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LIPID AND LIPOPROTEIN CHANGES IN KENYA URBAN WOMEN
USING TWO ORAL CONTRACEPTIVES //

BY

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A thesis Submitted in Fulfillment of the Requirement for the Degree

master of science of the

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DECLARATION

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*This book is dedicated to my wife Irene and my two daughters,
Wangui and Sekai.*

S U M M A R Y

One hundred and twenty five black Kenyan women attending the Family Welfare Clinic at Kenyatta National Hospital and seeking Oral Contraceptive advice were recruited to the study. They were randomly allocated two combined fixed dose Oral contraceptives; microgynon (150 ug levonorgestrel + 30 ug Ethinyl Oestradiol) and Eugynon (500 ug dl - norgestrel + 50 ug Ethinyl Oestradiol). The women were aged between 18 and 33 years (mean 25 + 7 years), a mean parity of 3 and a mean education of 8 years. They were all menstruating normally and in general good health. They had no history of liver disease, diabetes, renal failure, thyrotoxicosis, thromboembolic disease or any other contraindication to the use of sexual steroids. Fasting blood was taken on the day of recruitment and after 1, 3, 6, 9 and 12 months of Oral Contraceptive medication. Lipids and Lipoprotein-cholesterol were estimated each time. Height, weight, Systolic and diastolic pressure were also recorded for every visit.

The dl-norgestrel +EE regimen caused a significant increase ($P<0.01$) in body weight from the 9th cycle which remained so to the 12th cycle of treatment. The preparation levonorgestrel + EE caused a slight increase in body weight which was significant ($P<0.05$) from the 9th cycle. Both systolic and diastolic pressures were also significantly elevated ($P<0.01$) by both Oral Contraceptive preparations. Fasting lipid and lipoprotein cholesterol before therapy were estimated as: Total cholesterol 3.88 ± 0.73 mmol/l; triglycerides 0.68 ± 0.03 mmol/l; HDL-cholesterol 1.15 ± 0.22 mmol/l; LDL-cholesterol 2.41 ± 0.75 mmol/l and VLDL - cholesterol 0.33 ± 0.13 mmol/l/

The two preparations elicited a significant increase ($P/0.01$) in both the plasma lipids, total cholesterol and triglycerides. Lipoprotein cholesterol was also significantly elevated ($P/0.01$) except HDL - Cholesterol which was decreased significantly ($P/0.01$) by both preparations. However, dl - norgestrel + EE caused a slightly higher change than levonorgestrel + EE in both lipids and; lipoprotein cholesterol though the difference was not statistically significant.

ABBREVIATIONS

ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
Apolipo protein	Peptide on lipoprotein coat designated as A, B, C, D, E, and F.
ATP	Adenosine triphosphate
C-terminal	Carboxyl terminal
CDP	Cytidine diphosphate
CH	cholesterol
CHD	coronary heart disease
CVD	cardiovascular disease
CE	cholesteryl ester
d	density
Dc	dissociation constant
EDTA	ethylene diamine tetracetic acid
EE	ethinyl oestradiol
GLU	glutamic acid
HDL	high density lipoprotein (fraction 2 or 3)
HMGCoA	B-hydroxy-3 methylglutaryl coenzyme A
IHD	ischaemic heart disease
IDL	intermediate density lipoprotein
K _m	michaelis constant
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LDL-C	low density lipoprotein cholesterol
LP	lipoprotein
LPL	lipoprotein lipase
LCAT	lecithin cholesterol acyltransferase
MI	myocardial infarction
N-terminal	amino terminal
NAD	nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
O.C.	oral contraceptive
PEP	phosphoenolpyruvate
PK	pyruvate kinase
PHLA	post heparin lipolytic activity
POD	peroxidase
QC	quality control

<i>S</i>	<i>Svedberg unit</i>
<i>SD</i>	<i>standard deviation</i>
<i>SEM</i>	<i>standard error of the mean</i>
<i>SER</i>	<i>serine</i>
<i>SHBG</i>	<i>sex hormone binding globulin</i>
<i>steroid hormones</i>	<i>refer here to oestrogen and progesterone</i>
<i>TG</i>	<i>triglycerides</i>
<i>Type II lecithin</i>	<i>lecithin synthesised through CDP-ethanolamine pathway</i>
<i>U</i>	<i>unit (enzyme)</i>
<i>VLDL</i>	<i>very low density lipoprotein</i>
<i>W</i>	<i>omega—denotes the first -CH₃ group in fatty acid</i>
<i>W.H.O.</i>	<i>World Health Organization</i>

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INTRODUCTION1:1 STEROIDS AND LIPOPROTEINS

In childhood, girls have unusually higher plasma levels of triglycerides and cholesterol than boys. This reflects an increased level of very low density lipoproteins (VLDL) and low density lipoproteins (LDL). During puberty high density lipoproteins (HDL) decrease while VLDL and LDL levels increase in males (1). In premenopausal women, a 10% to 25% cyclic suppression of total plasma cholesterol, LDL cholesterol, and LDL apoprotein has been described in the luteal phase. However, HDL cholesterol increases slightly during the second half of the menstrual cycle (2). In postmenopausal women the lipid and lipoprotein changes in the direction of increased atherogenicity. During the sixth decade of life, women develop even higher LDL cholesterol levels than men (3).

Exogenous "natural" and synthetic oestrogens induce lipid and lipoprotein changes in women (4). From studies both of fertile and castrated women (5, 6) it has been inferred that this induced change depends on the type of oestrogens dose or mode of administration.

When a small daily dose (2 mg) of oestradiol is given, a pure oestrogenic response follows. Protein synthesis is stimulated which is seen as an increase in high density lipoproteins. The HDL-lipid content is little affected/for a small increase /but in phospholipids (4, 6).

Thus the number of HDL particles, including its enzymes increases resulting in enhanced catabolism of VLDL and increasing the plasma content of triglycerides. The exchange of apolipoprotein C from HDL-particles onto the surface coat of VLDL results in increased levels of LDL-cholesterol concentration in plasma. Due to the increased HDL particles, the slower rate of cholesterol ester formation is compensated and serum cholesterol level, therefore, remains unchanged (7).

Ethinyl oestradiol mimics oestrone though its effects are more pronounced (8). Thus there seems to be a difference between natural oestrogens (oestradiol) and synthetic ones (ethinyl oestradiol) with regard to lipid and lipoprotein metabolism. Natural oestrogens increase HDL cholesterol and reduce LDL and sometimes also VLDL fractions as found in earlier studies (5, 7, 9-11). Ethinyl oestradiol on the other hand reduced LDL and increased HDL and VLDL (4, 6). These findings have been confirmed by recent investigations (12, 13, 14).

As compared to oestrogens, the effect of progestogens on serum lipid and lipoprotein metabolism has received relatively little attention. Oliver and Boyd (15), Svanborg and Vikrot (16) reported

that progesterone administration has no significant effect on serum-lipid levels in man. However, other studies have reported a triglyceride lowering effect of progesterone in humans (17). Other studies have failed to demonstrate significant changes in serum triglyceride levels in progesterone-treated rats (2, 18, 19). Progesterone was, however, found to reverse oestrogen-mediated increase in serum triglyceride concentration in one study (20).

Oral administration of the 19 norsteroids (norethindrone and ; norgestrel) in pre-or postmenopausal women induced no significant changes in the levels of LDL-cholesterol (21). An increase in LDL-triglyceride was reported after treatment with dl-norgestrel, 1.8 mg/day (22). This triglyceride increase could account for the increase in total LDL mass reported by Valette et al (19).

The most consistent lipoprotein change observed in norgestrel users is a dose-dependent reduction in the level of HDL-cholesterol (21-26). The reduction in HDL has been predominantly in the HDL₂ subclass (6). This reduction is accompanied by a significant increase in the activity of heparin-released hepatic lipase (27, 28), an enzyme that has been implicated in the metabolism of HDL₂ (28). This reduction is more pronounced when these steroids are administered without added oestrogen (31), reflecting the reported increase in HDL levels associated with oestrogen use (5, 8, 23).

Some studies using levonorgestrel vaginal rings, have reported a reduction in HDL-cholesterol which persisted for up to 12 months of treatment (29). This was accompanied by reductions in total serum triglycerides, cholesterol (29, 30) and VLDL-cholesterol (29).

LDL cholesterol was reduced within the first three treatment cycles (29, 30, 31) but in one study rose towards the baseline levels by one year (29). Reductions in HDL and particularly HDL₂ were more pronounced than other lipoprotein classes. This resulted in increased total cholesterol/HDL-cholesterol and LDL/HDL ratios (29, 32)

1:2 EFFECT OF ORAL CONTRACEPTIVES

ON LIPIDS AND LIPOPROTEINS: CLINICAL IMPLICATIONS

The influence of oral contraceptives (O.C.) on lipid and lipoprotein metabolism has been the aim of many studies during the last decade (14,16, 17, 23, 33). This interest has been brought about by reports that lipid/lipoprotein changes have a significant importance in the etiology of cardiovascular complications associated with oral contraceptive use. Higher risks of such events as pulmonary, coronary thrombosis, cerebral embolism and myocardial infarction among young contraceptive pill users have been shown in several epidemiological studies (34, 35, 36, 37, 39).

Inmann and Vessey (40) reported that the risk of death from pulmonary embolism or cerebral thrombosis in pill users was seven to eight times higher than in non users. Mann and Inmann (34) showed that the risk of death from myocardial infarction was age related, being 2.8 times in age group 30-39 and 4.7 times in age group 40-44. The risk of non fatal myocardial infarction was also reported to be 2.7 times in age group 30-39 and 5.7 times in 40-44 (41). Beral (42) in an epidemiological study from 21 countries showed increased risk from 2.3 times to 5.2 times for all cardiovascular mortality in the use of oral contraception.

Hence it seems there is certainly a definite association between O.C. use and risk, morbidity and mortality from certain cardiovascular diseases.

Lewis and Chait (43) have demonstrated that in patients with ischaemic heart disease (IHD) several lipoprotein and lipid abnormalities are present. They found hyperlipoproteinaemia in 55% of the IHD patients with increased triglycerides, plasma cholesterol and VLDL levels. These were the most frequent abnormalities followed by increased LDL-cholesterol.

Many oral contraceptives are a progestogen - oestrogen combination and the net metabolic effect induced is not only dependent on the oestrogenic as well as the progestogenic component, but also on the total steroid dose administered. Synthetic oestrogens when combined with progestogens have been shown to cause an increase in LDL and a decrease in HDL (44,15). Earlier prospective studies yielded conflicting results.

Pincus (45) found no change in serum cholesterol or LDL. Aurell et al (46) found increased triglyceride and cholesterol levels. Brody et al (47) found no significant changes in any lipid levels using Anovlar (norethisterone acetate 5 mg and ethinyl oestradiol 50 ug). These investigators however, found increased triglycerides during use of ovulen (ethynodiol acetate 1 mg and mestranol 100 ug). Gershberg et al (48) found elevated triglyceride levels but showed that cholesterol remained unchanged. In relation to HDL, Aurell (46) and Wynn (49) found lowered HDL levels whereas Rossner (50) found slightly increased levels. Most workers found elevated LDL-cholesterol levels (51, 52, 53).

Larger cross-sectional and prospective studies have however indicated that triglycerides, total cholesterol and LDL are raised significantly (48, 54, 55). Briggs et al (56), Rossner et al containing levonorgestrel and ethinyl oestradiol decreased HDL-cholesterol.

1:3 ATHEROSCLEROSIS AND LIPOPROTEINS

The association between changes in the concentration of serum lipids and lipoproteins and the increased risk of cardiovascular disease (CVD) is now recognised (59-62). The lipid concentrations in the different classes of lipoproteins are correlated to atherosclerotic disease. Thus the low density cholesterol concentration are positively correlated to CVD (63). Very low density lipoprotein triglycerides have also been shown (64) to be positively correlated to CVD. In humans, an elevation of the LDL-C levels leads to a more rapid development of atherosclerosis (65). A lowering of the LDL-C can cause regression of induced atherosclerotic plaque in animals. Other studies on humans indicate that the rate of regression of an atheroma is either decreased or prevented when the LDL-C level is lowered (66).

The mechanism of the formation of the atheroma is not yet clear. However, recent work on cultured cells shows that certain lipoproteins are rapidly taken up by arterial smooth muscle and endothelial cells. (67, 68). Studies on cholesterol-fed rabbits show that arterial cholesterol is either initially derived from plasma cholesterol or is in rapid equilibrium with plasma cholesterol (69). Field et al (70) have concluded that in atherosclerotic patients, a major portion of arterial cholesterol is derived from arterial synthesis in situ. It is usually assumed that plasma LDL is taken up by the arterial wall by an LDL-receptor mediated mechanism (71). Recently apoproteins characteristic of VLDL and chylomicrons (B and C) have been identified in lesions of human arteries. Zilversmit (72) has shown that chylomicrons can be converted to remanants at the arterial surface. Chylomicrons and VLDL

of endogenous origin are bound to the vascular endothelial wall by sulfated mucopolysaccharides present in the arterial intima and media (73). After the action of the enzyme lipoprotein lipase (LPL) on the triglycerides (27) the indigestible remnants are partially taken up by endothelial or smooth muscle cells of the intima by phagocytic or pinocytic action (Fig. 8).

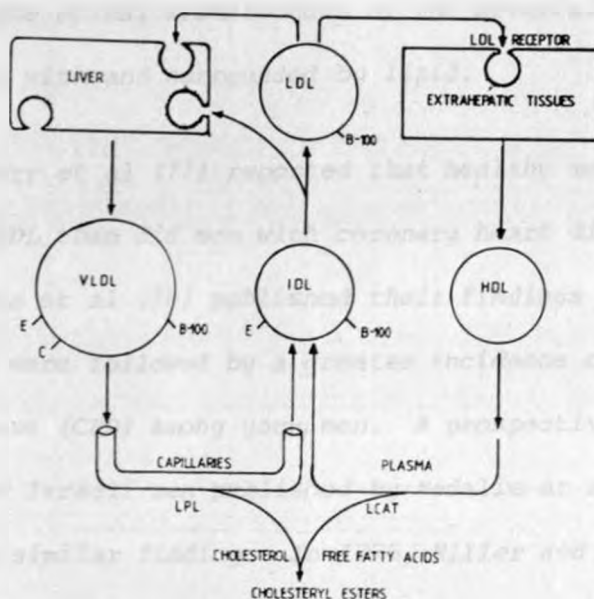


Figure 8 METABOLIC RECIPROCALITY BETWEEN LDL AND HDL

Fig 8.

VLDL is released from the liver with the three apoproteins B-100, C and E. On the capillary wall it undergoes hydrolysis by the enzyme LPL and is reduced to IDL and loses apo C to HDL. It is further reduced to LDL with loss of apo E also to HDL. The LDL is then taken up by receptors in the liver and extrahepatic tissues where it is catabolized. From Goldstein et al (71).

After the action of hydrolases (74) cell death occurs and the cholesteryl ester-rich contents are spilled into the extracellular spaces.

Smooth muscle cells (myo-intimal) of the media then invade the intima under the stimulus of the cholesterol deposited. These cells multiply, de-differentiate and migrate (75). An early event of atherosclerosis is the fragmentation of the internal elastic membrane (76) which allow the cells to escape from the usual environment of the media. They continue to proliferate and alter the normal architecture of the arterial wall which is soon filled with and surrounded by lipid.

In 1959, Barr et al (77) reported that healthy men had higher levels of HDL than did men with coronary heart disease. In 1966, Gofman et al (78) published their findings that lower HDL levels were followed by a greater incidence of coronary heart disease (CHD) among young men. A prospective study among middle aged Israeli men published by Medalie et al (79) in 1974 reported a similar finding. In 1975, Miller and Miller presented the 'HDL hypothesis' proposing the HDL particles were involved in the transport of cholesterol from the peripheral cells (80). This hypothesis was based on the inverse correlation between the concentration of HDL and the incidence of CHD as found in earlier studies (79, 81, 82). Recent prospective investigations support the hypothesis (83-85). It has also been sustained by the demonstration of xanthomas and early severe coronary atheromatosis in women with HDL deficiencies and normal LDL and low VLDL levels (86). In studies of the CHD patients, the HDL hypothesis has been strengthened by the negative correlation between the HDL-C level and the severity of atherosclerosis at coronary angiography (87).

The precise role of HDL in atherogenesis is not known but it has been suggested that HDL plays a protective role by blocking the uptake of cholesterol by smooth muscle cells and facilitating its removal from the arterial wall. This hypothesis developed from the work of Glomset (88) on the enzyme lecithin/cholesterol/ (LCAT). He showed that LCAT acts upon cholesterol and phosphatidyl choline in HDL to produce cholesteryl esters and lysolecithin. The lecithin is transferred to albumin, while cholesteryl esters leave the surface of the HDL particle and either enter its non-polar core or are transferred to the core of VLDL or LDL. The nascent discoidal HDL secreted by the liver acts as the substrate for LCAT and its action upon this HDL creates a gradient favouring movement of cholesterol from cells into the blood plasma and hence to the liver.

High density lipoprotein exists as two portions with different floatation constants (89). The most dense portion, HDL₃ ($d=1.125-1.21$) accepts free cholesterol from the tissues by exchange across cell membranes. This polar-free lipid on the HDL surface is converted to cholesterol ester via the enzyme LCAT. The neutral cholesterol ester migrates to the core where it accumulates creating a more lipid-rich form of HDL, designated HDL₂. This has a density range of 1.063-1.125 (90). The low HDL-concentration in men with CHD seems to depend mainly on the reduction of the HDL₂ portion (87) while the high HDL level in women is dependent on HDL elevation (91).

Oral contraceptives have been shown to induce metabolic changes including lipid/lipoprotein derangement. It has also been shown in the above discussion that atherosclerosis and myocardial infarction are closely related to impaired lipid/lipoprotein metabolism

2. THE STUDY

With the above discussion in mind, the changes reported in lipid and lipoprotein metabolism among oral contraceptive users have aroused considerable interest among researchers. The various studies are not always comparable due to different study designs, varying steroid dosage and failure of researchers to use suitable controls. Almost all the studies on this subject have been conducted in caucasian populations in affluent societies. A study of 131 Nigerian Women who had been using Noridy (1mg norethn-drone + 50 ug mestranol) for 1 - 60 months and 35 non users was reported by Taylor et al (92). These investigators reported a significantly higher mean HDL-cholesterol, and HDL-cholesterol/total cholesterol ratio. Another study was conducted in Egypt by Osman et al (93) on 16 women before and after continuous cyclic administration of Gyn-Anovlar (3 mg norethisterone and 50 ug ethinyl oestradiol) for six months. Another group of 20 subjects, who had never used contraceptive pills, was used as a control for the study. Triglycerides, LDL and LD /HDL ratio showed a statistically significant increase. In this study the subjects acted as their own controls eliminating the problem of using matched controls. The other discrepancy in many other reported studies was the use of different assay methods which resulted in very high coefficient of variation. Usually, small populations were studied and for short periods of oral contraceptive use. Comparatively, our study followed up the subjects for a period of one year and used fully enzymatic methods of lipid analysis which are relatively specific for the lipid parameters as compared to chemical methods. There is a need, therefore, to establish lipid and lipoprotein normal reference levels in this population since no data exist in the literature.

The data used in the hospitals has been based on caucasian levels which might/or not be reflective of our local population. This information will help the clinicians to make a better diagnosis and treatment of hyperlipideamias in women of reproductive age. The reported changes occurring during the use of steroid hormones instituted as contraceptive therapy on otherwise normal women makes it even more necessary to study the metabolic changes occurring during their use in this population.

There is increasing use of oral contraceptives in Kenya and this study may improve our knowledge as concerns lipid and lipoprotein metabolism in our population.

The objectives of the study were:-

1. To establish baseline levels of lipids and lipoprotein in healthy black women population in Kenya.
2. To evaluate the effect of two commonly used oral contraceptives on the effect of lipid and lipoproteins in the same population.
3. To analyse the data and find out which oestrogen/progestogen combination induced less lipid metabolic changes.

3 BACKGROUND

3:1 SEX STEROIDS

The sex steroids are divided into three groups according to the number of carbon atoms. The C-21 series include the corticoids and the progestins with the basic structure being the pregnane nucleus. The C-19 series include the androgens and are based on the androstane nucleus. The oestrogens based on the estrane nucleus belong to the C-18 steroids. For the purpose of this study we will concentrate on oestrogens and progestagens.

3.2 OESTROGENS

Oestrogens are dihydroxy alcohols of eostrone. In their natural form they exist as 17- β -oestradiol which due to the rapid, fast-pass, hepatic metabolism following intestinal absorption, are not active orally.

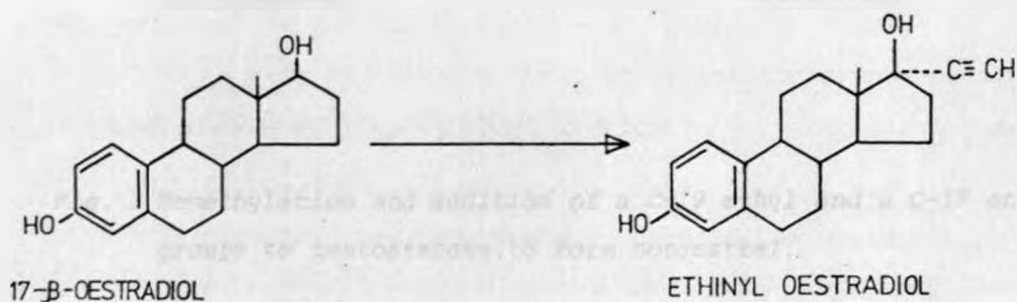


Fig. 2 The addition of an ethinyl group to 17- β -oestradiol

However, attachment of an ethinyl group at the 17- β position (Fig. 1) slows this hepatic uptake and metabolism sufficiently to produce a serum half-life compatible with oral administration (94).

Ethinyl oestradiol and its 3-methoxy form, mestranol are the two synthetic oestrogens most commonly used in currently available oral contraceptives. This study utilized oral contraceptives with an oestrogen dosage of 30 ug (microgynon) and 50 ug (Eugynon) combined with the progestagens 150ug levonorgestrel and 500ug dl-norgestrel respectively.

3.3 PROGESTOGENS

The synthetic oral progestogens are derived from testosterone by the demethylation of testosterone at C-19. This reduces the androgenic properties of testosterone thereby unmasking progestational activity (Fig.2).

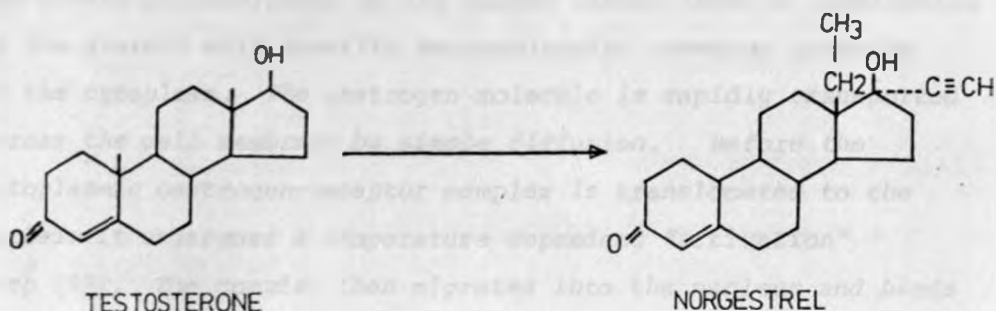


Fig. 3 Demethylation and addition of a C-19 ethyl and a C-17 ethynyl groups to testosterone to form norgestrel.

The presence of the ethyl group and the addition of the 17 ethynyl group make norgestrel a very potent oral progestagen. The binding of various norgestrel isomers indicate that the d-enantiomer is an active progestagen. The binding affinity of the dl-racemate is approximately half of the d-isomer while the l-form does not bind at all (95). The contraceptives under investigation had a dosage of 150ug (microgynon) levonorgestrel and 500 ug (Eugynon dl-norgestrel).

3.4. METABOLISM OF 17 α OESTROGENS

As noted above, ethinyl oestradiol is the synthetic product of 17-B oestradiol obtained by the addition of an ethinyl group at the α position. The effect of this 17- α ethinyl substitution is not localised but is transferred to the A ring resulting in a conformational and electronic change in the steroid molecule (96).

In plasma, ethinyl oestradiol exist as both the free form and as the 3-sulphate conjugate, the latter being formed in either the liver or the jejunal mucosa (97). The unbound free steroid is the physiologically active form. 17-B oestradiol has been shown to bind both albumin and the sex hormone binding globulin-(SHBG) (98). The 17-B group is important for the binding of steroids to SHBG but the lack of binding of ethinyl oestradiol may be attributed to the conformational and electronic deformation produced by the 17- α acetylation in the oestradiol molecule (96).

The effect of oestrogens on its target tissue involve combination of the steroid with specific macromolecular receptor proteins of the cytoplasm. The oestrogen molecule is rapidly transported across the cell membrane by simple diffusion. Before the cytoplasmic oestrogen-receptor complex is translocated to the nucleus it undergoes a temperature dependent "activation" step (99). The complex then migrates into the nucleus and binds to "acceptor sites" on the chromatin. Biological effect of synthetic steroid analogues must be mediated via interaction with specific hormone receptors (100).

The end product of ethinyl oestradiol metabolism is oestrone glucuronidate (85%) and the sulphate conjugate forms only 10% (100, 101, 102). The sulphate conjugate can bind to albumin with low levels of hormone in circulation (99). Under physiological conditions, about 85% of the sulphate conjugate is bound to the strong binding sites of albumin (97).

3.5. (2) PROGESTOGENS

The progestins of the 19-norsteroid series (gonanes) possess some androgenic properties unlike those of the pregnane group; and most of these steroids actually bind to the androgenic receptor. This is due to the removal of C-19 methyl group and the presence of Δ^4 double bond. The presence of a Δ^5 double bond as in norethynodrel results in the loss of the androgenic properties (103) and this compound actually exhibits some oestrogen activity.

A 3-keto-4-ene structure is a common feature for the effective binding of the ligand to the progestin receptor. Reduction of the double bond or the 3-keto group decreases the molecule's binding affinity (104-106). An unsaturated group, such as the ethinyl, increase the progestational potency of the product (107), as determined by bioassay,

When radioactive progesterone was injected into an oestrogen primed chick, the labelled hormone was detected in both the cytoplasm and the nucleus of the oviduct (108). The cytoplasmic receptor was found to sediment at approximately 4 S during sucrose gradient centrifugation in 0.3 M KCl and aggregate to 6S and 8S when ionic concentration was lowered (109).

This receptor appears to be a cigar-shaped dimeric molecule with a molecular weight of 225 000 daltons and is composed of two unequal subunits (A and B) with molecular weights of 100 000 and 117 000 daltons (109). They are unstable and very highly thermolabile.

Like in oestrogens, the cytoplasmic progesterone-receptor complex undergoes a two step "activation" mechanism in its translocation to the nucleus (110). Kinetic studies have shown that nuclear receptor sites are a class of high affinity ($D_C = 10^{-8} - 10^{-9} M$) saturable sites and more of them are available in target tissues than in non-target tissues. Some studies suggest the presence of other higher affinity sites ($D_C = 10^{-11} M$) (111) which may be the final "effector sites" for the steroid hormone action and may represent binding loci next to newly synthesized genes (112).

The binding of progesterone to the progesterone receptor appear to increase in response to estrogen stimulation (113).

3.6 LIPIDS

Lipids have been divided into four major classes. These are cholesterol, triglycerides, phospholipids and free fatty acids. The serum lipids are water insoluble and are transported in serum together with specific lipid-carrying proteins as spherical macromolecular complexes. These particles with a surface composed of apolipoproteins and polar lipids and a core of non-polar lipids are known as lipoproteins. Recently, their importance has increased due to their possible role in the pathogenesis of cardiovascular disease.

3.7 (a) TRIGLYCERIDES

These are esters of glycerol with three fatty acids. The fatty acids may be the same or different. Oleic acid is the fatty acid most abundant in fats with palmitate next. The melting point of the fats depends on the fatty acids, the lower the melting point the greater the proportion of unsaturation. Triglycerides are the major constituents of the adipose tissue which acts as the major storage organ of this fat.

Triglycerides have a very high caloric value relative to carbohydrates and proteins, hence its suitability for storage. They have a complex hormonal sensitivity (114, 115) which constitutes a feedback control of triglyceride levels in plasma. The plasma triglyceride concentration is also highly sensitive to changes in dietary composition and some drugs (114, 116). An increase in the proportion of carbohydrate or the ratio of saturated to polyunsaturated fats results in an increase in plasma triglyceride levels. This may represent an increased inflow of triglycerides from the liver or diminished outflow into fat stores (117, 119)

3.8

METABOLISM OF TRIGLYCERIDES

Exogenous triglycerides are hydrolysed in the gut to free fatty acids and glycerol by the action of pancreatic lipase and its cofactor colipase (120). Studies have shown that the lipase must adsorb to the lipid-water interface of its insoluble substrate (121). The presence of colipase is to bind to the lipid-water interface and provide an anchor to the binding of the lipase.

After hydrolysis, the dietary fat is absorbed as monoglycerides and fatty acids and resynthesized into triglycerides in the mucosal cells. The first direct evidence of *de novo* synthesis of free fatty acid by the intestinal mucosa was reported by Gould et al (122). The synthesis occurs almost exclusively in crypt cells (123, 124). Unsaturated fatty acids are esterified more rapidly than saturated fatty acids. (111). This is due to a presence of an intracellular carrier protein, with differing affinities for fatty acids which transport them from the microvillus membrane to the smooth endoplasmic reticulum (125, 126). In addition to its role in the synthesis of fatty acyl-Coenzyme A, the endoplasmic reticulum is also the primary site at which activated fatty acids are incorporated into triglycerides and phospholipids.

Two pathways for triglyceride synthesis have been indentified in the intestinal mucosa, the less significant α -glycerophosphate pathway (Fig. 3) is analogous to that demonstrated in the liver (127). Glycerophosphate reacts with 2 molecules of fatty acyl coA to form phosphatidic acid.

The phosphate ester then is cleaved, forming 1, 2-diglyceride which reacts with an additional fatty acyl CoA to form triglyceride. The α -glycerolp-osphate which enters this pathway is derived primarily from the glycolytic pathway (via reduction of dihydroxyacetone phosphate), but free glycerol entering the cell from the intestinal lumen or plasma may be phosphorylated directly (128).

The second pathway of intestinal triglyceride synthesis involves the direct acetylation of monoglyceride. The fat hydrolysate of the intestinal lumen (fatty acids and 2 monoglycerides) are incorporated into mixed bile salt micelles, and enter the absorptive cell, in the endoplasmic reticulum where the 2 monoglycerides are acetylated directly to form triglyceride - Fig. 3 (129, 130). There is strong evidence that this most direct, and efficient, pathway is also quantitatively more important (131). This importance in part reflects the fact that monoglycerides and fatty acids are the major products of intraluminal triglyceride hydrolysis, but in addition, Polheim et al (132) have shown recently that monoglycerides inhibit the α -glycerophosphate pathway of triglyceride synthesis.

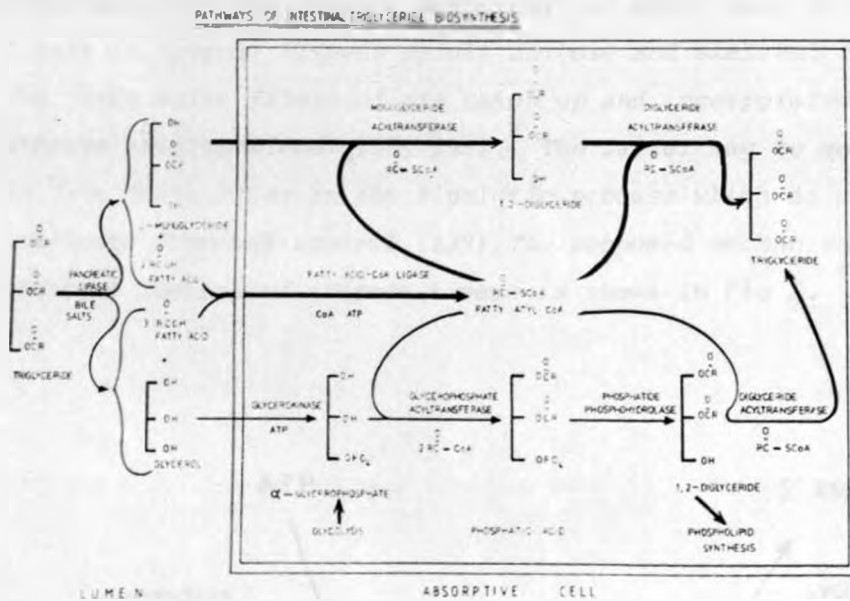


Fig 4. shows the major pathways of intestinal triglyceride biosynthesis. One is the direct acetylation of a monoglyceride to form a diglyceride which is acetylated again to form a triglyceride. The other which is not very significant involves reaction between α -glycerophosphate and two molecules of fatty acyl CoA. From Gangyl et al (117).

In both pathways 1, 2-diglycerides is synthesized as an intermediate, prior to final acetylation. There is however evidence that diglycerides synthesized by the two pathways do not mix, and constitute separate pools (133). The reason for this may be that, the two diglyceride pools are formed in distinct portions of the endoplasmic reticulum membrane and remain associated with the enzymes of the pathway by which they are synthesized (134).

In the liver, triglycerides may be formed from de novo synthesized fatty acids and from free fatty acids liberated by lipolysis of the adipose tissue under fasting conditions, and during ordinary dietary conditions, plasma free fatty acids are virtually the sole precursors for VLDL triglycerides (135). After ingestion of carbohydrate rich diets, hepatic fatty acids appear (136). The rate-limiting step in triglyceride synthesis is not known and interaction regarding mechanisms regulating triglyceride formation and its incorporation into VLDL is incomplete. What is clear is that chylomicrons and VLDL triglycerides undergo hydrolysis of ester bond by lipoprotein lipase in several tissues mainly adipose and skeletal tissue (131). The fatty acids liberated are taken up and incorporated into storage triglycerides (137, 138). The latter may be mobilized as free fatty acids in the lipolytic process which is under intricate hormonal control (139). The proposed mechanism of the hormonal control of adipose tissue is shown in Fig 5.

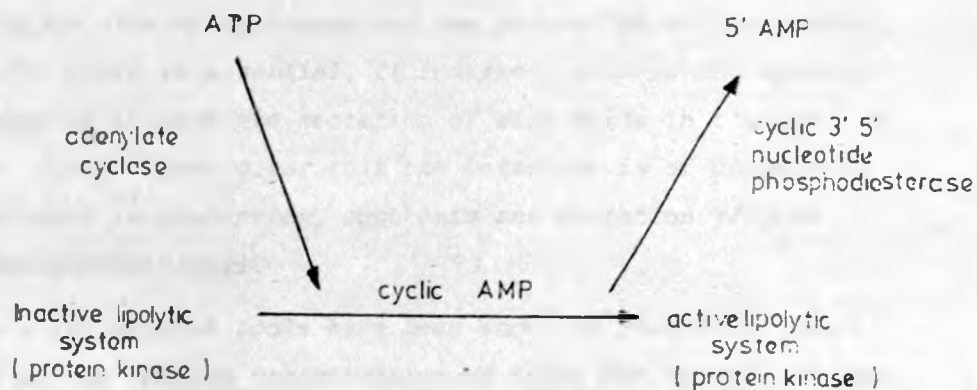


FIG 5 MECHANISM OF CONTROL OF LIPOLYSIS

Fig. 5 MECHANISM OF CONTROL OF LIPOLYSIS

The hormone unites with a receptor on the cell surface. The union activates the adenylase cyclase enzyme within the membrane wall leading to the conversion of Adenosine triphosphate (ATP) within the cell to cyclic adenosine monophosphate (AMP). The adenylase cyclase model involves "catalytic" and "regulator" constituents (140). The cyclic AMP released is specifically bound to a cytoplasmic receptor protein, and the CAMP-receptor protein complex activates a protein kinase. The protein kinase is also present in an inactive form composed of a regulatory and catalytic unit. Cyclic AMP releases the catalytic unit, and active phosphorylation ensues via the transfer of phosphate from ATP to a variety of substrate proteins by means of the kinase activity (141)

3.9

(b) CHOLESTEROL

Cholesterol is a 27-carbon structural modification of the basic unit perhydropentanophenanthrene ring. It is a monohydric alcohol which, in the body, forms ester bonds with free fatty acids. Due to this esterification 75% of cholesterol in plasma exists in esterified form. The rest is in the form of free cholesterol.

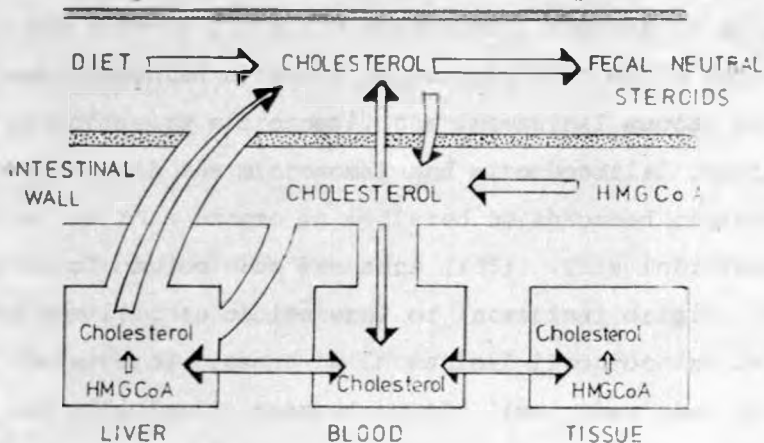
Cholesterol is a structural component of cellular membranes and a precursor of steroid hormones and bile acids. The liver is the major site of synthesis and the catabolism of cholesterol. It also plays an essential, if indirect, role in cholesterol absorption through the secretion of bile acids in the gut. Of late it has become clear that the intestine is of comparable importance in absorption, synthesis and excretion (Fig.5) of cholesterol (142).

Tissue cholesterol pools have been shown to correlate weakly (80) to the plasma concentration of total cholesterol in man. However, using the 2-pool analysis (143) it has been shown that tissue cholesterol pool size has a strong negative correlation to the high density lipoprotein.

Dietary cholesterol mixes in the intestinal lumen with cholesterol secreted in bile and from mucosal cells (144) (Fig. 5).

The micellar solubility of cholesterol which determines its mucosal uptake, depends on the local concentration of monoglyceride and fatty acids as these expand the micellar hydrocarbon core on which the sterols are dissolved (145). The hydrolysis of triglycerides provides fatty acids and monoglyceride for micellar expansion and it is probably by this mechanism that the feeding of fat enhances cholesterol absorption (145).

Before absorbed cholesterol appears in lymph, much of it is esterified with long chain fatty acids. The reaction is catalysed by mucosal cholesteryl esterase, an enzyme similar to the cholesteryl esterase of pancreatic origin (146).



CHOLESTEROL METABOLISM IN MAN

Fig 6 Shows the pool compartments of cholesterol in man.

The pools interchange readily. Cholesterol is synthesized from acetate in the liver and enters the blood stream which is also fed from dietary cholesterol through the intestinal wall. Some tissues also synthesize cholesterol and are also replenished from the blood stream. From Dietschy (123).

Although most tissues in man are capable of synthesizing cholesterol, hepatic and intestinal cholesterogenesis predominates (144, 127). There is ample evidence that intestinal cholesterol biosynthetic pathway is the same as that which has been demonstrated in the liver. Thus, three molecules of acetyl-CoA are condensed to form B-hydroxy-B-methylglutaryl COA (HMG-CoA) with subsequent non-reversible reduction of the latter to

mevalonic acid by the enzyme HMG-CoA reductase (148). This enzyme is considered rate-limiting though under certain circumstances HMG-CoA synthase (or HMG-CoA condensing enzyme) also becomes rate controlling (149, 150). The reaction proceeds via squalene which undergoes cyclization to lanosterol (30C) which is converted to the 27-carbon cholesterol by modification of the sterol nucleus and side-chain (151).

Virtually all of the measurable de novo synthesis of cholesterol is carried out in the crypt cells with cells located on the villi showing minimal activity (152). Other studies have shown, however, that tritiated mevalonate is incorporated into sterols, primarily in the villi (153). Dietschy (144) has shown that intestinal cholesterol synthesis is inhibited by bile salts at some point after the formation of acetoacetyl-CoA and prior to mevalonate. This shows that the rate-limiting step is probably the same in liver and intestine. Sheffer et al (154) has however presented evidence supporting that while hepatic HMG-CoA reductase is microsomal, the intestinal mucosa enzyme is present in both the microsomal and mitochondrial fractions. The control of this enzyme is mediated by absorbed cholesterol in the form of chylomicron remnants (135). This inhibitory effect is highly specific to cholesterol of intestinal origin. This is because cholesterol present in intestinal lipoproteins is more rapidly and efficiently trapped by the liver than that in other plasma lipoproteins (156).

In the liver of the normal rat, cholesterol synthesis shows a striking diurnal variation with a peak at midnight and a nadir during the light period (157). This appears to be mediated by changes in the synthesis and degradation of the microsomal HMG-CoA reductase (158). Other factors affecting cholesterol synthesis in the liver include cholesterol feeding which decrease synthesis and biliary obstruction which increase synthesis. Weiss and Dietschy et al (159) postulated that cholesterol of intestinal origin directly inhibits hepatic HMG-CoA reductase and as there is constant transfer of cholesterol from the intestine to the liver, this inhibitory effect would be operative. However, the question as to whether bile salts, cholesterol or both are physiological regulators remains unresolved. (160).

3:11

(C) PHOSPHOLIPIDS

Like triglycerides, phospholipids are derived from the trihydric alcohol glycerol. A phosphate group is attached to the hydroxyl group of the third carbon (Fig 6). To the phosphate moiety may then be attached a molecule of either choline, ethanolamine, serine or inositol depending on the type of phospholipid. Lecithin comprises roughly 70% of the phospholipids in human serum. Sphingomyelin constitutes 20%, lysolecithin 7% and cephalin 2-3%. Phospholipids are important components of cell wall membranes and serum lipoproteins.

3:12

METABOLISM OF PHOSPHOLIPIDS

In liver lecithin is synthesized through several pathways and there is a rapid equilibration between the liver and serum lecithin (161). In man, the relative fatty acid composition of liver and serum lecithin is roughly the same (162). One of the routes of synthesis—pathway 1 (Fig. 7) involves cytidine diphosphate (CDP) and is known as choline diglyceride pathway.

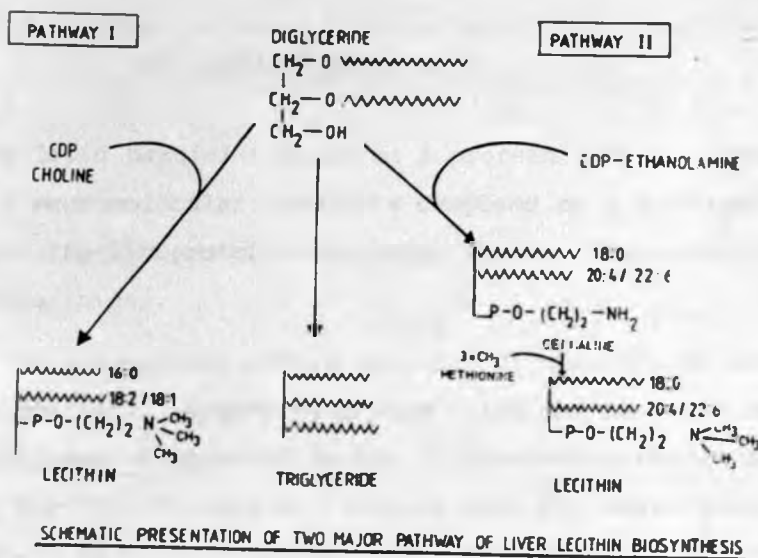


Fig 7 Shows the two major pathways of lecithin biosynthesis in the liver. The precursor is a diglyceride and pathway utilizing CDP-choline is the most common. Pathway utilizing CDP-ethanolamine contributes only to 10% of lecithin formation. From silverstolpe (165).

This pathway gives preferably palmitic acid in the 1-position and linoleic acid or oleic acid in the 2-position of the lecithin molecule (163).

Pathway II, involves cytidine-diphosphate and is known as the ethanolamine methylation pathway (Fig. 6). In vitro studies of human liver slices have shown the contribution of this pathway to be 10-15% of pathway I (164). This pathway has been shown to give a lecithin with predominantly stearic acid in position 1 and arachidonic acid in position 2.

Lecithin-lysolecithin shift is accomplished by phospholipase A1 activity which causes a deacylation of the fatty acid in position 1 of lecithin which is transformed into lysolecithin (165). In vitro, hepatic lipase possesses phospholipase A1-activity with lecithin and cephalin (166). Another mechanism liable to cause this shift would be an inhibition of the enzyme LCAT. The enzyme enhances transfer of a polyunsaturated fatty acid from the 2-position of lecithin to the 3-position of cholesterol giving lysolecithin and a cholesterol ester (167). This reaction takes place mainly in the high density lipoproteins.

3:13

(D) LIPOPROTEINS

These are lipid particles bound to a protein moiety. They are spherical macromolecular complexes composed of a hydrophilic surface of apo-lipoproteins and polar lipids (free cholesterol and phospholipids).

The core is hydrophobic and is composed of cholesteryl esters and triglycerides. Depending on the size and relative amount of the different components in the lipoprotein particle the hydrated density (d) varies. This is used for separation of lipoproteins by ultracentrifugation. The following classification system is commonly used; chylomicrons ($d=0.92 - 0.96$), very low density lipoproteins -VLDL ($d=0.96 - 1.006$), low density lipoproteins-LDL ($d=1.006 - 1.063$) and high density lipoproteins-HDL ($d=1.063 - 1.211$). The HDL fraction is often separated into two major factions, HDL₂ and HDL₃ (89).

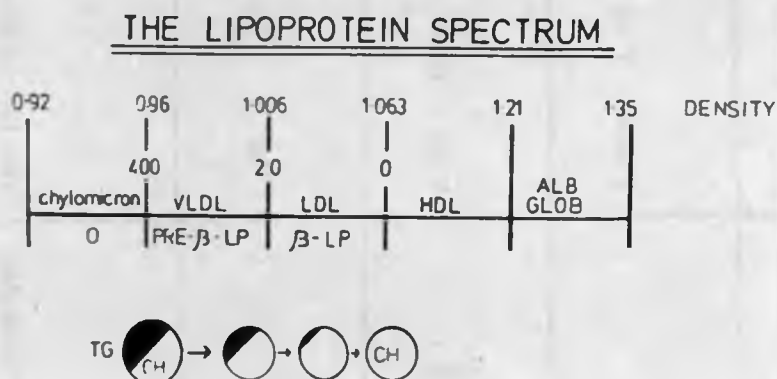


Fig. 8.: Adapted from Silverstolpe (I65).

Separated according to electrophoretic mobility, the lipoproteins are identified as α-lipoprotein (HDL), pre-β-lipoproteins (VLDL) and β-lipoproteins (LDL) as in Fig. 8. The relative content of lipids in the lipoprotein fractions of plasma from fasting individuals are shown in Table I. Table II shows the distribution of apolipoproteins in each lipoprotein class.

3: I 4 CHYLOMICRONS.

These are the largest lipoproteins with a diameter of 300-5000 Å and a density of less than 0.95 g/ml. They contain about 1% cholesterol, 0.5 - 2% protein, 80-95% triglycerides and 2 - 12% phospholipids (II8).

They occur postprandially in healthy individuals and are therefore not found in the plasma of fasting subjects. Chylomicrons are formed in the intestinal mucosa, and transport dietary fat, mainly triglycerides, via the lymphatic system to the blood stream. The chylomicrons are successively transformed into smaller particles by lipoprotein lipase (I67) to free fatty acids and glycerol. The free acids can be reassembled with glycerol to form triglycerides in the adipose tissue. This is stored as an energy reservoir or immediately utilized in muscles as energy source.

LIPID LIPOPROTEIN CLASS	CHOLESTEROL	TRIGLYCERIDE	PHOSPHOLIPID	PROTEIN	APOLIPOPROTEIN	DENSITY RANGE	ELECTROPHORETIC MOBILITY
CHYLOMICRON	1	80 - 95	2 - 12	1	C, A	.96	ORIGIN
VLDL	10.25	50 - 70	15 - 25	10 - 13	B, C, E	0.96 - 1.006	Pre - B
LDL	40 - 45	10	20 - 25	20 - 25	B	1.006 - 1.063	B
HDL	15	5	35	50	A, C, D, E	1.063 - 1.2	α

Table 1. Distribution of various lipids and apolipoproteins in each lipoprotein class.

APOPROTEIN	LOCATED	MW	CARBOHYDRATE	FUNCTION	ORIGIN
A - I	HDL, Chylomicron	28 300	-	LCAT activation	Intestine, liver
A - II	HDL, chylomicrons	17 000	-	?	Intestine, liver
B	VLDL, LDL	?	5%	i) TC transport ii) Binding to high affinity affinity LDL, Receptor leading to LDL uptake into cells	Intestinal, Liver
C - I	VLDL, Chylomicrons	6331	0	?	Liver
C - II	VLDL, Chylomicrons HDL	8837	0	Activates LPL	Liver
C - III 0, 1, 2	VLDL chylomicrons HDL	8764	-	? inhibits LPL	Liver
D	HDL	22700	-	? Regulates hepatic clearance of chylomicron remnant	Liver

Table II Characteristics of human serum lipoprotein apoproteins.

In conditions like type I hyperlipidaemia (hyperchylomicronemia) where lipoprotein lipase is absent there is an associated gross hypertriglyceridemia (167). In some cases of type V hyperlipoproteinemia a similar enzyme defect leads to an accumulation of chylomicrons and VLDL. In these cases the enzyme activity is reduced but not absent. Chylomicrons have been shown to be associated with the apolipoprotein C (108). The apo C-11 appears to activate the enzyme LPL reducing the K_m (Michaelis constant) of the enzyme for the triglyceride (169).

Dietary cholesterol is also incorporated into chylomicrons, whose other apolipoprotein B-48 is of lower molecular weight than apo B-100 of VLDL and HDL (Fig 8). After its TG are hydrolysed by endothelial LPL, the cholesterylester-rich chylomicron remnant is rapidly cleared by uptake into the liver through receptor-mediated endocytosis. None of these particles are converted to LDL. These remnant particles are thought to be highly atherogenic and its not clear as yet whether the inverse relationship between HDL concentration and CHD results from the anti-atherogenic property of HDL or from an atherogenicity of remnant particles (170).

A third apoprotein (Table II)-apo AI-is also present on the chylomicron surface coat. It enters into circulation partly as a component of nascent chylomicrons (171) and partly from small intestine as a component of discoidal nascent HDL (172). The mechanism by which the chylomicrons particle is assembled is not clear but studies of Cardell et al (173) showed that triglycerides which appear initially within the apical smooth endoplasmic reticulum, subsequently accumulated in the Golgi apparatus and are discharged into the lateral intracellular space. The structure of the chylomicron is a hydrophobic core (triglyceride and cholesterol esters), surrounded by a hydrophillic 'membrane' consisting of protein, phospholipid and free cholesterol. This concept of a spatial orientation of the components of the lipoprotein particle, may be regarded as analogous to the micelle (174).

Studies have shown that the triglycerides of chylomicrons are largely removed in extrahepatic tissues, whereas the cholesteryl esters are taken up almost entirely in the liver (175,176).

Nestel et al (177) found that component cholesteryl esters remained in the blood when chylomicrons were injected into functionally hepatectomized dogs, while triglycerides were removed rapidly as in intact dog. They suggested that triglycerides were removed first through the action of lipoprotein lipase in extrahepatic tissue and that cholesteryl esters, contained in a partially degraded 'skeleton' are subsequently removed by the liver (178). The concept of the involvement of high density lipoprotein in the chylomicron metabolism was supported by the works of Havel et al (178) and Eisenberg et al (179). The workers found that the group of small C apoproteins with rapid electrophoretic mobility, transfer to rat chylomicrons and VLDL from HDL after they are secreted, and return to HDL as triglycerides are taken up in extrahepatic tissue. Phospholipids also leave the surface and may be transferred similarly, as suggested by the substantial increase in HDL-phospholipids during alimentary lipemia in man (178). By contrast, the B-apoprotein is retained in the remnants.

3:15 (b) VERY LOW DENSITY LIPOPROTEINS (VLDL)

The major source of VLDL is the liver though lately the intestinal mucosa has been shown also to synthesize them (48). Intestinal and hepatic VLDL differ electrophoretically in that though both migrate to the pre-B regions, nevertheless intestinal lymph VLDL is slower than plasma VLDL since the former has a lower number of surface peptides which are increased as they enter the general circulation. (180).

The removal mechanism is similar to that of chylomicrons, the enzyme itself being activated by apolipoproteins CII which is carried by HDL (181). Upon entering the plasma compartment, these

TG-rich particles undergo a series of transformation by which they are converted progressively into smaller particles called intermediate density lipoproteins (IDL) or remnants and finally to low density lipoprotein-LDL (182, 142). The core of the VLDL particle contains large amounts of TG and relatively small amounts of cholesteryl ester (CE). The surface contains three apoproteins, Apo B-100, Apo E and Apo C. In the capillaries of adipose tissue and muscle the VLDL-TG are hydrolysed by epithelial LPL, but the CE remain intact (183). During hydrolysis, both apo C and E leave the VLDL particle but apo B remains with the remnants. During catabolism there is also a net loss of both the core TG and surface components i.e. phospholipids (60). The product of this conversion, IDL is relatively enriched in cholesteryl ester (183). Others escape hepatic uptake and remain in circulation where most of the residual triglycerides are removed and the core is reduced to nearly pure cholesteryl ester. It is during this final conversion that apo E and C are lost and the resultant particle is known as LDL (182, 183). The apoprotein CII is picked up again by HDL which acts as a shuttle for apo C (184). The VLDL fraction formed primarily in the liver can be decomposed to form LDL and HDL. A close metabolic relationship exists between VLDL and HDL through the conversion of cholesteryl esters Fig. 8 (185, 186). The cholesterol released from the VLDL fraction is esterified to cholesteryl esters. The free fatty acids necessary for esterification are split off from the lecithins in the HDL. The free fatty acids are released while some of the cholesterol esters are taken by the VLDL. Fig 8 shows the metabolic pathway of VLDL. It is observed that the LDL particle is transported back to the liver and extrahepatic tissue where the

particles are recognized by the LDL receptors through the apolipoprotein B (188).

3:16 (C) LOW DENSITY LIPOPROTEINS (LDL)

They have apo B as their major apolipoprotein and a beta mobility on electrophoresis. The N-terminal amino acid of the LDL apolipoprotein is glutamic acid with serine as the C-terminal amino acid, hence the designation Glu-apo LP-Ser. Most of the LDL particles originate from the metabolism of VLDL by the enzyme LPL and most of serum CH is transported in this fraction (185). Low density lipoprotein is catabolised in peripheral cell systems after binding to specific LDL receptors (186). This peripheral receptor like the hepatic LDL receptor which binds remnants (811) binds apo E with higher affinity than apo B, the binding of the two apoproteins is calcium dependent and is inhibited by EDTA and the receptors are subject to metabolic regulation by a variety of hormonal, nutritional and pharmacological factor (189).

The LDL-receptor is a heavily glycosylated transmembrane protein with molecular mass of 160,000 daltons and comprises five distinct structural domains (190). At its N-terminus on the exterior of the cell membrane there are seven cysteine-rich, highly ordered repeat sequences, which have been implicated in LDL binding. Proximal to these are a stretch of 350 amino acids. The next domain lies immediately adjacent to the cell membrane and is highly glycosylated. The membrane itself is spanned by a 22 amino acid hydrophobic region which leads into a 50 amino acid C-terminal sequence protruding into the cytoplasm. The entire protein molecule is responsible for the selective recognition of lipoproteins containing apoproteins B or E and for their concentration on particular sites (coated pit

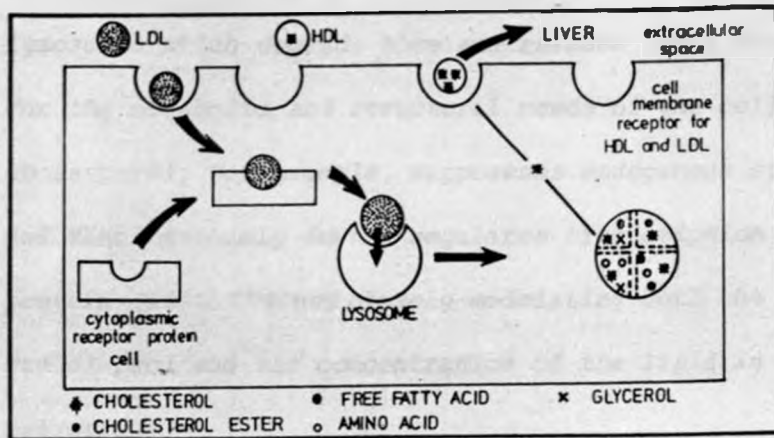


Figure 9 BREAKDOWN OF LDL IN THE CELL

Fig 9 The lipoprotein LDL is taken up by receptors on the cell wall of the liver. The particles is then internalized by endocytosis as the LDL-receptor complex and released into the lysosome. Here it is hydrolysed into its components. Some cholesterol esters are taken up by HDL and returned to the liver for catabolism. From Goldstein et al (71).

regions) on the cell surface (Fig 10). These facilitate membrane translocation of LDL within endocytic vesicles, directing their migration across the cytoplasm to the perinuclear lysosomes which degrade them and release their constituents for the metabolic and structural needs of the cell. The liberated cholesterol, for example, suppresses endogenous sterol synthesis and simultaneously down - regulates transcription of the receptor protein (186), thereby closely modulating both the intracellular sterol pool and the concentration of the lipid in the milieu exterieur.

3:17 (d) HIGH DENSITY LIPOPROTEINS (HDL)

This is an important carrier for cholesterol mainly in the esterified form. It contains apoproteins AI and AII in its coat (188). The synthesis and secretion of HDL takes place in the liver and intestine (191). They are secreted in the form of bilaminar discs composed of phospholipid surrounded on its hydrophobic perimeter by detergent - like apoproteins (192). Such nascent HDL particles have a capacity to take up additional cholesterol and are highly reactive with lecithin-cholesterol acetyltransferase. After entering the interstitial fluid nascent HDL particles acquire A peptides from cyclomicrons and become enriched with unesterified cholesterol molecules derived from the surface components of TG-rich lipoproteins liberated during their catabolism. The enzyme circulates in plasma in association with HDL and is probably activated by apoproteins AI and CII (193).

The enzyme esterifies the unesterified cholesterol within HDL with a proportion of the resultant cholesterol esters entering the central core of the HDL particle by virtue of their non-polar nature, leaving vacant binding sites on the

Figure 10 THE ROLE OF HDL IN FAT METABOLISM

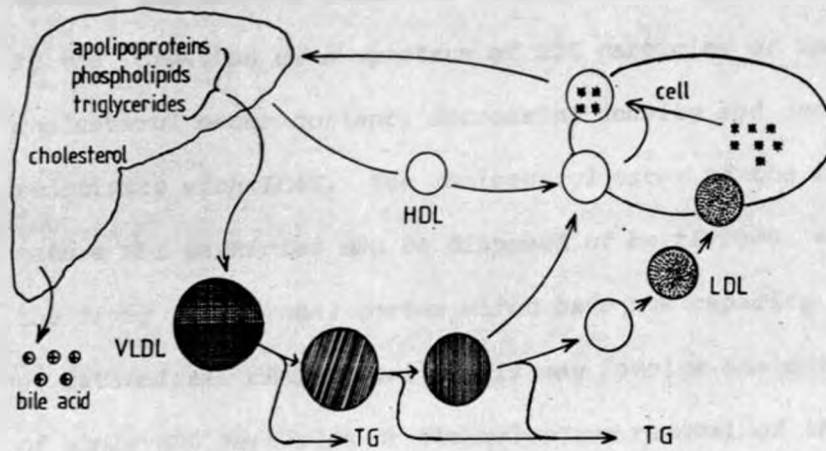


Fig 10. The liver is the source of HDL which is released as discoidal particles. During the catabolism of VLDL, apoproteins leave the HDL surface and onto VLDL at the same time taking up apoproteins and other lipids as VLDL is reduced to LDL. It therefore acts as a shuttle for apolipoproteins during lipoprotein metabolism. From Samsioe (under publication)

surface of the particle for uptake of further cellular unesterified cholesterol (194). This process is repeated between the intravascular and extravascular spaces, resulting in the formation of a spectrum of HDL particles of increasing cholesteryl ester content, decreasing density and decreasing reactivity with LCAT. The cholesteryl ester of the most mature HDL particles may be disposed of by tissues, such as the liver and adrenal cortex which have the capacity to excrete or catabolize cholesterol. This may involve the catabolism of whole HDL particles or the selective removal of the esterified cholesterol moiety. High density lipoprotein also contains another lipoprotein apo CII which may activate the enzyme lipoprotein lipase an enzyme secreted from parenchymal cells such as adipocytes (195) and acts at the capillary endothelium level to hydrolyse the triglyceride moiety of the chylomicrons and VLDL with accompanying hydrolysis of phospholipids (196).

4.0 MATERIALS AND METHODS

One hundred and fifty six women aged between 18 and 33 years (mean 25 ± 7 Years) attending the Family Welfare Clinic at Kenyatta National Hospital and seeking contraceptive advice were recruited to the study. The protocol had been earlier approved by the hospital ethical committee and verbal informed consent obtained from each subject.

All the subjects were menstruating normally and were in general good health. None of these women had been on any form of steroid contraception for at least six months prior to the study. They had no history of liver disease, diabetes, renal failure, thyrotoxicosis, or any other contraindication to the

use of OC. A detailed screening form was filled at the recruitment of each subject. The information showed age, education and occupation, number of pregnancies, last menstrual period, blood pressure, height and weight.

On the day of recruitment the subjects were asked to return the following day to the clinic after a 12 - 14 hours fast for blood sampling. The subject was asked to rest for about 5 minutes in a sitting position before the blood sample was taken. From each subject, 10 ml of blood was taken from the antecubital vein by venepuncture. Blood was collected into 10 ml bottles containing disodium ethylene diamine tetracetic acid salt (1.5g/l). After mixing, the samples were taken to the laboratory and centrifuged at 3000 rpm for 15 minutes at room temperature. Plasma was separated by suction and then transferred into plain dry vial for storage at 4°C.

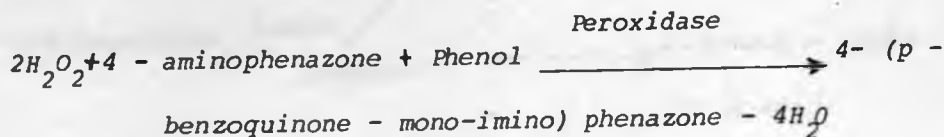
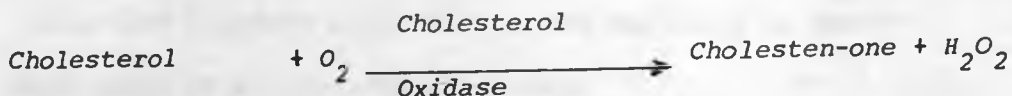
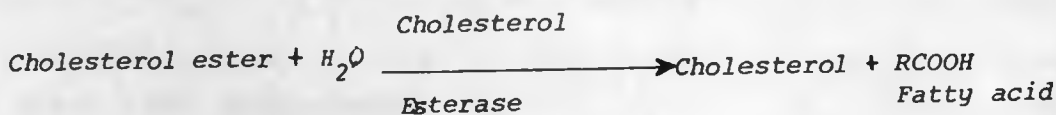
For total cholesterol and triglycerides, analysis was carried out within 24 hours of the blood collection. For HDL-cholesterol, precipitation of the B-lipoproteins was carried out the same day of blood collection and the supernatant stored at 4°C. Analysis for the HDL-cholesterol in the supernatant was carried out within 72 hours. A Pye Unicam SP-6 UV/VIS was used to read absorbances. Conventional statistical methods were used to calculate range, mean, SD and standard error of the mean (SEM). Paired Student's t -test using the two -tailed 't' test tables were used to calculate statistical significance between the pretreatment and treatment values. The Hewlett Packard HP-97 was partly used, but most of statistics were done using a microcomputer (memory 700) at the Kenya Medical Research Institute. The sample size had been previously

calculated using the assumption that 90% of the O.C. users would have increased lipid values by the 12th month of use. The Z value was obtained by using the 95% confidence limit and the formula gave an N value of 138.

4:1 ANALYTICAL METHODS

4:1:1 (a) TOTAL CHOLESTEROL

An enzymatic colorimetric method based on the Boehringer Mannheim Kit No. 172 626 was used. It uses the principle that cholesterol ester is hydrolysed to free cholesterol by cholesterol esterase and cholesterol to cholesten - one and hydrogen peroxide by cholesterol oxidase. Hydrogen peroxide is then coupled to aminophenazone and phenol in presence of peroxidase to produce a pink chromogen.



Cholesterol esters (75% of the total cholesterol) are converted by esterase to free cholesterol. In presence of molecular oxygen and cholesterol oxidase, cholesterol is converted to

Δ^4 - cholestenone and hydrogen peroxide. Two moles of the hydrogen peroxide in presence of 4 - aminophenazone and phenol are coupled in the catalytic action/peroxidase

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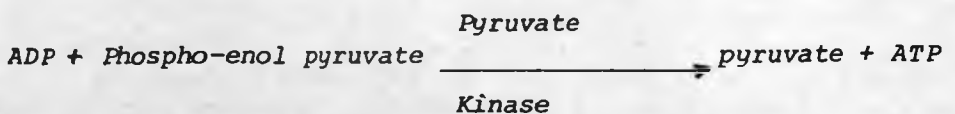
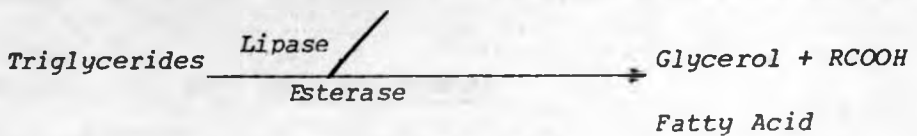
to form the phenazone chromogen. This chromogen is measurable colorimetrically having a maximum absorption peak at 500nm.

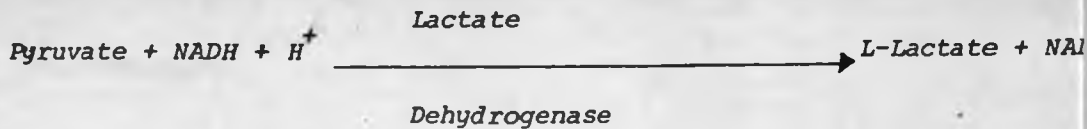
REAGENT AND PROCEDURE

Solution 1 (Potassium phosphate buffer 0.4 mmol/l, ph 7.7; Phenol 20 mmol/l; methanol 1.85 mmol/l) was added to an equal volume of solution 2 (Potassium phosphate buffer 4-amino phenazone 2mmol/l, methanol 1.85 mmol/l and hydroxypolyethoxydodecane 0.4). Solution 3 (cholesterol esterase 40 u/ml, cholesterol oxidase 12 u/ml and peroxidase 8 u/ml) was added as one-hundredth of the volume of one of the two solutions to the mixture. The new mixture is solution 4. To 20 ul of the sample was added 2 ml of solution 4. This was vortex mixed and allowed to incubate for 30 minutes at 25°C. The absorbance was read at 500 nm after one hour.

4:1:2 (b) TRIGLYCERIDES

Boehringer Mannheim's kit no. 138 355 for fully enzymatic hydrolysis of triglycerides was used.





Triglycerides in plasma are hydrolysed by lipase/esterase to glycerol and free fatty acids. Glycerol in presence of adenosine triphosphate (ATP) and the enzyme glycerol kinase is converted to glycerol-3-phosphate and adenosine diphosphate (ADP). The latter in presence of phosphoenol pyruvate (PEP) and pyruvate kinase is converted to pyruvate and ATP. Pyruvate reacts with reduced NAD^+ . Decrease in absorbance was proportional to the concentration of plasma triglycerides at 340 nm.

Into each test-tube pipette 2.50 ml of solution 1 (60mmol/L tris/tartaric acid buffer Ph 7.2, magnesium sulphate, 41 mmol/L cholic acid sodium salt, .9 mmol/L, Triton X-100, 2 mmol/L, ATP 0.5 mmol/L, PEP 0.3 mmol/L, NADH 0/2 mmol/L, Lipase 24 u/ml esterase, U/ml, PK 2.5U/ml. LDH 2.5 U/ml) To each test tube 50 ul of the sample material or standard was added. This was then mixed with a spatula and incubated for 10 minutes at 20-25°C, and the absorbance read 365 nm. Suspension 2 (Glycerol kinase kinase 2.5 U/ml) was then added (10 ul) to the reaction mixture and after 10 minutes the absorbance was read again at 365 nm.

4:1:3 (c) HDL-CHOLESTEROL

The method is based on the principle that polyanions and divalent cation are capable of precipitating chylomicrons, very low density lipoproteins and low density lipoproteins. Centrifugation leaves only the high density lipoproteins in the supernatant and their cholesterol content is determined.

enzymatically.

REAGENTS AND PROCEDURE

To 500 ul of the sample and control material, 1 ml of the precipitant Cat. No. 545 004 (Phosphotungstic acid 0.55 mmol/L, magnesium chloride 25 mmol/L) was added. This was vortex mixed and allowed to stand for 10 minutes at room temperature and then centrifuged for 10 minutes at 4000 rpm. From the clear supernatant, 200 ul was pipetted in each test tube. To this was added 2.0 ml of the cholesterol reagent (as in total cholesterol) and vortex mixed. This mixture was incubated for 20 minutes at room temperature and the absorbance of the sample measured at 500 nm against the reagent blank after 30 minutes.

4:1:4 (d) LDL-CHOLESTEROL

This was calculated from the estimated levels of total cholesterol, triglycerides and HDL-cholesterol according to the Friedwald formula (197).

$$\text{LDL-cholesterol} = \text{Total cholesterol} - \frac{(\text{TG} + \text{HDL-cholesterol})}{2.2}$$

4:1:5 (e) VLDL-CHOLESTEROL

VLDL-cholesterol = Total cholesterol - (LDL-cholesterol + HDL-cholesterol).

4:1:6 ULTRACENTRIFUGATION OF LIPOPROTEIN LIPIDS

4:1:6:1 (e) VLDL, LDL AND HDL

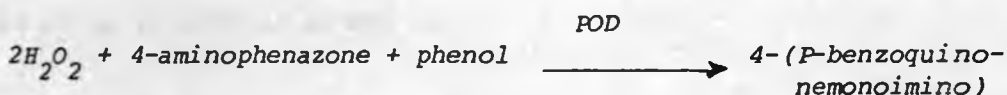
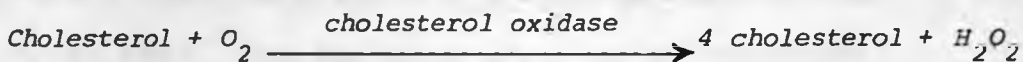
Into a ultracentrifugation tube 5 ml of 2M sodium chloride was added.

Then 4 ml of serum was layered upon the 2M sodium chloride after which 3 ml of 1M NaCl was added. The corks were crewed well and the tubes were transferred into a cold rotor. Ultracentrifugation was performed at 4°C and 40 000 rpm for 22 hours in one step with a modification of a gradient method describes by Walton et al (198). The tubes were later cut at two levels according to the separation of lipoprotein bands. Each fractions were isolated- VLDL d=1.006 g/ml, LDL d=1.063 g/ml and HDL d=1.063 - 1.2. g/ml. The samples were then frozen for further analysis of total cholesterol, triglycerides as described above. Further analysis was done to estimate free cholesterol and phospholipids.

4:1:6:2

(f) FREE CHOLESTEROL

Fully enzymatic cholesterol method based on the Boehringer Mannheim Kit No. 310 328 was used.



The principle is similar to that for the estimation of total cholesterol, The difference is that the cholesterol esterase step is omitted. The pink chromogen absorbs maximally at 500 nm.

4:1:6:3 (g) PHOSPHOLIPIDS

The Boehringer Mannheim colorimetric method kit no. 124 974 which is based on the molybdate/vanadate method of Zilversmit et al (199) was used.

Phospholipids are precipitated with trichloroacetic acid and oxidized to phosphate with perchloric acid and hydrogen peroxide. The released phosphate form a coloured complex with molybdate and vanadate in the presence of nitric acid.

To 100 ul of the sample 2.0 ml of trichloroacetic acid (1.2mol/l) was added. This was mixed gently and then allowed to stand for 10 minutes at room temperature. It was then centrifuged at 3000 rpm for 10 minutes at 4°C. The supernatant was decanted and the precipitated retained. To the latter, 500 ul of perchloric acid (70%) and 300 ul of hydrogen peroxide (30%) was added. This was mixed and the tubes placed into a sand bath and heated to 190°C. They were allowed to remain at this temperature for 15 minutes. When the contents were clear, they were allowed to cool to room temperature.

To each tube was added 2.0 ml redistilled water and 1.0 ml solution 1 (ammonium molybdate 40 mmol/L and sulphuric acid 2.5N) was added. This was vortex mixed and the absorbance read after 10 minutes at 405 nm.

4:1:6:4 (b) HDL₂ AND HDL₃

Into a cellulose acetate tube 4 ml of serum was mixed with 8 ml potassium bromide (KBr) of approximate density 1.64. The metal cap was screwed and the tube transferred into a 50 Ti rotor. This was centrifuged at 4°C and 40,000 xg for 22 hours. Each tube was cut in the middle the upper layer being the HDL₂ and the lower layer the HDL₃ fraction. All the above lipids except triglycerides were estimated in each fraction.

4:1:7:0

INTERNAL QUALITY ASSURANCE

In each assay batch, two quality assurance material was included and treated as the samples. These materials were purchased from Boehringer Mannheim and were designated as Precilip Lot. No 2-374 and Precilip E. L., Lot No. 4-305. The Lyophilized material was reconstituted with distilled water and divided into 1 ml aliquots into microtest tubes.

These were labelled, capped and frozen to -20°C . Before the assay, one microtest tube of each lot was thawed and included into each batch. The result of each analyte was plotted in a different day graph every time as assay was performed (Fig.13a, b, c, and 14a, b, c).

4:1:7:1

EXTERNAL QUALITY ASSUARANCE

The laboratory (lab 5) is involved in an external quality control programme - the W. H. O. lipid EQA Scheme. From the Queen Elizabeth Research Laboratories in Birmingham, U. K. Six vials of lyophilized material were sent to the participating laboratories and analysed for total cholesterol, total glycerol and phospholipids. The results were sent back as soon as possible and the means and the coefficient of variation for each laboratory was calculated from the running mean of all participants appendix I)

5:0

RESULTS

Table III shows that 156 subjects were recruited on a continuous basis for 16 months to be able to get a good number for the study..

Out of these 77 were given levonorgestrel + EE and 79, dl-norgestrel + EE. By the end of the study only 125 subjects were left in the study with 31 subjects having dropped out. This constitutes a drop out rate of approximately 20%. After the 9th cycle of use of the former preparation there was a drop out rate of 10.2% compared to 11.4% of the latter (dl-norgestrel + EE) preparation. This rate increased to 17% and 23% respectively after one year of use. The total number of drop outs for both preparations was 12 after the sixth cycle compared to 31 by the twelveth cycle.

TABLE III NUMBER OF PATIENTS WHO COMPLETED VARIOUS MONTHS OF STUDY

Duration of O.C Use in months	0	3	6	9	12
Subjects on dl- norgestrel + EE	79	78 (1.3)	71 (10)	70 (11.4)	61 (23)
Subjects on levonorgestrel + EE	77	75 (3)	73 (5.2)	69 (10.2)	64 (17)
Total No. of Subjects by Month	156	153 (2)	144 (7.7)	139 (11)	125 (20)

EE - Ethinyl Oestradiol

Figures in bracket are per cent drop our rate

Table IV shows the various reasons of discontinuation from the study. Change from O.C. use to intrauterine devices was most prevalent. Alone it was responsible for almost 50% of method change. The major reason was poor compliance. Increased blood pressure was next, contributing to 25.8% of drop outs.

Five other subjects dropped out from the study and never returned to the clinic and gave no reason at all. However, one came back later seeking a safer method of contraceptive because friends explained to her the dangers of using an oral contraceptive.

Two subjects also from the levonorgestrel + EE group became pregnant and dropped out. One returned to the clinic on the third cycle complaining of amenorrhea since starting on O.C. A pregnancy test was done and a positive result obtained. The other subject became pregnant on the ninth cycle. The reason was attributed to poor compliance for the pill in both subjects.

TABLE IV. REASONS FOR DROPPING OUT OF THE STUDY

REASON	d1-NORGESTREL	LEVONORGESTREL + EE	no. OF SUBJECTS
High Blood Pressure	5	3	8
Pregnancy	-	2	2
Change to other methods	9	5	14
Nausea and vomiting			
No obvious reason	1	4	5
Total number of drops outs	17	14	31
% Drop out Rate	11	9	20

Table V shows subject information, mean parity, education and age. The two groups did not show any significant differences for the three parameters. The two groups were therefore closely related.

Table V SUBJECTS GENERAL CHARACTERISTICS

PARAMETERS	d1-NORGESTREL + EE (n=61)	LEVONORGESTREL + EE (n=64)
AGE (MEAN + SD) Years	24.4+8.6	25.9+7.4
EDUCATION (NUMBER OF YEARS IN SCHOOL)	8.1+4.1	8.6+3.5

Table VI shows the effect of levonorgestrel + EE on height, body weight and blood pressure. This preparation elicited a significantly elevated ($P < 0.05$) body weight after the 9th cycle. Systolic blood pressure was significantly higher ($P < 0.01$) after six months of use and remained so to the 12 cycle. Diastolic pressure was significantly elevated ($P < 0.01$) after the 9th month of O.C. use and remained so to the end of the study but the values were still within the normal range.

TABLE VI. Effect of Levonorgestrel (150 Uq) + EE (30 Uq) on Height Body Weight and Blood Pressure

	PRE TREATMENT	MONTHS OF 'MICROGYNON' USE			
		3	6	9	12
n	77	75	73	69	64
Height (cm)	155.1+3.3	154.6+4.8	155.7+2.9	155.3+3.7	155.3+4.1
Body Weight(Kg)	56.1+6.2	56.7+6.9	57.9+7.4	58.8+6.1*	58.4+4.4
Systolic Blood Pressure (mmHg)	122+9 (124.9)	124+7	126+8**	127+11**	127+7
Diastolic (mmHg)	72+5	71+4	73+8	75+6**	76+9**
Blood Pressure (mmHg)	(78)				

Values in Brackets are normal Caucasian Levels from Stern et al (202)

* $P < 0.05$

** $P < 0.01$

Table VII shows the effect of dl-norgestrel + EE on height, body weight and blood pressure. Body weight was significantly increased ($P/0.01$) on the 9th cycle. Diastolic pressure was significantly higher ($P/0.01$) after 3 months of the use of the preparation to the 12th month but still within the normal range. Systolic pressure was significantly elevated ($P/0.05$) by the sixth month to the end of the study.

It is therefore apparent that both preparations definitely affect both body weight and blood pressure. However, dl-norgestrel + EE seems to cause significant elevations ($P/0.01$) in body weight and diastolic blood pressure earlier than levonorgestrel + EE though the latter shows a similar trend for systolic blood pressure.

TABLE VII. EFFECT OF dl-NORGESTREL (500 Ug) + EE (50 ug) on height, Bod and Blood Pressure

	MONTHS OF 'EUGYNON' USE				
	PRETREATMENT	3	6	9	12
n	79	78	71	70	61
Height (cm)	154.6+4.7	154.4+3.7	154+4.8	154.7+2.7	154.5+3
Body Weight (Kg)	55.6+5.8	56.3+6.3	56.9+5.8	58.7+8.8	59.8+7.
Systolic Blood Pressure (mmHg)	122+7	120+8	124+4	129+ 11	128+6
Diastolic Blood pressure (mmHg)	71+4 (78)	73+3	75+3	76+2	79+6

Values in brackets are normal Caucasian levels from stern et al (202)

* $P/0.05$

** $P/0.01$

5:2 Fasting Lipids and Lipoproteins

Table VIII shows the mean fasting lipids and Lipoprotein cholesterol in a population of 125 normal black Kenya women. The mean level of total cholesterol was 3.89 ± 0.73 (ISD) mmol/l, triglycerides 0.68 ± 0.03 mmol/l, HDL-cholesterol 1.15 ± 0.22 mmol/l, LDL-cholesterol 2.41 ± 0.75 mmol/l and VLDL-cholesterol 0.33 ± 0.13 mmol/l. Comparison is made with results from other studies of Osman et al in Egypt '(93), Rossner et al in Sweden (50) and Friedwald et al (197) in the U.S.

TABLE VIII. FASTING LIPID AND LIPOPROTEIN CHOLESTEROL (MEAN + SD)

STUDY	TOTAL CHOLESTEROL mmol/L	TRIGLY- CERIDES mmol/L	HDL- cholesterol mmol/L	LDL CHOLESTEROL mmol/L	VLDL CHOLESTEROL mmol/L
THIS STUDY RANGE	3.89 ± 0.73 (2.80-5.60)	0.68 ± 0.30 (0.15-1.38)	1.15 ± 0.22 (0.70-1.62)	2.45 ± 0.75 (1.60-4.40)	0.33 ± 0.13 (0.07-0.58)
OSMAN et al (93)	4.52	0.61	1.12	2.20	-
ROSSNER et al (150)	4.7 ± 0.21	0.83 ± 0.21	1.68 ± 0.08	2.73 ± 0.15	0.23 ± 0.12
Friedwald et al (197)	4.9 ± 0.86	0.73 ± 0.07	1.38 ± 0.34	3.17 ± 0.73	0.36 ± 0.23

5:3 LIPID COMPOSITION OF FASTING SERUM AND LIPOPROTEINFRACTIONS IN 4 WOMEN

Table IX shows the lipid composition of fasting serum and lipoproteins fractions in 4 women. The four specimens were analysed for their lipid contents as shown in the table below. They were then fractionated by ultracentrifugation and the lipid content of the three fractions analysed. The levels are compared to caucasian levels derived from Samsioe (248)

TABLE IX. LIPID COMPOSITION IN FASTING SERUM AND LIPOPROTEINLIPOPROTEIN FRACTIONS IN 4 WOMEN

PARAMETER	n=4 MEAN * (mmol.L	ISD	MEAN (mmol/L
Total cholesterol	4.40	0.67	4.41
Free cholesterol	1.64	0.38	1.40
Triglycerides	0.61	0.22	0.96
Phospholipids	2.77	0.40	2.85
HDL-Cholesterol	1.20	0.11	1.15
LDL-cholesterol	2.01	0.65	1.90
VLDL-cholesterol	0.29	0.14	0.44
HDL- Free cholesterol	0.54	0.32	0.26
LDL-Free cholesterol	0.66	0.18	0.52
VLDL-Free cholesterol	0.28	0.16	0.14
HDL-Triglycerides	0.13	0.04	0.08
LDL-Triglycerides	0.16	0.06	0.16
VLDL-Triglycerides	0.32	0.14	0.40
HDL-Phospholipids	1.24	0.54	1.13
LDL-Phospholipids	0.57	0.27	0.64
VLDL-Phospholipids	0.18	0.06	0.22

* These values are derived from Samsioe (248)

5,4 CHANGES IN LIPIDS AND LIPOPROTEIN LEVELS WITH O.C. USE(i) Cholesterol

Table X shows changes in total cholesterol with use of the two oral contraceptive preparations with time of use. In the group using levonorgestrel + EE regimen, there was a significant increase ($P/0.01$) in total cholesterol from the third cycle to the 12th month (Figs 11a and 11b). For the group using dl-norgestrel + EE the significant elevation ($P/0.01$) in total cholesterol was also observed from the 3rd month of O.C. use to the end of the study. The mean increase due to two oral contraceptive regimens is however not significant. However, the dl-norgestrel + EE group show persistently higher total cholesterol levels than the levonorgestrel + EE group.

TABLE X CHANGES IN TOTAL CHOLESTEROL AT VARIOUS STAGES OF USE WITH THE TWO PREPARATIONS

DURATION IN MONTHS		0	1	3	6	9	12
TOTAL CHOLESTEROL	n Levonorgestrel	(77)	(77)	(75)	(73)	(69)	(64)
	+ EE	3.83 +	4.23 +	4.33 +	4.36 +	4.49 +	
		0.62	0.89	0.65	0.65	0.63	0.53
MMOL/L	dl-norgesterl +		3.97	4.39	4.31	4.54	4.64
	EE	3.92 +	+	+	+	+	+
		0.81	0.57	0.47	0.60	0.63	0.50
	n	(79)	(79)	(78)	(71)	(70)	(61)

Fig. 11 a The change in plasma total cholesterol with O.C. Use.

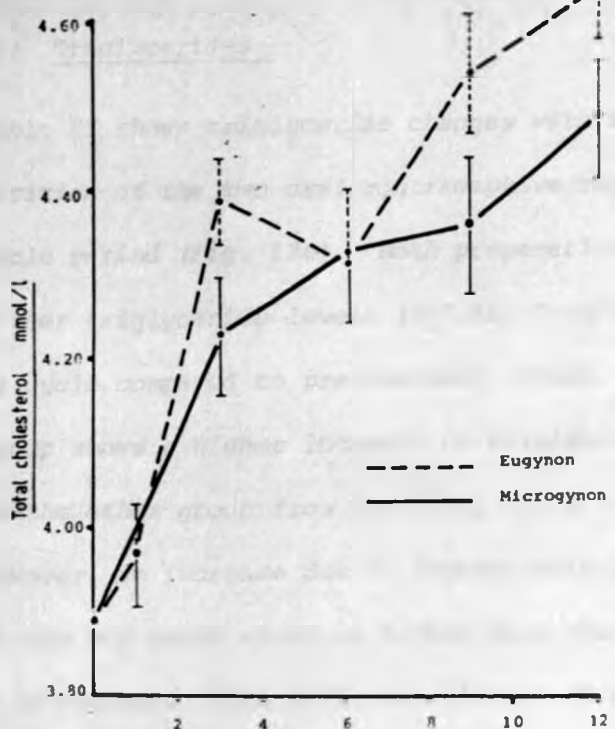


Fig. 11 a changes in plasma total cholesterol with months of levonorgestrel/use plus EE () and dl-norgestrel + EE () use.

Total cholesterol was increased by the two O.C. regimens from pre-treatment levels throughout the 12 cycles of use. The elevation was more pronounced in the dl-norgestrel + EE users than in levonorgestrel + EE users.

Fig. 11 b. Percent change in total cholesterol over the pre-treatment cycle in women using oral contraceptives.

DISTRIBUTION OF LIPIDS/LIPOPROTEINS WITH DURATION OF O.C. USE

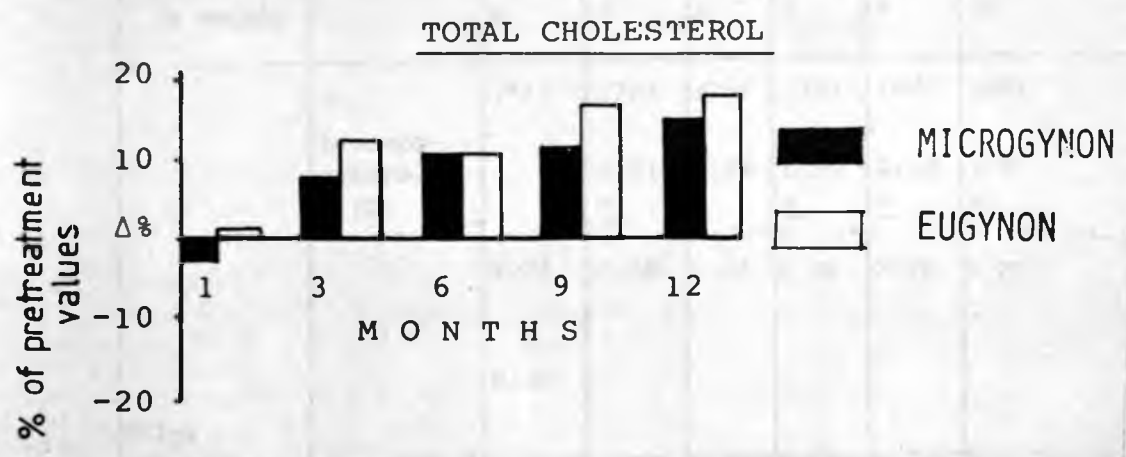


Fig 11 b. The increase in total cholesterol from the pre-treatment

ii) Triglycerides

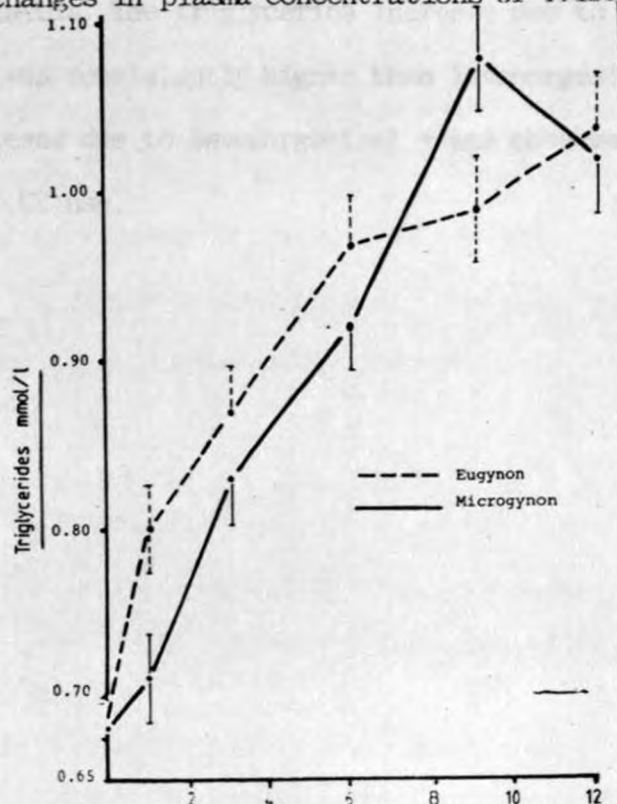
Table XI shows triglyceride changes with time after the administration of the two oral contraceptive regimens over the 12 cycle period (Fig. 12a). Both preparations elicited significantly higher triglyceride levels ($P < 0.01$) from the 3rd cycle to the 12 cycle compared to pretreatment values. The dl-norgestrel + EE group shows a higher increase in triglyceride levels compared to the other group from the first cycle to the 6th month. However, an increase due to levonorgestrel + EE is observed on the 9th month which is higher than that of the dl-norgestrel + EE regimen. This difference is not statistically significant. A slight increase due to dl-norgestrel + EE is apparent through to the 12 month compared to levonorgestrel + EE. Dl-norgestrel + EE increased triglycerides by 53% compared to 50% increase due to levonorgestrel + EE at the end of the study. It is noted that these changes were still within the normal ranges but approaching the upper limit of normal range by the 12th month.

TABLE XI CHANGES IN TRIGLYCERIDES AT VARIOUS STAGES OF USE WITH THE TWO PREPARATIONS

Duration in Months		0	1	3	6	9	12
Trigs	n	(77)	(77)	(75)	(73)	(69)	(69)
	Levonorgestrel + EE		0.71 +	0.83 +	0.92 +	1.08 +	1.02 +
		0.71 +	0.25	0.23	0.26	0.27	0.27
		0.36					
mmol/L	dl-norgestrel + EE		0.80 +	0.87 +	0.97 +	0.98 +	1.04 +
		0.65 +	0.24	0.24	0.22	0.28	0.29
	n	(79)	(79)	(78)	(71)	(70)	(61)

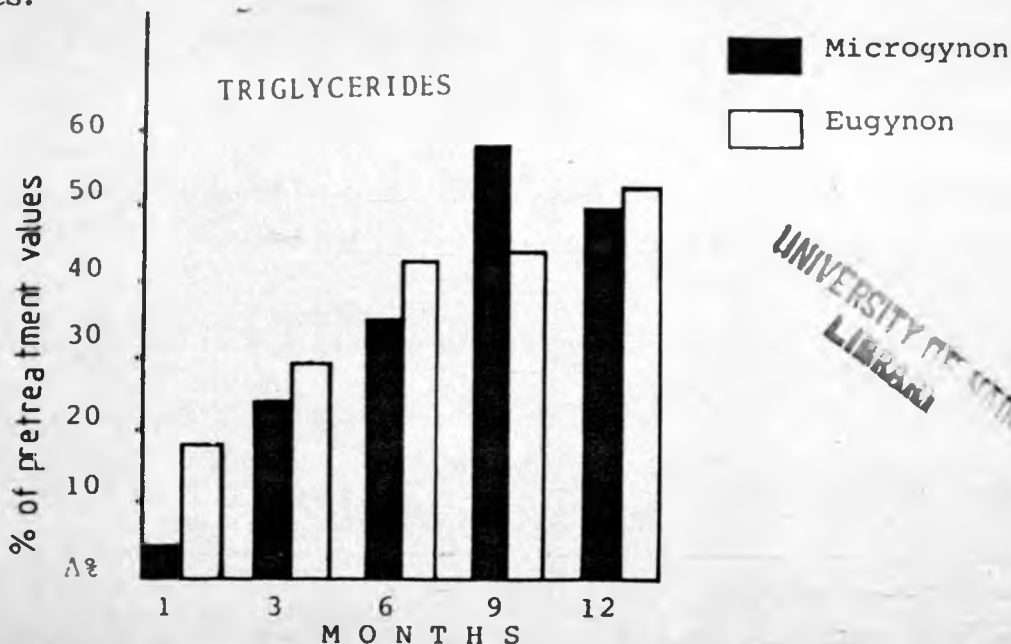
levels in plotted over the period of O.C. use dl-norgestrel + EE shows a higher increase than levonorgestrel + EE.

Fig. 12 a. The changes in plasma concentrations of Triglycerides with O.C. use



Changes in plasma triglycerides with months/use of levonorgestrel + EE (_____) and dl-norgestrel + EE (- - - - -) use. Triglycerides rose from pre-treatment levels and continued to increase through the treatment cycles in both groups of O.C. users. The increase was more marked in the dl-norgestrel + EE group than in the levonorgestrel + EE group.

Fig 12 b. Percent change in triglycerides over the pre-treatment cycles.



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Fig. 12 b. Triglyceride increase was plotted as a percentage of the pre-treatment levels. The triglyceride increase due to dl-norgestrel + EE was consistently higher than levonorgestrel + EE. A spurious increase due to levonorgestrel + was observed at nine months of O.C. use.

Treatment	Time (months)	Triglyceride Increase (%)					
		0	3	6	9	12	15
dl-norgestrel + EE	Pre-treatment	100	100	100	100	100	100
	3	118	123	126	129	130	131
	6	128	132	135	138	140	141
	9	135	140	143	146	148	150
	12	145	150	153	156	158	160
levonorgestrel + EE	Pre-treatment	100	100	100	100	100	100
	3	105	110	115	120	125	130
	6	110	115	120	125	130	135
	9	115	120	125	130	135	140
	12	120	125	130	135	140	145

iii) HDL-cholesterol

Changes in HDL-cholesterol are shown in Fig. 13a and Table

XII. HDL-cholesterol was depressed significantly ($P/0.01$) from the 6th cycle of therapy with the two O.C. regimens. The preparation dl-norgestrel + EE decreased HDL-cholesterol by 17% compared to that of levonorgestrel + EE of 15% at the end of the study. This difference was however, not significant (Fig. 13b).

Table XII CHANGES IN HDL-CHOLESTEROL WITH TIME

Duration in Months		0	1	3	6	9	12
	Levonor- gestrel + EE	(77) 1.18 1.18 <u>+</u> 0.26	(77) 1.15 <u>+</u> 0.20	(75) 1.08 <u>+</u> 0.19	(73) 1.04 <u>+</u> 0.22	(69) 1.00 <u>+</u> 0.20	(64) 0.98 <u>+</u> 0.21
mmol/L	dl norges- trel + EE n	0.17 <u>+</u> 0.24 (79)	1.11 <u>+</u> 0.23 (79)	1.08 <u>+</u> 0.18 (78)	1.03 <u>+</u> 0.17 (71)	1.01 <u>+</u> 0.19 (70)	0.96 <u>+</u> 0.20 (61)

Fig. 13 a. The changes of concentrations of HDL-cholesterol with O.C.

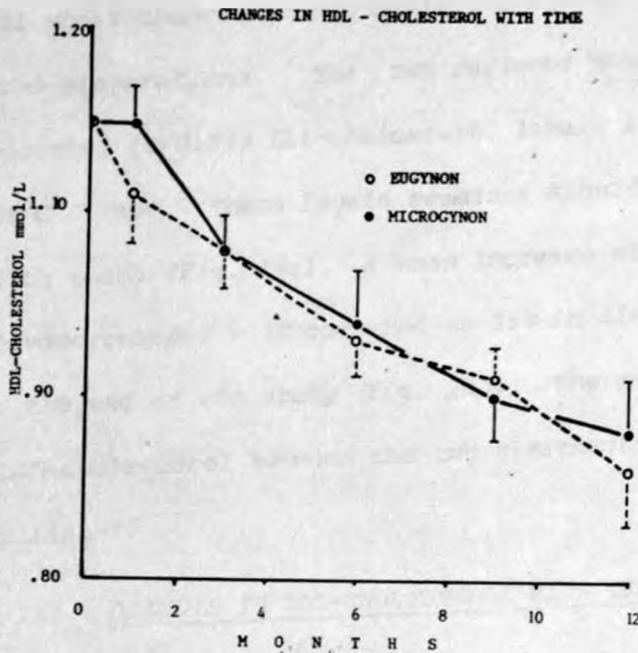
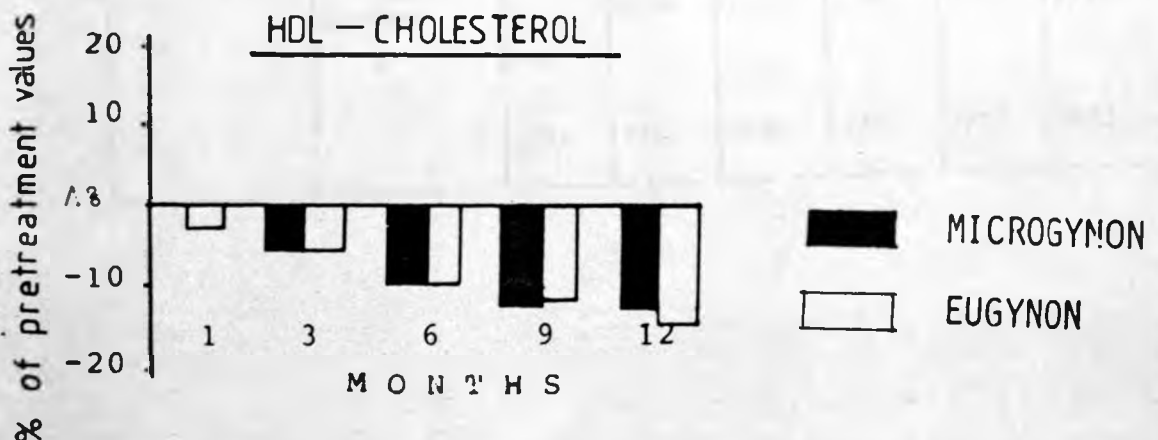


Fig. 13 a. The two O.C. caused decrease in HDL-cholesterol from the pre-treatment levels. This decrease was observed to the 12th cycle and was more pronounced in dl-norgestrel + users than in the levonorgestrel + EE group.

Fig. 13 b. Percent changes in HDL-cholesterol over the pre-treatment cycle.

DISTRIBUTION OF LIPIDS/LIPOPROTEINS WITH DURATION OF O.C. USE



iv) LDL-cholesterol

Table XIII shows changes in LDL-cholesterol with time of use for the two preparations. The two regimens showed a significantly elevated ($P < 0.01$) LDL-cholesterol levels after three months of O.C. use. These levels remained significantly high to the 12th month (Fig. 14a). A mean increase of 31% was observed in the levonorgestrel + EE compared to 35% in dl-norgestrel + EE users at the end of the study (Fig. 14b). The mean difference of the LDL-cholesterol between the two treatments was, however, not significant.

TABLE X III CHANGES IN LDL-CHOLESTEROL WITH TIME

Duration in Months		0	1	3	6	9	12
LDL- Chol	n	(77)	(77)	(75)	(73)	(69)	(64)
	levonor- gestrel	2.39	2.58	2.74	2.86	2.92	3.16
+ EE		0.64	0.63	0.56	0.76	0.60	0.60
dl- norgestrel		2.48	2.48	2.80	2.84	3.08	3.26
	+ EE	0.81	0.56	0.53	0.60	0.55	0.54
		(79)	(79)	(78)	(71)	(70)	(61)

Fig. 13 b. Decrease in LDL-cholesterol was higher in dl-norgestrel ± EE users than levonorgestrel ± EE. This decrease was consistent for the whole period of O.C. use.

Fig. 14 a. The changes in plasma LDL cholesterol

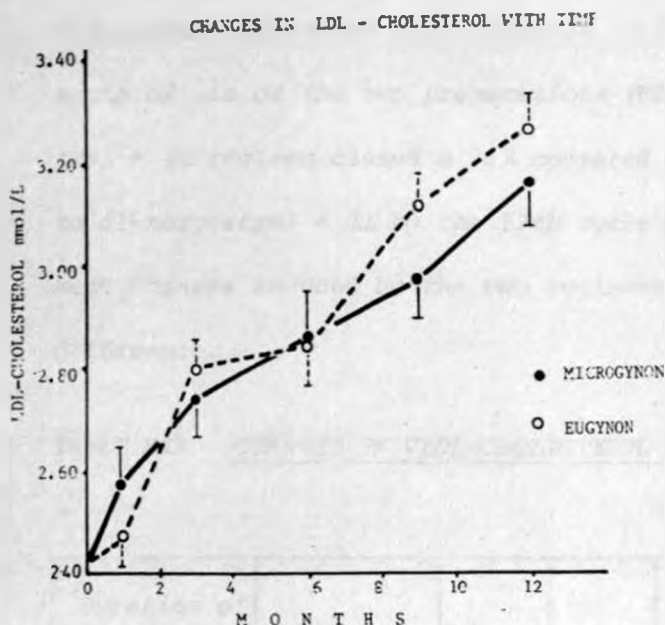


Fig 14 a. LDL-cholesterol as in total cholesterol was increased up to the 12th cycle after the use of the two O.C. regimens. However, the increase due to levonorgestrel + EE was less compared to dl-norgestrel + EE.

Fig 14 b. Percent change in LDL-cholesterol over the pre-treatment cycle.

DISTRIBUTION OF LIPIDS/LIPOPROTEINS WITH DURATION OF O.C. USE

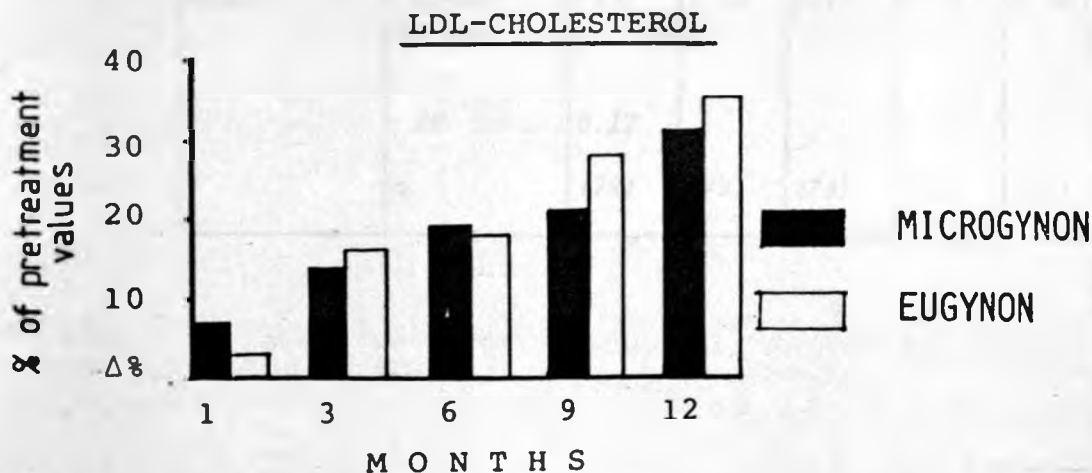


Fig. 14 b. LDL-cholesterol increased from the pre-treatment levels to the 12th cycle of O.C. use. The increase due to dl-norgestrel + EE was higher than in levonorgestrel + EE users.

v) VLDL-cholesterol

Table XIV shows the change in VLDL-cholesterol with the duration of use of the two oral contraceptive preparations. This lipoprotein increased significantly ($P/0.01$) after the 3rd month of use of the two preparations (Fig. 15a). The levonorgestrel + EE regimen caused a 36% compared to 39% increase due to dl-norgestrel + EE by the 12th cycle of use. The between-treatment changes induced by the two regimens showed no significant difference.

TABLE XIV CHANGES IN VLDL-CHOLESTEROL WITH TIME

Duration of Time		0	1	3	6	9	12
VLDL	n	(77)	(77)	(75)	(73)	(69)	(64)
	levonorgestrel + EE	0.30	0.32	0.37	0.42	0.48	0.45
		\pm	\pm	\pm	\pm	\pm	\pm
		0.12	0.11	0.11	0.12	0.14	0.13
Chol mmol/L	n		0.34	0.39	0.41	0.45	0.46
	dl-norgestrel + EE	0.35	0.13	0.11	0.11	0.14	0.14
		\pm	\pm	\pm	\pm	\pm	\pm
		0.17					
	n	(79)	(79)	(78)	(71)	(70)	(61)

Fig 15 a. Change of VLDL-cholesterol with duration of O.C.

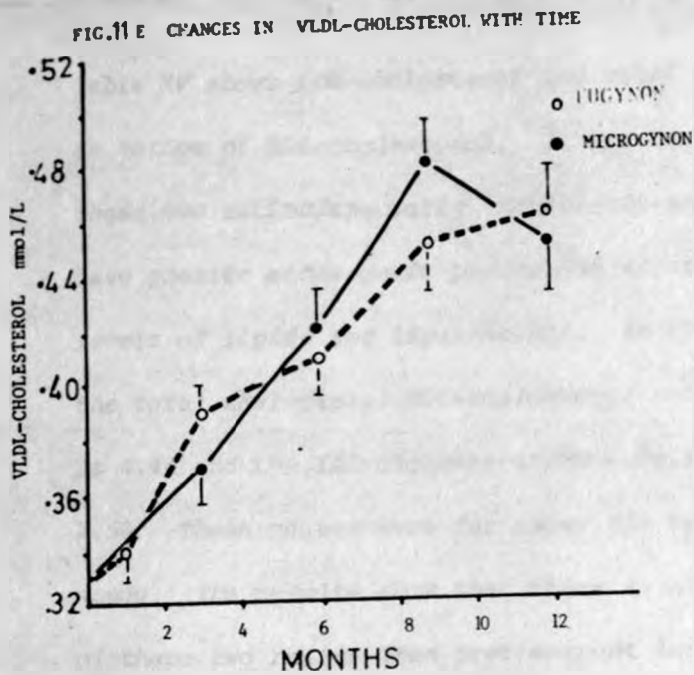


Fig 15 a. The two O.C. preparations increased VLDL-cholesterol from the pre-treatment levels. This increment remained so to the 12th cycle of treatment. DI-norgestrel + EE increased VLDL-cholesterol more than levonorgestrel + EE.

Fig 15 b. The percent change of VLDL-cholesterol over the pre-treatment cycle.

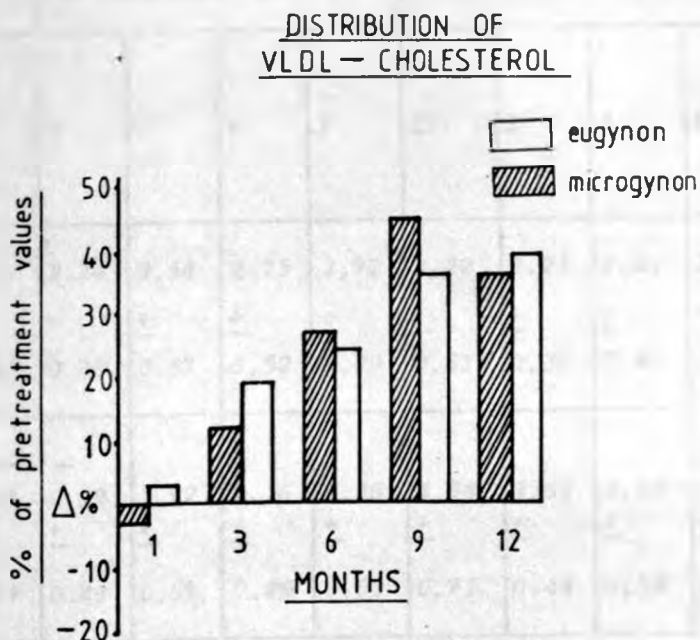


Table XV shows LDL-cholesterol and total cholesterol expressed as ratios of HDL-cholesterol. It has been postulated that these two ratios/specially LDL-cholesterol/HDL-cholesterol /e have greater atherogenic predictive value than either absolute levels of lipids and lipoproteins. In the Framingham study (61) the total cholesterol/HDL-cholesterol ratio was estimated as 4.44 and the LDL-cholesterol/HDL-cholesterol ratio as 2.50. These values were far above the values found in this study. The results show that there is a gradual increase of these two ratios from pretreatment levels to the 12th cycle. The ratios however, tend to be higher with the use of dl-norgestrel + EE compared to levonorgestrel + EE.

Table XVI. RATIOS LDL-CHOLESTEROL/HDL-CHOLESTEROL AND TOTAL CHOLESTEROL/HDL-CHOLESTEROL

DURATION OF USE IN MONTHS	RATIO	Levonorgesterl + EE					dl-norgesterl + EE				
		0	1	3	6	9	12	1	3	6	9
LDL-CHOLESTEROL	2.10	2.24	2.54	2.75	2.92	3.22	2.23	2.54	2.78	2.89	3.40
	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm
HDL-CHOLESTEROL	0.42	0.36	0.57	0.52	0.39	0.51	0.39	0.44	0.61	0.43	0.17
TOTAL CHOLESTEROL	3.38	3.29	3.92	4.16	4.36	4.58	3.58	4.06	4.18	4.50	4.83
	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm
HDL-CHOLESTEROL	0.24	0.48	0.61	0.89	0.69	0.71	0.44	0.59	0.72	0.43	0.61

5:5 QUALITY ASSURANCE DATA

Within batch coefficient of variation was estimated as 2.89%, 3.61% and 4.10%, for total cholesterol, triglycerides and HDL-cholesterol respectively in 20 determinations of precilip E. L. carried out in one assay. The between batch coefficient of variation was determined in two internal quality assurance materials, Precilip E. L. and the low level control precilip. The coefficient of variation for precilip E. L. was 3.50% 4.33% and 4.87% for total cholesterol, triglycerides and HDL-cholesterol. The values for precilip were 3.53%, 4.28% and 5.74% respectively for the three parameters in 47 determinations carried out over the study period.

Levy Jennings' Quality Control Charts

Fig. 16a.

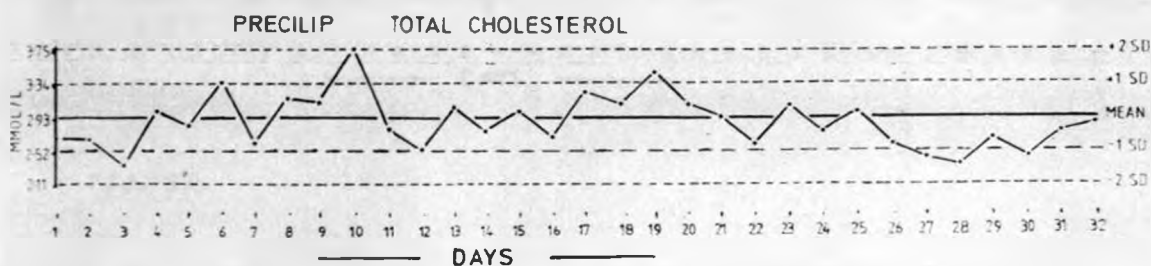


Fig. 16b.

Fig. 13b

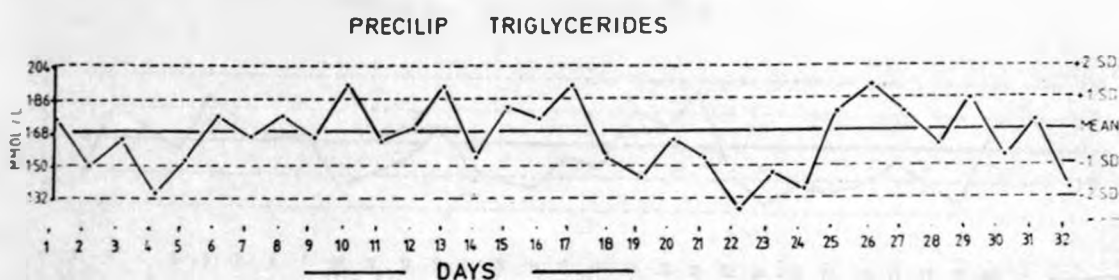


Fig. 16c.

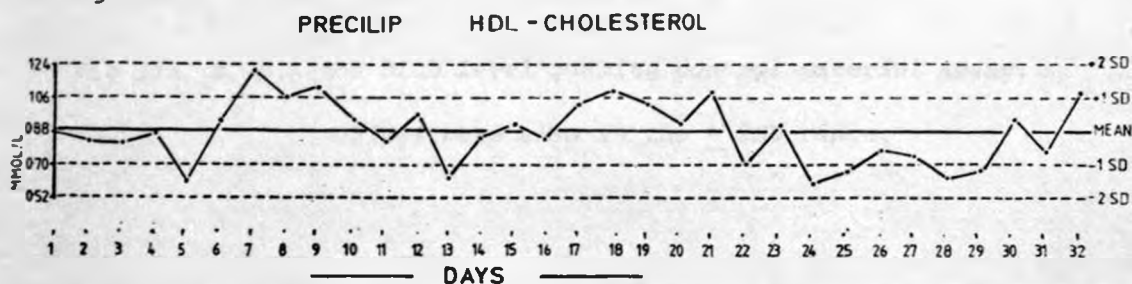


Fig. 16 a, b, c. The low level quality control assay values were within $\pm 2SD$ and therefore within acceptable limits.

Levy Jenning's Internal Quality Control Charts

Fig. 17a

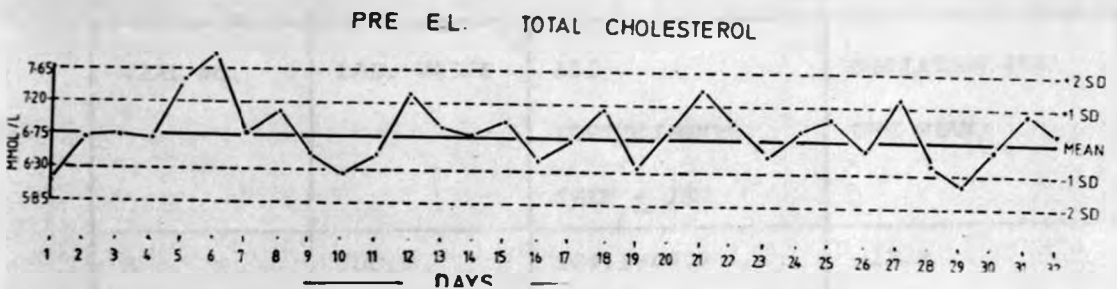


Fig 17b

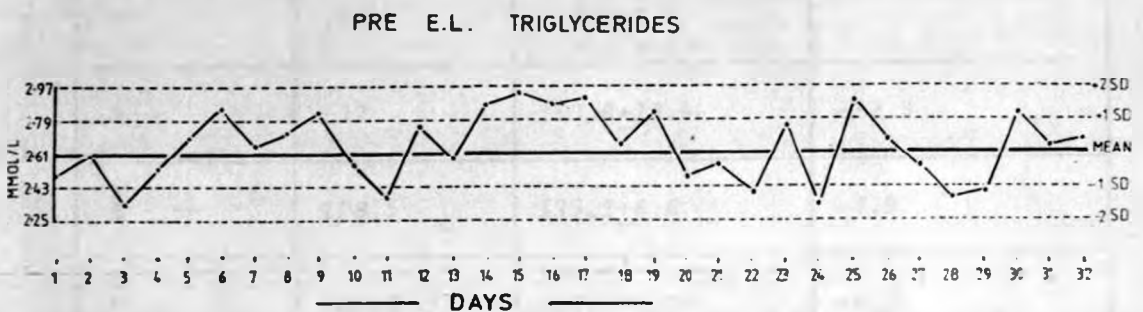


Fig. 17c

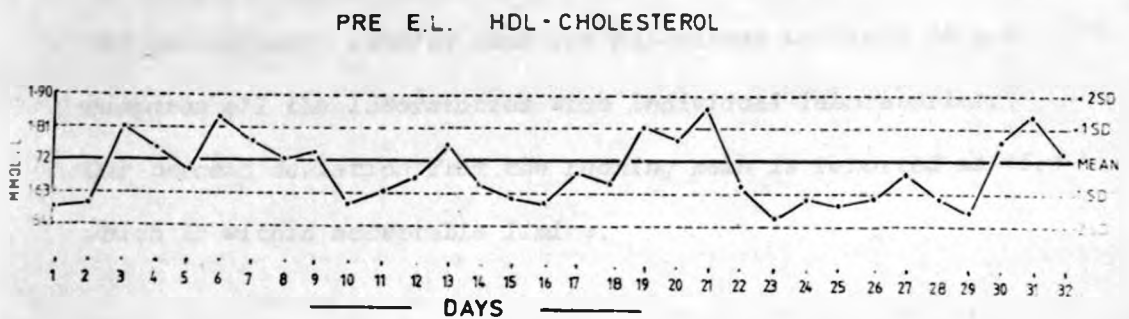


Fig 17a, b, c. The high level quality control material assay values were also in the $\pm 2SD$ range.

TABLE XVI

E Q A DATA (LAB. NO. 5)

i) Total cholesterol in mg/dl

VIAL NO.	LAB. VALUE	ALL PARTICIPANTS MEAN \pm 1SD	DEVIATION FOR THE MEAN
A	300.0	344.1 \pm 49.9	-12.8
B	252.0	289.4 \pm 26	-12.9
C	50	64.1 \pm 7.6	
D	132	150.8 \pm 13.8	-12.5
E	128.5	135.1 \pm 8.6	-4.9
F	122.5	113.3 \pm 5.9	+8.1
G	201.5	195.9 \pm 10.8	+2.9
MEAN			-5.4

The participants running mean was calculated within 1 SD and compares all the laboratories with individual laboratories.

Our percent deviation from the running mean is reported as -5.4 which is within acceptable limits.

ii) HDL-Cholesterol in mg/dl

Vial No.	Lab. Value	All Participants mean \pm 1 SD	% of Deviation from the mean
A	144	195.0 \pm 59.2	
B	85,5	76.6 \pm 52.3	+ 11,6
C	30	32.0 \pm 12.8	- 6,2
D	17,5	24.2 \pm 16.8	- 27,7
E	52,5	54.1 \pm 12.7	- 3,0
F	30	24.3 \pm 12.2	+23,5
G	88,5	66.3 \pm 39.8	+ 33,5
MEAN			+ 5,3

iii) Triglycerides in mg/dl

Vial No.	Lab Value	All Participants Mean \pm 1 SD	% Deviation from the mean
A	79	69.6 \pm 26.3	- 13.5
B	155.5	195.6 \pm 22.0	+ 20.5
C	13	25.6 \pm 8.3	-
D	66	74.0 \pm 6.2	- 10.8
E	119	145.1 \pm 18.2	- 18.0
F	79	88.7 \pm 7.3	- 10.9
G	198	192.8 \pm 11.8	+ 2.7
MEAN			- 7.3

6:0 DISCUSSION

The family Welfare Clinic at Kenyatta National Hospital opened its doors for the very first time in 1966 and 1666 women attended the clinic seeking advice for family planning in that year. Out of this number, 48% sought the contraceptive pill as the method of choice (200). This number has since increased to 40.000 by 1983 nationwide.

This tremendous increase in the number of users has come about due to the awareness for the need to control fertility which has been a result of Government initiative to educate the public on maternal and child health. Unfortunately, this apparent increase in the number of users has been matched by an equally high drop out rate. A study carried out six years after the beginning of the programme showed that the drop out rate was as high as 73.5% for the oral contraceptive pill (200). Sanghvi (201) in 1983 reported a termination rate of 45% during the first six month of use which increased to 60% after one year of pill use. He found no significant difference in the discontinuation rates between dl-norgestrel + EE and levonorgestrel + EE.

This study has found a relatively lower overall discontinuation rate of 20% which can be explained by the fact that incentives in the form of busfare were offered to the subjects as compared to national level. However, there is agreement that the difference in termination rates between the two oral contraceptive regimens were not significant (201). A higher but non-significant drop out rate was found in the dl-norgestrel + EE group compared to the levonorgestrel + EE group.

The present study has shown that the largest number of drop outs sought other methods of contraception. About 50% of these subjects changed to intrauterine devices showing dissatisfaction with the pill. It has been reported in another study (201) that 59.9% of oral contraceptive users discontinued the pill to seek alternative methods of contraception, the largest changing to intrauterine devices.

Raised blood pressure was also found to be responsible for high discontinuation rates. In this study it contributed to 29% of the total number of drop outs. In the case of dl-norgestrel + EE group, weight gain is almost paralleled by the rise in diastolic pressure which shows a significant increase from the third month of pill use. Systolic blood pressure shows a significant increase in the levonorgestrel + EE group only. These results corroborate the study of Stein et al (202) who found that both systolic and diastolic blood pressures were higher for Mexican-American women who took combination agents. Ramcharan et al (203) in a prospective study involving 11672 women found that women on oral contraceptives were six-fold more prone to develop hypertension than non-users.

An even larger study involving 46000 women by the Royal College of General Practitioners (37) reported a 2 < 2 1/2 times more likelihood to develop hypertension in O.C. users than in non users.

There was a definite and significant weight gain ($P < 0.01$) in the dl-norgestrel + EE users from pretreatment levels after 9 months of use. A significant ($P < 0.05$) weight gain was observed after 9 months of levonorgestrel + EE use but did not

persist to the 12th month of use. This is in agreement with an earlier study (204). In the levonorgestrel + EE users significant increase ($P/0.01$) manifests on the 9th month of pill use.

In this study the higher progestagen content oral contraceptive dl-norgestrel + EE is associated with earlier change in blood pressure especially diastolic pressure. Synthetic progestagens used in oral contraceptives have been associated with salt retention (205). Thus from the study of Royal College of General Practitioners the incidence of hypertension on these agents appeared to correlate with the amount of progestagen in the preparation and not with the amount of oestrogen (37). Another observation in this study is the pronounced increase in systolic blood pressure in association with levonorgestrel + EE than with dl-norgestrel + EE. A similar observation was reported from an earlier study (201). It is not clear why this is so but the metabolism of any O.C. regimen with its oestrogen/progestagen ratio and the pharmaco-kinetics of each individual steroid may differ in any different population (203).

The pretreatment systolic pressure in both groups of users was 122 mmHg. A study by Williams (206) reported an average systolic blood pressure level of 121.3 mmHg among the Kikuyu women. Sharper et al (207) reported a mean systolic pressure of 123 mm Hg among Baganda females. These authors concluded that environment factors are the major determinants of blood pressure levels and prevalence of hypertension in a community. Data from a study by Parry (208) supported this

contention when he found a mean systolic blood pressure of 112.4 mmHg. among Ethiopian female Highlanders in the same age group as the Kikuyu and Baganda females.

Diastolic blood pressure in this study was recorded as 72 mmHg. An earlier study on the Ganda and American Negroes gave their mean diastolic pressures as 71 mmHg and 72 mmHg respectively (207). Since the risk to CVD is thought to be a continuous function of blood pressure even within normal limits range, the increase in blood pressure due to oral contraceptive should be regarded as a health concern.

This study recorded an average increase of 5 mmHg and 6 mmHg in systolic pressure after 12 months of levonorgestrel + EE and dl-norgestrel + EE use respectively. In a study by Fisch et al (209) systolic blood pressure averaged 5 mmHg higher and diastolic pressure 1.3 mmHg in O.C. users, compared to non-users. In another study, average systolic blood pressure was 7.2 mmHg higher in white contraceptive users, compared with non-users (203). Among Mexican - American women, average systolic blood pressure was 19.9 mmHg higher and average diastolic blood pressure 9.4 mmHg higher in users compared to non-users. Our data gives an increase of 8 mmHg and 4 mmHg in diastolic blood pressure after the 12th cycle of dl-norgestrel + EE and levonorgestrel + EE respectively from pretreatment levels. If a progestin is the culprit as reported, then the increase due to dl-norgestrel compared to levonorgestrel parallels the almost double dose of the progestin in the former preparation.

The relatively lower levels of lipids in this study especially total cholesterol and triglycerides compared to levels from other populations (1, 44, 46, 49, 64) probably reflect differences in dietary patterns.

Several studies by Sharper and Jones have now confirmed that the African has a low serum cholesterol (211). They reported a mean fasting total cholesterol of 4.2 ± 0.7 mmol/L in a study involving 101 participants drawn from East African countries. Onitiri, Sander and Boyo found a mean total cholesterol of 3.93 mmol/l in 53 female nurses (mean age 21 years) in Nigeria (212). Blood cholesterol level is related to the amount of dietary saturated animal fat. Another study carried out in Nigeria reported a fasting mean total cholesterol of 4.1 mmol/l. However, the author pointed out that only 50% of his subjects were fasting (92). An earlier study carried out in Egypt reported a fasting mean total cholesterol of 4.52 mmol/l in 20 women controls (93). These levels are lower compared to caucasian levels where the fat intake is higher (50, 98).

The majority of the subjects who attended the clinic belong to the lower middle class with an average education of 8 years. Their diet comprise basically of white polished maize flour eaten with vegetables, potatoes, legumes and wheat flour with occasional beef or fish. There is evidence that fibres of cereals and legumes have a cholesterol lowering effect (213). This is consistent with the low levels of cholesterol found in this study both in plasma total cholesterol and lipoprotein cholesterol.

Another study has shown that plasma cholesterol levels were lowered by a mixed diet containing fruits, vegetables and legumes (214) and beans (215) in the diet. In a study by Durrington et al (216) 12 males fed on pectin showed a decrease in total serum cholesterol and apolipoprotein B though serum triglyceride levels did not change. This local diet is also likely to contain relatively higher levels of polyunsaturated fatty acids which have been reported to depress plasma cholesterol concentrations and the concentration of LDL. Omega - 3 fatty acids (levels especially high in leafy vegetables) appear to have a major second point of action in lowering plasma triglycerides and in particular VLDL (217). The mechanism by which polyunsaturated fatty acids in diet lower plasma lipid levels is not clear. It has however, been found that large amounts of linoleic acid, an ω - 6 fatty acid,

in diet alters the fatty acid composition of plasma lipoproteins (218). This change may lessen their capacity to carry lipids, in particular cholesterol, with concomitant lower plasma cholesterol. These factors may therefore lead to lower levels of cholesterol in our normal population. These together with studies carried in societies whose cholesterol intake is lower than in Western societies have suggested a significant relationship between diet, lipid levels and CHD (219, 220). Plasma cholesterol levels were significantly lower than those from Western countries (221).

This study reveals that, both oral contraceptive preparations significantly elevated plasma total cholesterol levels in the African women from pre-treatment. The regimen levonorgestrel + EE increased plasma total cholesterol by 19% at the end of the study period of one year. This agrees with the work of

other investigators who reported plasma total cholesterol elevation by combined contraceptives, was of the order of between 10 - 20% of the pre-treatment levels (31). In another study total cholesterol levels were depressed after one month of O.C. use in subjects taking 20 EE/250 or 30 EE/150 regimens (25). This observation was also apparent in this study. Total cholesterol was depressed after one month of use but increased after 3 months to the end of the study. This is probably a progestagen phenomenon which neutralizes the cholesterol elevating action of oestrogen to actually cause a depression. The relatively higher oestrogenicity of the preparation is apparent though the oestrogen/progestin ratio is maintained as in the levonorgestrel + EE. Miller et al (222) maintains that it is unlikely that there are any biological differences when compared to a 50ug preparation.

Fasting plasma triglyceride levels in this study compare with other levels reported in African (212) and caucasian (198) studies. Blood triglyceride concentration is related to the amount of carbohydrates in the diet (114). A study on the Alaskan Arctic Eskimos showed that their native diet had unusually low serum triglycerides and VLDL (223). Those on a higher carbohydrate diet exhibited significant elevations of serum triglycerides. As stated earlier in the text the diet is supplemented by large quantities of leafy vegetables which contain w-3 fatty acids. The fatty acids are known to lower values reported in Africans (98) compared to Caucasian levels (198).

Significantly elevated concentrations of plasma triglycerides were observed after use of both preparations. There was a slightly higher increase due to dl-norgestrel compared to levonorgestrel + EE from the 3rd month of oral contraceptive therapy. This observation has been reported in earlier studies which showed elevated plasma triglyceride concentration during treatment with oral contraceptives (11, 14, 47, 48, 49, 55).

A triglyceride lowering effect has been widely reported in progestins of the 19-nortestosterone as a class (25, 31). Ethinyl oestradiol on the other hand is associated with an increase in serum triglycerides (202) and VLDL - triglycerides (6, 22, 31). In a crossover design study on 15 healthy volunteers on levonorgestrel + EE significantly elevated total serum triglycerides were reported. The two contraceptives used in this study differ and it is therefore difficult to interpret the results. The gradual triglyceride elevation may be an oestrogen effect (16, 31) which is dose dependent. The oestrogen dose in dl-norgestrel + EE is higher than in levonorgestrel + EE and may contribute to the increased triglyceride levels in the former preparation. A study by Stokes et al involving 676 women found elevated mean fasting serum triglyceride levels on all women on O.C. preparations (224). Most oestrogenic pills gave the highest triglyceride levels. In another study triglyceride concentration in whole plasma, VLDL and LDL were elevated in response to increasing oestrogen potency as were triglyceride concentrations in the LDL (14). Rossner et al estimated fasting level of triglycerides on 12 healthy young women before and after use of an oral contraceptive. He found that fasting level of triglycerides increased by about 200% after O.C. use (31).

The mechanisms leading to hypertriglyceridaemia are not clear. Although both overproduction and defective removal of VLDL-triglycerides may play a role in causation of hypertriglyceridaemia, controversy rages as to which mechanism is most commonly responsible.

A defective removal mechanism is however gaining popularity. Havel et al (225) and Boberg et al (226) came to the conclusion that patients with elevated plasma triglycerides did not have enhanced influx of VLDL-triglycerides and advanced the view that hypertriglyceridaemia rarely originates from overproduction of VLDL-triglycerides but more often from defective degradation.

Oral contraceptives in this study elevated plasma triglycerides. It is well established that these agents decrease lipoprotein lipase an enzyme which plays an important role in metabolic clearance of plasma triglycerides (137). There is also evidence indicating that oral contraceptives increase triglyceride turnover (227). It seems that this is due to the oestrogen component of these agents, since when progestins are given alone, the opposite effects are observed on postheparin lipoprotein lipase activity (228) and triglyceride turnover. Indeed, progesterone partially reduces the oestrogen effect on triglyceride entry into the plasma component (227) and this reduces the hypertriglyceridaemic action of oestrogens.

Oral contraceptives have also been reported to have a diabetogenic effect (49). As a result of the altered carbohydrate metabolism, the plasma free fatty acids levels are elevated. The elevation could lead to increased hepatic VLDL formation and release of triglycerides with consequent hypertriglyceridaemia rather than an impaired removal mechanism (229).

The levels of normal HDL-cholesterol in this study compare well with those reported in other African studies (93, 230) but slightly lower than levels reported in more affluent societies (31, 64, 197). The lower levels of HDL-cholesterol in our women are consistent with the low total plasma cholesterol in the same population. The lower calorie intake exemplified by the quantity of food consumed per day could result in the relatively low levels of HDL-cholesterol. Taskinen and Nikkila using a restriction of calories to 400 per day for up to seven days in healthy females, reported lowered levels of HDL-cholesterol with concomitant reduction of LPL activities in adipose tissue and skeletal muscle (231). Vegetarians on the other hand have been shown to have low levels of serum HDL-cholesterol (232). Concurring with our results is the finding that diets rich in carbohydrates also lower plasma HDL-cholesterol. Similar studies on Tarahumara Indians, who feed on low fat, high carbohydrate and high fibre has shown remarkably low levels of HDL-cholesterol by Western standards (223).

Both oral contraceptive preparations used induced a significant decrease in plasma HDL-cholesterol. It is however, evident from the data that there is a slightly higher increase due to dl-norgestrel + EE use than levonorgestrel + EE. The two preparations contain different amounts of oestrogen and progestagen. Androgens have been reported to decrease HDL-cholesterol while oestrogens increase this parameter, (31). It is difficult to interpret these results well.

However, it is possible that though both levels of progestagen reduce the HDL-cholesterol levels, the higher dose in di-norgestrel + EE further reduces the effect of 50 ug oestrogen in the regimen. This finding confirms earlier studies on the gonane norgestrel (21-26).

It has also been reported that levonorgesterl + Ee induces a decrease in HDL-cholesterol which is probably reflective of the intrinsic androgenicity of levonorgestrel (56, 25). Bergink et al reported a reduction in HDL-cholesterol in 27 young and healthy women on levonorgestrel (150 ug) and ethinyl oestradiol (30 ug) after 3, 6 and 12 months of use (234). This observation is consistent with our findings.

The mechanism by which oral contraceptives decrease HDL-cholesterol is not known. What is clear, is that oestrogens increase HDL -cholesterol and on the other hand 19-nortestosterone compounds exhibit androgenic properties (23). Levonorgestrel has strong progestational and therefore antioestrogenic activity and thus lowers HDL-cholesterol (235). The decrease in HDL-cholesterol in this study could therefore be attributed to progestagen effect which acts to reverse the beneficial effect of the oestrogen.

Graft and Peters have reported significant decreases in plasma concentrations of the amino acids proline, glycine, alanine, leucine and tyrosine in women taking oral contraceptives (236). This decrease in amino acids possibly result in a reduced HDL-protein synthesis with consequential decrease in HDL mass.

Indeed, Crona et al reported a decrease in Apo-A1 in ten oophorectomized women after administration of levonorgestrel + EE (237). The progestagen may therefore interfere with the individual amino acid incorporation in the HDL-protein or the actual hepatotoxicity of the C-17 alkylated compounds, resulting in reduced biosynthesis of the proteins and a decrease in the HDL mass.

The fasting levels of LDL-cholesterol observed in this study are lower than those reported in Caucasian populations which may be reflected in the lower total cholesterol levels reported in our population. Wilson et al (238) and Burslem et al (239) have shown that high carbohydrate and vegetarian diets lower both LDL and HDL-cholesterol levels. Another study by Spritz et al (240) showed that major diet cholesterol induced changes in total cholesterol were due to LDL-cholesterol change. Thus it is possible that the low LDL-cholesterol levels in our women may be attributed to diet.

The levels of LDL-cholesterol tend to increase from pre-treatment levels after the use of the two oral contraceptive regimens. The levels are significantly higher after three months of use of the two preparations. The two steroids have antagonistic properties as far as LDL-cholesterol is concerned. It is clear though, that the intrinsic androgenic effect of levonorgestrel overrides the oestrogenic effect and raises this lipoprotein fraction. The higher the dose of levonorgestrel in the dl-norgestrel + EE fraction contributes to a slight but non-significant increase.

This effect may be due to the progestin or combined oestrogen/progestin activity.

VLDL-cholesterol was also significantly elevated in this study by the two oral contraceptive regimens. This has been the most consistently elevated lipoprotein as reported by other similar studies (49, 50). Oestrogens have been known to stimulate VLDL lipid synthesis and secretion (9) and in a study involving use of sequential oral contraceptive agents VLDL-cholesterol was reported elevated (24). The effects were related largely to the oestrogenic dominance of these agents. In another study by Wallace et al (11) most of the lipoprotein fractions including VLDL-cholesterol were elevated in oral contraceptive users compared to controls. The increase of these parameters was positively associated with the oestrogen dose content of the oral contraceptive preparation.

Previous studies have shown that levonorgestrel will cause hyperinsulinemia in doses of 150 ug or more (241). Insulin is thought to interfere with VLDL metabolism by stimulating triglyceride synthesis (242). Endogenous hypertriglyceridaemia is often associated with hyperinsulinaemia by stimulating hepatic formation of fatty acids from carbohydrate sources. According to Kissebah et al (243) increased VLDL production in endogenous hypertriglyceridaemia is a manifestation of enhanced lipolysis secondary to insulin resistance in adipose tissue.

Both total cholesterol/HDL-cholesterol and LDL-cholesterol/HDL-cholesterol ratios tend to be elevated with the use of both oral contraceptive regimens. It is evident from the results that the elevation is more pronounced in the higher oestrogen and progestagen regimen than in the lower. The predictive value of these indices in the development of cardiovascular disease have recently been emphasized by Baggett et al (244). In case control studies from five populations, Castelli et al (83) reported that HDL-cholesterol has an inverse relationship with CHD prevalence. This relationship is independent of total and LDL cholesterol. Later, Miller et al (80) speculated that a decrease in HDL and a concomitant elevation of LDL may accelerate development of atherosclerosis. Studies by other investigators found that a low LDL-cholesterol/HDL-cholesterol/HDL-cholesterol had a greater predictive value than HDL-cholesterol alone (245). A study by Glueck et al (246) on 58 subjects with familial hyperalpha lipoproteinemia, 20 subjects with familial hypobeta lipoproteinemia and 153 controls the LDL-cholesterol/HDL-cholesterol ratio was 2.8 and 1.95 times higher ($P < .001$) in controls than in familial hypobeta and familial hyperalpha subjects. Morbidity and death from IHD was examined in eight kindreds with familial hypobeta lipoproteinemia and compared with the data in 18 kindreds with familial hyperalpha lipoproteinemia. It was demonstrated that kindreds with familial hypobeta and familial hyperalpha lipoproteinemia have a diminished incidence of MI. A relatively low ratio of LDL-cholesterol/HDL-cholesterol may thus be associated with longevity.

In our population we found a total cholesterol/HDL-cholesterol ratio of 3.38 and an LDL-cholesterol/HDL-cholesterol ratio of 2.10. In the Framingham study (61) the average total cholesterol/HDL-cholesterol in women was reported as 4.44, while the LDL-cholesterol/HDL-cholesterol ratio was reported as 2.50 in another study. The use of oral contraceptives in this study shows a progressive elevation of both ratios with the month of use. Thus it is shown that O.C.'s increased LDL-cholesterol and reduce HDL-cholesterol thus elevating the two ratios. The trend is therefore probably toward a cardiovascular disease risk over a long period of use. The preparation dl-norgestrel + EE shows a higher elevation which is not significant compared to levonorgestrel + EE.

This study has therefore found a definite change in lipid and lipoprotein cholesterol in women on oral contraceptives. This change continued to the end of the study. Some investigators have reported a return of the lipid parameters to pretreatment levels by the 9th to 12th month of oral contraceptive use. (55). Others have reported a consistently high lipid and lipoprotein levels upto the 12th month (46). This pattern might progress in continuous use of oral contraceptives to levels close or even as high as in the WEstern industrialised societies where myocardial infraction is prevalent. Wynn et al (247) has indicated that changes in serum lipids and lipoproteins found with oral contraceptive use resemble the pattern described by Fredrickson as type IV hyperlipoproteinemia (118).

But it is apparent that dl-norgestrel + EE (Biginon) produced more pronounced change in all parameters studied than levonorgestrel + EE (Microgynon). Whether the effects are oestrogenic or progestagenic is difficult to interpret at this juncture. However, the high oestrogen and progestagen dose regimen shows a more pronounced change in the subjects.

While there is no agreement as to the direct connection between type - IV hyperlipoproteinemia and accelerated development of atherosclerosis there is evidence of a positive association (64). This oral contraceptive induced hyperlipidemia together with tendencies toward obesity, elevated blood pressure, impaired glucose tolerance are additional factors toward development of atherosclerosis. We have reported the increase in weight and blood pressure. Another factor which was reported by Ross and Glomset (67) as regards atherosclerosis is the importance of platelet activity, smooth muscle proliferation in the light of abnormal lipid levels. It has been observed that abnormal serum lipids increased platelet hyperaggregability.

It is therefore obvious that the pathogenesis of atherosclerosis is less than clear. The ^{known} aetiological factors are multifactorial. There is however a consensus that oral contraceptive use causes a derangement of lipids and lipoproteins in otherwise normal women. Hyperlipidaemia and hypoalpha lipoproteinemia induced by O.C. use has now been regarded as a risk factor for IHD (61, 64, 68, 72). The oestrogen component of the combined oral contraceptive has been shown to elevate HDL-cholesterol, triglycerides and lower LDL-cholesterol and total cholesterol. The progestagen component lowers HDL-cholesterol and elevates LDL-cholesterol. They therefore act antagonistically probably at molecular level. A balance in their effects is therefore necessary such that the dose is effective to prevent ovulation and causing minimal side effects. The two preparations in this study exhibited different oestrogen doses and different progestagen doses. The results are therefore interpreted cautiously.

CONCLUSION

This study has found that:-

1. The normal fasting lipid and lipoprotein levels in a Kenya urban women population are as follows-

Total cholesterol 3.89 ± 0.73 (280 - 5.60), triglycerides 0.68 ± 0.30 (0.15 - 1.38), HDL-cholesterol 1.15 ± 0.22 (0.70 - 1.62), LDL-cholesterol 2.41 ± 0.75 (1.60 - 4.40) and VLDL-cholesterol 0.33 ± 0.13 (0.07 - 0.58). These values are relatively lower than in their caucasian counterparts which could explain the lower incidence of IHD reported in this population.

2. The oral contraceptive regimen dl-norgestrel + EE increased body weight and diastolic blood pressure. These two parameters are associated with increased risk of cardiovascular disease. The two regimens also elevated total cholesterol, serum triglycerides, LDL-cholesterol and VLDL-cholesterol. The preparation dl-norgestrel + EE caused a higher though not significant increase as compared to levonorgestrel + EE. HDL-cholesterol was decreased by the two preparations though again dl-norgestrel + EE elicited a higher but not significant decrease. These changes in lipids and lipoproteins though not significant in the period of study cannot be ignored in the light of a long term use of these agents.

3. The two oral contraceptive preparations contain different doses of both the oestrogen and progestogen. *Dl-norgestrel + EE* contain almost twice the amount of levonorgestrel compared to levonorgestrel + EE. It is not possible to ascertain which component causes the changes in the parameters measured but whether the components act alone or in combination, the high doses in *dl-norgestrel + EE* might not be suitable. Most western countries have dropped most of the preparations containing 50 ug or more ethinyl oestradiol in favour of 30 ug or the newer triphasic preparations. The emphasis is now on effective low oestrogen/progestin dose preparation.

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APPENDIX I

APPENDIX I

COUNTRY	SPECIAL FUND	AGE STANDARDIZATION	
		1980	1985
	110	100.0	100.0
	115	100.0	100.0
	120	100.0	100.0
	125	100.0	100.0
	130	100.0	100.0
	135	100.0	100.0
	140	100.0	100.0
	145	100.0	100.0
	150	100.0	100.0

A P P E N D I X

WORLD HEALTH ORGANISATION LIPID

QUALITY ASSURANCE SCHEME

	1	100.0	100.0
	2	100.0	100.0
	3	100.0	100.0
	4	100.0	100.0
	5	100.0	100.0
	6	100.0	100.0
	7	100.0	100.0
	8	100.0	100.0
	9	100.0	100.0
	10	100.0	100.0
	11	100.0	100.0
	12	100.0	100.0
	13	100.0	100.0
	14	100.0	100.0
	15	100.0	100.0
	16	100.0	100.0
	17	100.0	100.0
	18	100.0	100.0
	19	100.0	100.0
	20	100.0	100.0
	21	100.0	100.0
	22	100.0	100.0
	23	100.0	100.0
	24	100.0	100.0
	25	100.0	100.0
	26	100.0	100.0
	27	100.0	100.0
	28	100.0	100.0
	29	100.0	100.0
	30	100.0	100.0
	31	100.0	100.0
	32	100.0	100.0
	33	100.0	100.0
	34	100.0	100.0
	35	100.0	100.0
	36	100.0	100.0
	37	100.0	100.0
	38	100.0	100.0
	39	100.0	100.0
	40	100.0	100.0
	41	100.0	100.0
	42	100.0	100.0
	43	100.0	100.0
	44	100.0	100.0
	45	100.0	100.0
	46	100.0	100.0
	47	100.0	100.0
	48	100.0	100.0
	49	100.0	100.0
	50	100.0	100.0
	51	100.0	100.0
	52	100.0	100.0
	53	100.0	100.0
	54	100.0	100.0
	55	100.0	100.0
	56	100.0	100.0
	57	100.0	100.0
	58	100.0	100.0
	59	100.0	100.0
	60	100.0	100.0
	61	100.0	100.0
	62	100.0	100.0
	63	100.0	100.0
	64	100.0	100.0
	65	100.0	100.0
	66	100.0	100.0
	67	100.0	100.0
	68	100.0	100.0
	69	100.0	100.0
	70	100.0	100.0
	71	100.0	100.0
	72	100.0	100.0
	73	100.0	100.0
	74	100.0	100.0
	75	100.0	100.0
	76	100.0	100.0
	77	100.0	100.0
	78	100.0	100.0
	79	100.0	100.0
	80	100.0	100.0
	81	100.0	100.0
	82	100.0	100.0
	83	100.0	100.0
	84	100.0	100.0
	85	100.0	100.0
	86	100.0	100.0
	87	100.0	100.0
	88	100.0	100.0
	89	100.0	100.0
	90	100.0	100.0
	91	100.0	100.0
	92	100.0	100.0
	93	100.0	100.0
	94	100.0	100.0
	95	100.0	100.0
	96	100.0	100.0
	97	100.0	100.0
	98	100.0	100.0
	99	100.0	100.0
	100	100.0	100.0

WHO LIPID EQA SCHEME - CYCLE 2Mean results (mg/dl)

	UKEQAS MEAN	ALL PARTICIPANTS+			
		n	mean	SD	CV
<u>Total cholesterol</u>					
A	318.1	6	344.1	49.9	14.9
B	290.6	6	289.4	26.0	9.0
C	61.9	6	64.1	7.6	11.9
D	147.4	6	150.8	13.8	9.2
E	135.5	6	135.1	8.6	6.4
F	112.2	6	113.3	5.9	5.2
G	189.2	6	195.9	10.8	5.5
AVERAGE				17.5	8.9
<u>Free Cholesterol</u>					
A	-	6	86.0	28.2	32.8
B	-	6	67.4	13.9	20.7
C	-	6	23.7	18.1	76.5
D	-	6	40.3	3.1	7.7
E	-	6	38.4	11.1	28.8
F	-	6	36.7	22.9	62.2
G	-	6	60.8	31.8	52.3
AVERAGE				18.4	40.1
<u>HDL Cholesterol</u>					
A	-	4	195.0	59.2	30.3
B	-	4	76.6	52.3	68.3
C	-	4	32.0	12.8	40.0
D	1	4	24.2	16.8	69.4
E	-	4	54.1	12.7	23.2
F	-	4	24.3	12.2	50.1
G	-	4	66.3	39.8	60.1
AVERAGE				29.4	48.8
<u>Triglyceride</u>					
A	-	6	69.6	26.3	37.8
B	-	6	195.6	22.0	11.2
C	-	6	25.6	8.3	32.6
D	-	6	74.0	6.2	32.6
E	-	6	145.1	18.2	12.5
F	-	6	88.7	7.3	8.2
G	-	6	192.8	11.8	6.1
AVERAGE				14.3	16.7

WHO LIPID EQA SCHEME - CYCLE 2Mean of duplicate Results

Lab	1	2	3	4	5	6	7	9
<u>Total Cholesterol</u>								
A	302.2	363	297	321.4	300	422.7	319.5	-
B	281.7	315.3	282	284.6	252	323.2	283.0	-
C	67.5	63.3	64	63	50	72.5	67	-
D	147.1	169.8	152	151	132	162.6	141.5	-
E	129.1	138.6	130	154.1	128.5	150.9	133.5	-
F	109.9	117.3	105.5	117.9	122.5	111.7	113	-
G	183	211.3	185	192.4	201.5	201.	193.5	-

Free Cholesterol

A	65	85.4	62	79	140	79.6	84	-
B	59	64.7	60	61.7	95.5	62.9	62	-
C	12.8	18.7	12	23.4	60	17.3	21.5	-
D	39.3	45.6	42	41.5	37.5	37.5	40	-
E	29.5	34.8	35	35.7	60.5	34.5	36	-
F	24.8	28.7	24	29	83	27.8	32	-
G	41	51.2	41	57.9	124.5	52.9	54	-

HDL Cholesterol

A	+	-	163	196	144	194.8	278.	-
B	+	-	92	68	85.5	2.8	126	-
C	+	-	34	68	30	16.4	47.5	-
D	+	-	23.5	21.8	17.5	8.2	47.5	-
E	+	-	51	43.4	52.5	41.5	71.5	-
F	+	-	22.5	26.7	30	8.2	36.5	-
G		-	71	78.3	88.5	8.8	97	-

Triglyceride

A	64	45.5	35.5	60.8	79	104.2	89.5	-
B	192.3	191.3	208	189.5	155.5	215.4	211	-
C	28.4	19.9	33	25	13	35.2	24	-
D	72.2	67.9	77.5	67.2	66	81.1	79	-
E	136.2	145	173.5	134.7	119	153.9	143	-
F	86	84.6	95.5	86.4	79	98.8	88	-
G	176.2	182.4	207	181.3	198	201.6	191.5	-

Total Phospholipids

A	328.1	355.7	-	305.4	192.5	11.5	307	-
B	384.9	365.7	-	353.5	247	13.6	381	-
C	113.7	112.9	-	173.6	130.5	19.7	115	-
D	177.9	182.6	-	173.6	130.5	19.7	-	-
E	187.5	194.2	-	185.3	180.5	9.9	184.5	-
F	145.7	140	-	138.8	127.5	7	142.	-
G	262.5	256.9	-	254.3	230.5	10.4	268	-

+ Not detected

WHO LIPID EQA SCHEME - CYCLE 2PERCENTAGE DEVIATION FROM ALL PARTICIPANT MEAN:

Lab	1	2	3	4	5	6	7	9
<u>Total Cholesterol</u>								
A	-12.2	+5.5	-13.7	-6.6	-12.8	+22.8	-7.1	-
B	-2.7	+8.9	-2.6	-1.7	-12.9	+11.7	-2.2	-
C	-	-	-	-	-	-	-	-
D	-2.5	+12.6	+0.8	+0.1	-12.5	+7.8	-6.2	-
E	-4.4	+2.6	-3.8	+4.9	+11.7	-1.2	-	-
F	-3.0	+3.5	-6.9	+8.1	-1.4	-0.3	-	-
G	-6.6	+7.9	-5.6	-1.8	+2.9	+2.6	-1.2	-
Average	-5.2	+6.8	-5.3	+1.4	-5.4	+9.2	-3.0	-
<u>Free Cholesterol</u>								
A	-24.4	-0.7	-27.9	-8.1	+62.8	-7.4	-2.3	-
B	-12.5	-4.0	-11.0	-8.5	+41.7	-6.7	-8.0	-
C	-	-	-	-	-	-	-	-
D	-2.5	+13.2	+4.2	+3.0	-6.9	-6.9	-0.7	-
E	-23.2	-9.4	-8.9	-7.0	+57.6	-10.2	-6.2	-
F	-32.4	-21.8	-34.6	-21.0	+126.2	-24.3	-12.8	-
G	-32.6	-15.8	-32.6	-4.8	+104.8	-13.0	11.2	-
AVERAGE	-21.3	-6.4	-18.5	-7.7	+64.4	-11.4	-6.9	-
<u>HDL Cholesterol</u>								
A	-	-	-	-	-	-	-	-
B	+	-	+20.1	-11.2	+11.6	-96.7	+64.5	-
C	+	-	+6.3	-13.7	-6.2	-48.7	+48.4	-
D	+	-	-2.0	-9.9	-27.7	-66.1	96.3	-
E	+	-	-5.7	-19.8	-3.0	-23.3	+32.2	-
F	+	-	-7.4	+9.9	+23.5	-66.3	+50.2	-
G	+	-	+7.1	+18.1	+33.5	-86.7	+46.3	-
AVERAGE			+2.9	-4.4	+5.3	-64.6	+56.3	-
<u>Triglyceride</u>								
A	-9.0	-34.6	-49.0	-12.6	+13.5	+49.7	+28.6	-
B	-1.7	-2.2	+6.3	-3.1	-20.5	+10.1	+7.9	-
C	-	-	-	-2.3	-	-	-	-
D	-2.4	-8.2	+4.7	-9.2	-10.8	+9.6	+6.8	-
E	-6.1	0.0	+19.6	-7.2	-18.0	+6.1	+1.4	-
F	-3.0	-4.6	+7.7	-2.6	-10.9	+11.4	-0.8	-
G	-8.6	-5.4	+7.4	-6.0	+2.7	+4.6	-0.7	-
AVERAGE	-5.1	-9.2	-5.5	-6.1	-7.3	+15.3	+6.7	-

Total Phospholipids

A	-	5	239.0	141.5	59.2
B	-	5	278.4	158.6	57.0
C	-	5	87.4	43.6	49.8
D	-	5	139.4	70.7	50.7
E	-	5	151.3	79.2	52.3
F	-	5	112.4	59.3	52.8
G	-	5	205.7	110.1	53.5
AVERAGE				94.7	53.6

+ excluding Lab 4

A	+13.7	+48.8	-	+27.8	-19.5	-95.2	+28.5	-
B	+38.3	+31.3	-	+27.0	-11.3	-95.1	+36.9	-
C	+30.1	+29.2	-	+21.5	-6.2	-84.6	+31.6	-
D	+27.6	+31.0	-	+24.5	-6.4	-85.9	+33.8	-
E	+23.9	+28.4	-	+22.5	+19.3	-93.5	+21.9	-
F	+29.6	+24.6	-	+23.5	+13.4	-93.8	+26.3	-
G	+27.6	+24.9	-	+23.6	+12.1	-94.9	+30.3	7
Average	+27.3	+31.2	-	+24.3	+0.2	-91.9	+29.9	

+ Not detected

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