indicates the extent of absorption) provides further support for this as most group 2 subjects have higher AUC's compared to the group 1 subjects (Table 22).

With respect to the composition of the edible portion of the egg, 50 g contains 4.137 g of fatty acids (as saturated, monounsaturated and polyunsaturated fatty acids) as opposed to 0.213 mg of cholesterol (Appendix 6).

This amount of fatty acid is converted to triglyceride after absorption and is carried in the circulation in chylomicrons. This is responsible for the increase in the triglyceride levels noting that 90% of the chylomicron consists of triglycerides (Ganong, 1983). These chylomicrons are cleared from the circulation as they pass through the capillaries of the liver and adipose tissue by the action of the enzyme lipoprotein lipase which hydrolyzes the triglyceride component of the chylomicrons into glycerol and free fatty acids. These free fatty acids are then stored, used for energy, or for the formation of adenosine triphosphate (ATP) (Brown and Goldstein, 1985).

Subject 6 has been classified as a patient with type I hyperlipidaemia on the basis of his response in the lipoprotein electrophoresis procedure i.e. the presence of an intense chylomicron band in the lipogram and a creamy supernatant in the serum sample that was left standing overnight. Further support for this diagnosis comes from a "closer observation" of his plasma triglyceride concentrations. As is evident in Table 14 this subject's plasma triglyceride level was elevated both during the preliminary testing and on the day of the test.

In addition to this, an analysis of the area under the curve (which is a measure of the extent of the absorption of triglyceride) demonstrates that this subject has a high (in fact the highest) AUC, (Table 22).

As is evident in Fig. 15, this subject's triglyceride concentration peaks at 4.5 hours but does not return to the baseline level at the end of the test period.

This could be related to a problem with the enzyme lipoprotein lipase, since this enzyme is responsible for the hydrolysis of the chylomicron core triglycerides into free fatty acids and glycerol (Brown and Goldstein, 1985). Thus, if this enzyme is inefficient the triglyceride levels will remain elevated.

This subject consumed 5.5 eggs per week and 448.87 mg of cholesterol per day. Although his plasma cholesterol level was 8.62 mmol/l during the preliminary testing, this dropped to 6.60 mmol/l on the day of the test (Table 14) after a drastic change in his diet pattern due to a fear of having a "heart attack".

Six of the group 2 subjects were categorized as patients with type 2(a) hyperlipidaemia. In this condition the serum cholesterol concentration is elevated. Only two of these subjects, i.e. subjects 1 and 5 had increased baseline cholesterol levels on the day of the test.

Note should be taken of the fact that the normal cholesterol range should be age-specific (Rossouw et al., 1988).

However, because of the uncertainty surrounding the clinical significance of an isolated mild-to-moderate increase in triglyceride values and their considerable biological variability, age-specific cut-points were not recommended for triglycerides (Rossouw et al., 1988).

CONCLUSION

Regardless of the dietary intake all subjects showed minor changes (less than 8.66 % coefficient of variation) in plasma cholesterol levels and hence it is concluded that dietary egg has no significant effect on plasma cholesterol levels over a post-ingestive period of 6 hours.

Normal subjects (group 1) showed an elevation in triglyceride concentration which returned to basal levels within 6 hours.

However, individuals with elevated plasma cholesterol and/or triglyceride levels (group 2) have a similar but exaggerated response. Their change in triglyceride concentration was much greater, and although showing a tendency to return to the basal concentrations, they do not return to their basal concentrations within the 6 hour period.

SUMMARY

An elevated blood cholesterol level is a well-known risk factor for atherosclerotic heart disease.

The effect of dietary cholesterol on plasma cholesterol is, however, still being investigated. Numerous studies have shown the differences between individuals in plasma cholesterol response on ingestion of a cholesterol-rich meal.

A number of researchers have suggested that differences in response are due to random fluctuations. These studies appear to concentrate on chronic or long-term investigations. No study could be found that addressed the effect of the intake of dietary cholesterol - in the form of egg - on plasma cholesterol levels over the short-term post-ingestive period of 6 hours .

The purpose of this study was to determine the effect that dietary egg has on human plasma cholesterol and triglyceride levels.

For this study subjects were divided into 2 groups, depending on their basal cholesterol and/or triglyceride concentrations i.e. those with normal levels (group 1) and those with elevated levels (group 2). Group 2 consumed whole egg on their trial day whilst group 1 consumed whole egg and egg yolk on two different trial days.

It was found that both groups handled the dietary cholesterol similarly. Although their plasma concentrations fluctuated throughout the six hour period, these fluctuations were not statically significant.

As far as the triglyceride component was concerned, the subjects with elevated cholesterol and/or triglyceride concentrations generally showed an exaggerated response. Their triglyceride concentrations rose to a greater level, compared to the normal subjects.

Although in most of the normal subjects the triglyceride concentrations returned to basal levels, most of those subjects with elevated basal levels merely showed a tendency to return.

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APPENDIX 1 : "Drug Studies Unit Informed Consent Form"

STUDY NO. : CHOL 1/89

VOLUNTEER CONSENT FORM

I, ... hereby volunteer to take part in the above-mentioned study. I realise that I may withdraw at any time.

I was informed that the study involves:

1. Physical examination and blood analyses two weeks before the experiment commences.

2. The completion of a 7 day record food intake from a week prior to the actual experiment.

3. On the trial day, after a fasting period of 12 hours a catheter will be inserted in a vein in my arm. This will remain in the blood vessel for 6 hours (the duration of the experiment). Two initial blood samples will be drawn (5ml) then I will be requested to eat 100g boiled egg yolk with 200ml tap water. Blood samples will then be drawn at various intervals.

4. That I will be allowed to drink 200ml of tap water every 2 hours.

5. No drug will be administered.

6. For each trial day and for filling in the 7 day food intake record form I will be paid R75,00 (Note : Tax will be deducted from University of Durban-Westville staff members).

Signed:

.....
INVESTIGATOR

APPENDIX 2 : Group 1 : Subject 1 : Nutrient Intake

ITEM	REF	TOTCHO G	VITA-RE	MOIST %	FIBRE-G	THIA-MG	KILOCAL	SUGAR-G	RIBOF-MG	KILOJOUL	CA-MG	NICOT MG	PROT-G	FE-MG	VITB6-MG	PLPROT	MG-MG	FOL-UG	ANPROT	P-MG	B12-UG	T/FAT-G	K-MG	PANT-MG	SATFAT-G	NA-MG	BIOT-UG	MUFAT-G	ZN-MG	ASCRB-MG	PUFAT-G	CU-MG	VITD-UG	CHOL-MG	VITA-IU	VITE-MG
HOLE	1.00	37.60	248.00	703.20	0.00	0.32	488.00	0.00	1.28	2056.00	952.00	0.80	26.40	0.80	0.34	0.00	104.00	40.00	26.40	744.00	3.20	26.40	1216.00	2.48	16.64	392.00	16.00	7.68	3.04	8.00	0.96	0.16	0.24	112.00	1008.00	0.80
T	cc	52.15	38.50	272.30	0.00	0.11	336.00	45.15	0.49	1403.50	434.00	0.35	15.05	0.35	0.12	0.00	42.00	24.50	15.05	343.00	1.40	7.35	556.50	1.40	4.10	168.00	0.00	1.75	2.10	3.50	0.18	0.21	0.00	14.00	154.00	0.21
INK,CARBONATED	2.00	321.30	0.00	2747.88	0.00	0.00	1193.40	321.30	0.00	5140.80	122.40	0.00	0.00	0.00	0.00	0.00	30.60	0.00	0.00	459.00	0.00	0.00	30.60	0.00	0.00	244.80	0.00	0.00	0.00	0.00	0.00	0.92	0.00	0.00	0.00	0.00
RE,FRUIT JUICE	c1	313.50	330.00	2409.00	30.25	1.65	1320.00	0.00	0.83	5582.50	220.00	8.25	11.00	5.50	0.74	11.00	192.50	522.50	0.00	220.00	0.00	2.75	4015.00	2.48	0.28	27.50	11.00	0.55	2.48	1375.00	0.55	1.65	0.00	0.00	3520.00	0.55
WHITE	9.00	179.64	0.00	133.20	10.08	0.86	957.60	0.00	0.25	4014.00	180.00	5.76	29.16	5.04	0.15	29.16	133.20	100.80	0.00	417.60	0.00	9.00	619.20	1.12	2.09	1191.60	3.60	5.18	3.78	0.00	1.55	0.58	0.00	0.00	0.00	0.00
BROWN	9.00	271.80	0.00	222.00	31.80	1.92	1590.00	0.00	0.54	6654.00	350.00	16.20	53.40	13.20	0.48	53.40	348.00	222.00	0.00	936.00	0.00	17.40	1404.00	1.86	0.00	2004.00	18.60	0.00	8.28	0.00	0.00	1.56	0.00	0.00	0.00	0.00
INE	52.00	0.00	62.40	3.00	0.00	0.00	45.92	0.00	0.00	192.00	0.48	0.00	0.02	0.00	0.00	0.00	0.08	0.00	0.02	0.72	0.00	4.88	0.72	0.00	0.72	29.84	0.00	1.44	0.00	0.00	2.64	0.00	0.26	0.00	208.00	3.60
	c1	71.36	0.00	220.80	2.24	0.06	470.40	0.00	0.03	1977.60	28.80	1.28	6.08	0.64	0.14	6.08	9.60	12.80	0.00	83.20	0.00	16.32	83.20	0.61	2.02	1136.00	2.88	2.94	1.09	0.00	10.27	0.06	0.00	0.00	3.20	11.49
,GOUDA	1.00	2.64	208.80	49.80	0.00	0.04	427.20	0.00	0.40	1789.20	840.00	0.12	29.88	0.24	0.10	0.00	34.80	25.20	29.88	655.20	1.80	32.88	144.00	0.37	21.13	982.80	1.92	9.30	4.68	0.00	0.79	0.04	0.23	136.80	772.80	1.00
,CHOPS	2.00	0.00	0.00	212.04	0.00	0.54	799.20	0.00	1.08	3340.80	32.40	25.92	100.08	7.56	0.79	0.00	100.80	14.40	100.08	864.00	7.20	44.28	1368.00	2.52	21.56	270.00	7.20	16.78	14.76	0.00	2.09	0.68	0.00	396.00	0.00	0.36
N,ROAST	1.00	0.00	70.50	89.25	0.00	0.09	358.50	0.00	0.26	1500.00	22.50	12.75	40.95	1.95	0.60	0.00	34.50	7.50	40.95	273.00	0.45	20.40	334.50	1.55	5.69	123.00	0.00	8.01	2.91	0.00	4.46	0.11	0.00	132.00	241.50	0.00
AND VEG.	c1	29.50	1240.00	364.50	5.50	0.25	735.00	0.00	0.45	3075.00	55.00	7.50	55.50	5.50	0.88	4.50	75.00	40.00	51.00	475.00	4.00	42.50	930.00	1.95	15.35	520.00	3.00	16.00	13.00	10.00	2.10	0.75	0.00	220.00	12425.00	0.55
HITE FRIED	c1	0.00	0.00	69.50	0.00	0.08	165.00	0.00	0.10	692.00	19.00	3.20	19.60	0.50	0.28	0.00	12.00	12.00	19.60	0.00	1.80	10.70	332.00	0.18	1.39	189.00	4.00	1.93	0.67	0.00	6.65	0.19	0.00	61.00	3.00	7.49
HITE BAKED	c1	0.00	2625.00	2488.50	0.00	2.80	5215.00	0.00	3.50	21805.00	770.00	112.00	689.50	21.00	9.77	0.00	420.00	420.00	689.50	70.00	63.00	308.00	11690.00	6.30	162.05	9520.00	140.00	74.20	23.45	0.00	14.00	6.65	2.80	2905.00	10675.00	22.40
BAKED	2.00	10.30	0.00	73.60	7.30	0.07	66.00	5.20	0.05	270.00	45.00	0.50	5.10	1.40	0.12	5.10	31.00	29.00	0.00	91.00	0.00	0.50	300.00	0.00	0.08	480.00	0.00	0.05	0.70	0.00	0.25	0.21	0.00	0.00	0.00	0.60
T,VEG.	c2	146.25	7.50	523.50	13.50	0.30	1035.00	0.00	0.23	4335.00	150.00	3.00	26.25	6.75	0.50	26.25	67.50	37.50	0.00	405.00	0.00	33.00	735.00	1.58	3.98	1762.50	5.25	5.78	3.75	0.00	20.10	0.53	0.00	0.00	112.50	22.43
LENER	1.00	8.40	4.00	370.00	10.00	0.24	96.00	0.00	0.20	408.00	108.00	2.40	7.60	1.60	0.81	7.60	44.00	204.00	0.00	140.00	0.00	0.80	1292.00	0.48	0.16	24.00	5.60	0.08	0.96	220.00	0.48	0.36	0.00	0.00	56.00	0.56
,MASHED	c1	1.62	3.30	8.61	0.10	0.01	10.78	0.00	0.01	45.54	3.96	0.11	0.23	0.02	0.02	0.13	1.87	0.77	0.10	5.72	0.01	0.36	30.14	0.05	0.21	4.40	0.06	0.09	0.03	0.55	0.01	0.01	0.00	1.10	13.53	0.02
,FRIED IN OIL	c1	16.90	0.00	69.80	1.10	0.09	165.00	0.00	0.02	694.00	7.00	1.20	1.50	0.30	0.24	1.50	18.00	8.00	0.00	36.00	0.00	10.10	295.00	0.46	1.29	4.00	0.00	1.85	0.24	6.00	6.45	0.15	0.00	0.00	3.00	7.09
,IN CURRY	c1	26.85	0.00	110.40	1.65	0.14	187.50	0.00	0.03	790.50	10.50	1.80	2.40	0.45	0.38	2.40	28.50	12.00	0.00	57.00	0.00	7.65	466.50	0.72	0.99	6.00	0.00	1.38	0.39	9.00	4.86	0.24	0.00	0.00	1.50	5.39
,RAW	1.00	1.12	45.20	37.60	0.60	0.02	7.60	0.00	0.02	32.40	2.80	0.24	0.36	0.20	0.02	0.36	4.40	3.60	0.00	9.20	0.00	0.08	82.80	0.10	0.01	3.20	0.60	0.01	0.04	7.20	0.04	0.03	0.00	0.00	453.20	0.14
	1.00	4.56	1687.80	52.68	1.50	0.06	25.80	0.00	0.04	108.60	16.20	0.54	0.60	0.30	0.08	0.60	9.00	8.40	0.00	26.40	0.00	0.12	193.80	0.12	0.02	21.00	0.42	0.01	0.12	5.40	0.05	0.03	0.00	0.00	16877.40	0.26
EAM	2.00	47.61	0.00	151.11	0.00	0.09	379.50	27.60	0.35	1589.30	276.00	0.23	7.59	0.69	0.05	0.00	25.30	4.60	7.59	207.00	0.00	18.86	345.00	0.00	9.64	161.00	0.00	6.95	0.92	0.00	1.29	0.07	0.00	25.30	0.00	2.76
CHOCOLATE	c2	92.32	260.80	33.12	1.12	0.08	616.00	62.56	0.11	2587.20	56.00	0.48	7.68	1.60	0.09	4.32	38.40	17.60	3.36	132.80	0.32	25.12	196.80	0.51	6.62	296.00	5.44	12.02	0.98	0.00	4.85	0.30	0.98	105.60	880.00	4.14
MADEIRA	2.00	29.20	0.00	10.10	0.70	0.03	196.50	18.25	0.06	826.00	21.00	0.25	2.70	0.55	0.00	1.00	6.00	0.00	1.70	60.00	0.00	8.45	60.00	0.00	4.40	100.00	0.00	2.82	0.25	0.00	0.73	0.05	0.00	0.00	0.00	0.00
S,COMM.,PLAIN	1.00	26.16	0.00	1.04	2.24	0.01	192.00	8.80	0.02	803.60	14.80	0.16	2.04	0.28	0.02	0.44	13.20	0.00	1.60	65.20	0.00	8.08	26.80	0.00	4.05	146.00	0.00	2.74	0.24	0.00	0.84	0.09	0.00	0.00	32.00	0.00
S,COMM.,WITH FILLING	1.00	30.65	0.00	0.99	0.54	0.02	222.75	11.25	0.02	932.40	11.70	0.23	2.16	0.32	0.02	0.81	5.85	0.00	1.35	108.45	0.00	10.13	17.10	0.00	5.67	217.35	0.00	3.12	0.23	0.00	0.72	0.03	0.00	0.00	0.00	1.53
	6.00	16.41	0.00	1.26	0.06	0.02	153.60	0.00	0.01	642.90	0.00	0.06	1.56	0.00	0.00	1.56	0.00	0.00	0.00	0.00	0.00	9.39	32.70	0.00	2.30	193.50	0.00	0.39	0.00	0.00	6.70	0.00	0.00	0.00	44.10	0.00
ATE	2.00	10.10	1.19	0.37	0.00	0.02	89.93	7.50	0.04	376.38	37.40	0.03	1.43	0.27	0.00	0.19	9.35	1.70	1.24	40.80	0.00	5.15	71.40	0.10	3.00	20.40	0.51	1.65	0.03	0.00	0.18	0.05	0.00	0.00	11.39	0.09
VER OIL	49.00	0.00	0.00	0.00	0.00	0.00	397.80	0.00	0.00	1664.55	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	45.00	0.00	0.00	5.67	0.00	0.00	8.32	0.00	0.00	28.85	0.00	0.00	0.00	13.50	31.50
AISE	6.00	1.32	0.00	25.20	0.00	0.01	291.00	0.90	0.02	1218.00	7.20	0.00	0.72	0.24	0.00	0.00	0.00	0.00	0.72	22.80	0.00	32.10	4.80	0.00	2.82	234.00	0.00	1.80	0.00	0.00	27.48	0.00	0.00	61.20	0.00	0.00
SAUCE	1.00	1.30	19.60	17.82	0.14	0.01	6.00	0.00	0.01	25.40	2.80	0.22	0.26	0.16	0.00	0.26	3.80	0.00	0.00	6.40	0.00	0.04	74.20	0.06	0.00	121.00	0.00	0.01	0.05	2.60	0.01	0.04	0.00	0.00	195.80	0.00
DRESSING	cc	0.99	0.00	4.56	0.00	0.00	87.00	0.75	0.00	364.20	0.75	0.00	0.00	0.03	0.00	0.00	1.05	0.00	0.00	1.50	0.00	9.45	0.75	0.00	1.20	0.00	0.00	1.75	0.00	0.00	6.08	0.00	0.00	0.00	2.85	6.60
UT	2.00	39.04	0.00	21.12	0.00	0.00	279.20	12.00	0.00	1173.60	56.00	0.00	4.80	1.52	0.00	4.80	12.80	0.00	0.00	44.00	0.00	12.64	88.00	0.00	1.69	48.00	0.00	2.78	0.00	0.00	7.30	0.09	0.00	0.00	0.00	0.00
	1.00	10.20	4.00	37.15	1.50	0.03	46.00	0.00	0.05	192.00	3.00	0.25	0.50	0.15	0.29	0.50	14.50	9.50	0.00	10.00	0.00	0.25	198.00	0.13	0.10	0.50	0.00	0.02	0.08	4.50	0.05	0.05	0.00	0.00	40.50	0.09
D	1.00	5.30	61.00	74.30	2.10	0.11	161.00	0.00	0.12	674.00	11.00	1.90	2.00	1.00	0.28	2.00	39.00	62.00	0.00	41.00	0.00	15.30	599.00	0.97	2.44	10.00	2.60	9.61	0.42	8.00	1.96	0.27	0.00	0.00	612.00	2.63
S	1.00	6.12	0.00	1.20	4.98	0.17	348.00	0.00	0.06	1455.60	51.60	8.88	16.08	1.14	0.24	16.08	112.80	63.60	0.00	303.60	0.00	29.52	421.80	1.25	5.97	259.20	0.00	12.02	3.97	0.00	10.04	0.76	0.00	0.00	0.00	4.99
	2.00	31.40	34.00	155.20	7.20	0.08	120.00	24.00	0.06	516.00	16.00	1.80	0.80	1.00	0.00	0.80	12.00	0.00	0.00	22.00	0.00	0.00	240.00	0.00	0.00	14.00	0.00	0.00	0.80	432.00	0.00	0.20	0.00	0.00	332.00	0.00
	1.00	15.40	389.00	81.70	1.60	0.06	65.00	0.00	0.06	273.00	10.00	0.60	0.50	0.10	0.13	0.50	9.00	0.00	0.00	11.00	0.00	0.30	156.00	0.16	0.07	2.00	0.00	0.10	0.04	28.00	0.05	0.11	0.00	0.00	3894.00	1.12
G	c1	31.70	70.00	54.70	0.80	0.05	219.00	10.90	0.08	924.00	49.00	0.30	3.90	0.50	0.06	3.00	23.00	7.00	0.90	73.00	0.10	9.00	155.00	0.16	2.12	73.00	0.70	4.56	0.25	0.00	1.82	0.10	0.18	3.00	241.00	1.89
TOTAL		1890.70	7410.59	11902.11	138.60	10.44	19563.18	556.16	10.85	82144.57	4974.29	219.31	1175.38	82.85	17.74	184.34	2067.40	1910.97	991.04	7460.59	83.28	834.25	28806.31	29.66	317.50	21089.59	229.38	225.66	94.73	2119.75	177.40	17.33	4.69	4173.00	52826.77	142.25
TOTAL / 7		270.10	1058.66	1700.30	19.80	1.49	2794.74	79.45	1.55	11734.94	710.61	31.33	167.91	11.84	2.53	26.33	295.34	273.00	141.58	1065.80	11.90	119.18	4115.19	4.24	45.36	3012.80	32.77	32.24	13.53	302.82	25.34	2.48	0.67	596.14	7546.68	20.32

KEY : TOTCHO - TOTAL CARBOHYDRATE; VITA - VITAMINE A; MOIST -MOISTURE; THIA - THIAMINE; KILOCAL - KILOCALORIE; RIBOF - RIBOFLAVIN; KILOJOUL - KILOJOULE; CA - CALCIUM; NICOT - NICOTINIC ACID; PROT - PROTEIN; FE - IRON; VITB6 - VITAMIN B6; PLPROT - PLANT PROTEIN; MG - MAGNESIUM; FOL - FOLIC ACID; ANPROT - ANIMAL PROTEIN; P - PHOSPHOROUS; B12 - VITAMIN B12; T/FAT - TOTAL FAT; K - POTASIUM; PANT - PANTOTHENIC ACID; SATFAT - SATURATED FAT; NA - SODIUM; BIOT - BIOTIN; MUFAT - MONOUNSATURATED FAT; ZN - ZINC; ASCRB - ASCORBATE; PUFAT - POLYUNSATURATED FAT; CU - COPPER; VITD - VITAMIN D; CHOL - CHOLESTEROL; VITE - VITAMIN E; G - GRAM; RE - RETINOL EQUIVALENCE; MG - MILLIGRAM; UG - MICROGRAM; IU - INTERNATIONAL UNITS.

APPENDIX 3 : Change in triglyceride concentration of group 1 subjects over a 6 hour period, after ingestion of whole egg

TIME (HOURS)	TRIGLYCERIDE CONCENTRATION (mmol/l)											
	SUBJ. 1	SUBJ. 2	SUBJ. 3	SUBJ. 4	SUBJ. 5	SUBJ. 6	SUBJ. 7	SUBJ. 8	SUBJ. 9	SUBJ. 10	SUBJ. 11	SUBJ. 12
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.00	0.35	0.80	0.25	0.24	0.26	0.27	0.26	0.53	0.48	0.20	0.56	0.26
2.00	0.47	0.58	0.40	0.22	0.62	0.37	1.25	0.52	0.59	0.21	0.61	0.28
2.50	0.40	0.64	0.72	0.37	1.01	0.36	1.37	0.57	0.93	0.18	0.55	0.38
3.00	0.41	0.59	0.88	0.52	1.07	0.35	1.96	0.80	1.10	0.24	0.35	0.24
3.50	0.44	0.71	1.00	0.39	1.54	0.45	2.18	0.90	0.59	0.15	0.11	0.05
4.00	0.31	0.52	0.96	0.20	1.83	0.50	2.04	0.51	0.21	0.11	-0.01	-0.13
4.50	0.19	0.64	0.80	0.12	1.90	0.53	1.81	0.31	0.07	0.14	-0.05	-0.12
5.00	0.19	0.37	0.42	0.06	1.81	0.40	1.32	0.18	0.07	0.05	-0.13	-0.29
6.00	-0.10	0.13	0.18	0.00	0.81	0.41	0.47	-0.18	-0.05	-0.01	-0.09	-0.38

APPENDIX 4 : Change in triglyceride concentration of group 2 subjects over a 6 hour period, after ingestion of whole egg

TIME (HOURS)	TRIGLYCERIDE CONCENTRATION (mmol/l)											
	SUBJ. 1	SUBJ. 2	SUBJ. 3	SUBJ. 4	SUBJ. 5	SUBJ. 6	SUBJ. 7	SUBJ. 8	SUBJ. 9	SUBJ. 10	SUBJ. 11	SUBJ. 12
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.00	0.29	-0.11	-0.05	0.11	0.19	0.14	0.37	0.49	0.16	0.36	0.17	-0.31
2.00	0.61	0.31	0.65	0.61	0.56	0.67	1.27	0.99	1.07	0.68	1.45	0.99
2.50	0.95	0.40	1.06	1.12	0.69	1.36	1.63	1.32	1.28	0.72	1.89	1.56
3.00	1.33	0.46	1.88	1.65	1.34	2.13	1.94	1.86	1.45	0.88	2.74	2.04
3.50	1.35	0.44	2.47	1.76	1.58	2.40	1.98	2.10	1.71	1.01	0.67	0.48
4.00	0.66	0.40	2.50	1.76	1.47	2.37	1.66		1.31	1.30	2.13	2.63
4.50	1.15	0.12	2.58	1.88	1.71	2.50	1.52	1.46	1.22	1.09	2.07	2.79
5.00	0.81	0.19	2.16	1.78	1.56	2.15	0.97	0.55	1.01	1.07	2.33	2.63
6.00	0.44	-0.17	1.71	1.02	0.81	1.39	0.39	-0.06	0.25	0.73	0.92	1.66

APPENDIX 5 : Change in triglyceride concentration of group 1 subjects over a 6 hour period, after ingestion of egg yolk

TIME (HOURS)	TRIGLYCERIDE CONCENTRATION (mmol/l)											
	SUBJ. 1	SUBJ. 2	SUBJ. 3	SUBJ. 4	SUBJ. 5	SUBJ. 6	SUBJ. 7	SUBJ. 8	SUBJ. 9	SUBJ. 10	SUBJ. 11	SUBJ. 12
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.00	0.17	0.10	0.25	0.07	0.43	0.02	0.01	0.70	0.49	0.06	0.31	0.03
2.00	0.58	0.37	0.43	0.16	0.59	0.32	0.41	0.86	0.50	0.13	0.57	0.27
2.50	0.66	0.44	0.62	0.26	0.86	0.65	0.64	0.80	0.82	0.22	0.59	-0.02
3.00	0.78	0.57	0.81	0.20	1.83	0.83	0.86	0.69	0.70	0.31	0.42	-0.12
3.50	0.91	0.52	0.88	0.34	2.10	1.03	0.94	0.34	0.32	0.21	0.38	-0.18
4.00	0.88	0.14	1.03	0.17	2.06	1.08	0.51	0.02	0.11	0.20	0.04	-0.26
4.50	0.75	0.40	0.73	0.10	2.08	0.94	0.52	-0.13	-0.02	0.12	-0.04	-0.36
5.00	0.56	-0.07	0.67	0.09	1.15	0.76	0.72	-0.23	-0.01	0.06	-0.13	-0.29
6.00	0.27	-0.16	0.44	-0.03	0.34	0.37	0.47	-0.23	-0.08	-0.07	-0.17	-0.36

APPENDIX 6 : NUTRIENT CONTENT OF EDIBLE PORTION (50 g) OF ONE LARGE EGG

Nurients and units	Handbook No. 8-1 (1989)	Handbook No. 8-1 (1976)
Proximate:		
Water....g	37.66	37.28
Food energy....kcal	75	79
....kJ	313	330
Protein (Nx6.25)....g	6.25	6.07
Total lipid (fat)....g	5.01	5.58
Carbohydrate, total....g	0.61	0.60
Crude fiber....g	0	0
Ash....g	0.47	0.47
Minerals:		
Calcium....mg	25	28
Iron....mg	0.72	1.04
Magnesium....mg	5	6
Phosphorus....mg	89	90
Potassium....mg	60	65
Sodium....mg	63	69
Zinc....mg	0.55	0.72
Copper....mg	0.007	—
Manganese....mg	0.012	—
Vitamins:		
Ascorbic acid....mg	0	0
Thiamin....mg	0.031	0.044
Riboflavin....mg	0.254	0.150
Niacin....mg	0.037	0.031
Pantothenic acid....mg	0.627	0.864
Vitamin B-6....mg	0.070	0.060
Folacin....mcg	23	32
Vitamin B-12....mcg	0.50	0.773
Vitamin A....RE	95	78
....IU	317	260
Lipids:		
Fatty acids:		
Saturated, total....g	1.550	1.67
14:0....g	0.017	0.02
16:0....g	1.113	1.23
18:0....g	0.392	0.43
Monounsaturated, total....g	1.905	2.23
16:1....g	0.149	0.19
18:1....g	1.736	2.04
20:1....g	0.014	—
Polyunsaturated, total....g	0.682	0.72
18:2....g	0.574	0.62
18:3....g	0.017	0.02
20:4....g	0.071	0.05
20:5....g	0.002	—
22:6....g	0.018	—
Cholesterol....mg	213	274
Amino acids:		
Tryptophan....g	0.076	0.097
Threonine....g	0.300	0.298
Isoleucine....g	0.341	0.380
Leucine....g	0.534	0.533
Lysine....g	0.449	0.410
Methionine....g	0.195	0.196
Crystine....g	0.145	0.145
Phenylalanine....g	0.332	0.343
Tyrosine....g	0.255	0.253
Valine....g	0.381	0.437
Arginine....g	0.375	0.388
Histidine....g	0.148	0.147
Alanine....g	0.348	0.354
Aspartic acid....g	0.628	0.602
Glutamic acid....g	0.816	0.773
Glycine....g	0.210	0.202
Proline....g	0.249	0.241
Serine....g	0.465	0.461

(Agriculture Handbook No. 8 - 1 (1989))