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Effects of levonorgestrel, ethinylestradiol and norethisterone on plasma cholesterol and triglycerides of wistar albino rat (*Rattus rattus)*

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Abstract
The effects of Microgynon, a combined oral contraceptive pill (0.15mg levonorgestrel and 0.03mg ethinylestradiol) and Primolut –N, a progestogen only pill (5mg norethisterone) were analysed for their in-vivo effects on rat (*rattus rattus*) plasma cholesterol and triglycerides. The drugs decreased plasma cholesterol levels in a concentration dependent manner. Microgynon had the most effect, and a highest percentage decrease of 93.33% (0.002 ± 0.001 mmol/l ($P < 0.05$)) was observed for the highest dose of 3.6µg/100g body wt. Primolut had lesser effects with figures (0.008 ± 0.001 mmo/l and 0.009 ± 0.001mmol/l respectively). The drugs increased the levels of triglyceride with Microgynon showing the highest value (0.114 ± 0.006 mmol/l). This result indicates that lipoprotein profile are needed for women before using these drugs.
Keywords: Levonorgestrel, Ethinylestradiol, Norethisterone , Cholesterol, Triglycerides

Introduction

Oral contraceptives (OC) first became available to women in the early 1960s. The convenience, effectiveness, and reversibility of action of these birth control pills (popularly known as "the pill") have made them the most popular form of birth control (Henderson *et al.*, 1991). Microgynon a combined pill (0.15mg levonorgestrel and 0.03mg ethinylestradiol) and Primolut- N a mini pill (5mg norethisterone) are among the most common drugs used in Nigeria for contraception and for other non contraceptive benefits. Like any other drug they have some side effects such as nausea, breast tenderness, weight gain, irregular menstrual bleeding as well as thrombosis. Initial oral contraceptive formulations contained very high levels of synthetic estrogen and progesterone, based on the assumption that these levels were necessary to prevent pregnancy (Skouby & Jesperson, 1990). Over the years however, hormone levels have continually decreased in order to provide formulation with maximum efficiency and minimum side effects (Grimes *et al.,* 1993; Briggs, 1992).

The estrogen/progestin combination is the most effective type of OC formulation, because these preparations consistently inhibit the midcycle gonadotropin surge, and thus prevent ovulation. Such formulations also act on other aspects of the reproductive process. They alter the cervical mucus, making it thick, viscid and scanty, which retards sperm penetration. They also alter motility of the uterus and oviduct, thus impairing transport of both ova and sperm. Furthermore, they alter the endometrium so that its glandular production of glycogen is diminished and less energy is available for the blastocyst to survive in the uterine cavity. Finally, they may alter ovarian responsiveness to gonadotropin stimulation. Nevertheless, neither gonadotropin production nor ovarian steroidogenesis is completely abolished. Levels of endogenous estradiol in the peripheral blood during ingestion of combination OCs is similar to those found in

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the early follicular phase of the normal cycle (Mishell *et al.,* 1982).

Contraceptive steroids prevent ovulation mainly by interfering with release of gonadotropin-releasinghormone (GnRH) from the hypothalamus. The combination OCs probably does have a direct inhibitory effect on the gonadotropin-producing cells of the pituitary, in addition to affecting the hypothalamus. This effect occurs in about 80% of women ingesting combination OCs. It is unrelated to the age of the patient or the duration of OC use, but is related to the potency of the preparations. The effect is more pronounced with formulations containing a more potent progestin and with those containing 50 µg or more of estrogen than with 30- to 35 µg formulations (Scott *et al.,* 1978). There are data showing that the delay in the resumption of ovulation after discontinuation of OC use is shorter in women ingesting preparations with less than 50 µg of estrogen than I those ingesting formulations with 50 µg of estrogen or more (Bracken *et al.,* 1990).

World Health Organization (1982) reported that the daily progestin-only preparations do not consistently inhibit ovulation, because of the inconsistent ovulation inhibition, their effectiveness is significantly less than that of the combination type of OCs.

Cholesterol, triglyceride and phospholipids, together with apoproteins, are linked in varying proportions to form several lipoproteins. Cholesterol is derived partly from the diet and it is also synthesized in most tissues. The greater part of the cholesterol in the blood is in LDL. In a normal man, LDL is produced by the metabolism of VLDL, one VLDL particle yielding one LDL particle. Many cells of the body have surface receptors which take up and internalize LDL, thus supplying cholesterol to the cell, this uptake turns off cholesterol synthesis within the cell. HDL takes up free cholesterol from the tissues for transport to the liver for re-use. Triglyceride in VLDL and chylomicra is taken up fro storage or oxidation by adipose tissue, muscle and other organs after hydrolysis by the enzyme lipoprotein lipase at the endothelial surface (Wootton & Freeman, 1982).

Materials and method

Microgynon was bought from Schering AG Germany. Primolut- N was bought from Medipharm (Pvt) Ltd., Lahore.Licencee of Schering AG. Federal Republic of Germany.The cholesterol reagent kit was bought from Randox Laboratories Ltd. Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom BT29 4QY. While the triglyceride reagent kit was from Human Gesellschaft fur Biochemica und Diagnostica mbH, Max Planck-Ring 21, D-65205 Wiesbaden, Germany.

108 albino rats (average weight 100.00 ± 10.00g) were used for the tests. These were obtained from the animal house of the Biochemistry department, faculty of Science, University of Port Harcourt. The rats were divided into three groups of 54 rats each for the different drugs. The drugs were administered orally, the initial weight of the drugs fed to the rats were scaled down to a ratio of the normal dosage taken by an average woman of 55kg. The animals were on their normal diets (standard commercial feed) before the drug administration and were continued on this diet after that five doses of the contraceptive drugs (microgynon: 0.36, 0.72, 1.40, 1.80 and 3.60 µg/100g body weight and primolut-N: 10.00, 20.00, 40.00, 50.00 and100.00 µg per 100g body weight were administered for each analysis. A set of 9 rats were used as controls for each drug analysis and no contraceptive drugs were administered to them. The tests were monitored for 24 hours ranging from 2hours, 4 hours and 24 hours.18 rats from each drug group were sacrificed after each time interval (3 rats from each dose group). This was done by cardiac puncture, with the animal under anesthesia (chloroform) in a desiccator. The blood collection was done immediately and was stored in a lithium heparin sample containers. The blood was centrifuged at 3000 rotations per minute for 3 minutes and the blood plasma were separated and used for the analysis.

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The plasma cholesterol levels were determined by enzymatic endpoint method.The principle of this method is that cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoeimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase (Trinder,1969).

Reagent kit contained 4-Aminoantipyrine (0.30mmol/l), phenol (6mmol/l), peroxidase (≥0.5 u/ml)cholesterol esterase (≥0.15 u/ml), cholesterol oxidase (≥0.1 /ml), pipes Buffer (80mmol/l; pH 6.8) and standard (5.17 mmol/l (200mg/dl)).

1.00ml of reagent was mixed with 10µl of the sample. The standard tube contained 1.00 ml of reagent and 0.01 ml of the standard. The blank tube had 1.00 ml of reagent and 10µl of distilled water.. The mixtures were incubated for 10 minutes at 37 ^{o}C. The absorbance of the samples were read against the reagent blank within 60 minutes at 546nm with spectronic – 20 spectrophotometer.

Calculations

Concentration of cholesterol in sample = $\frac{\Delta A_{unknown}}{\Delta A_{standard}}$ X $C_{standard}$

Normal values < 5.17 mmol/l (NCEP, 2001).

Enzymatic Colorimetric Test determined triglyceride levels for Triglycerides with Lipid clearing Factor (LCF). The Principle of this method is that the triglycerides were determined after enzymatic hydrolysis with lipases. Indicator is quinoneimine formed from hydrogen peroxide, 4-aminoantipyrine and 4-chlorophenol under the catalytic influence of peroxidase (Schettler & Nussel, 1975; Jacobs NJ & VanDemark PJ, 1960).

Reagent kit contained pipes buffer(pH 7.5) (50mmol/l), 4-chlorophenol (5mmol/l), 4-aminoantipyrine (0.25mmol/l), magnesium ions (4.5 mmol/l), ATP (2mmol/l), lipases (≥1.3u/ml), peroxidases (≥0.5u/ml), glycerol kinase (≥1.5u/ml), glycerol-3-phosphate oxidase (≥1.5U/ml) and 3ml standard (200mg/dl).

1000µl of reagent was mixed with 10µl of the sample. The standard tube contained 1000µl of reagent and 10µl of the standard. The blank tube had 1000µl of reagent. The mixtures were incubated for 5 minutes at 37 ^{o}C. The absorbance of the samples were read against the reagent blank within 60 minutes at 546nm with spectronic – 20 spectrophotometer.

Calculations

$\frac{\Delta A_{unknown}}{\Delta A_{standard}}$ X 2.28[mmol/l]

Normal values <1.71mmol/l

Results and discussion

Fig 1-2 shows the mean results ± SD of plasma cholesterol determination. Plasma cholesterol levels were decreased by the drugs in a concentration dependent manner with Microgynon having the most effect (0.002 ± 0.001 vs control 0.03 ± 0.000 mmo/l) followed by Primolut-N (0.008 ± 0.001 and 0.009 ± 0.001 respectively vs control 0.03 ± 0.000 mmol/l). A highest percentage decrease of 93.33% was observed for the highest dose of 3.6µg/100g body wt. The decrease was concentration dependent, but showed the same percentage decrease for both the 2 hours and the 4 hours intervals. The decreasing effects of the cholesterol levels by increasing drug dosage are statistically significant at 95.0% confidence level ($P < 0.05$).

Investigations by Smith and Sizto (1983) showed that oral contraceptives containing dl-norgestrel and ethinyl estradiol significantly increased the levels of cholesterol in women who had not taken OCs for 3 or more months. However they also observed that preparations containing levonorgestrel reduced levels of cholesterol. Cholesterol is group of fats vital to cell membranes, nerve fibers and bile salts, and a necessary precursor for the sex hormones. High levels indicate diet high in carbohydrates/sugars. Low levels indicate low fat diet, malabsorption, or carbohydrate sensitivity. Cholesterol measurements are used in the diagnosis and treatments of lipid lipoprotein metabolism disorders. Lipids play

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Fig.1. Effect of microgynon on cholesterol (mmol/l)

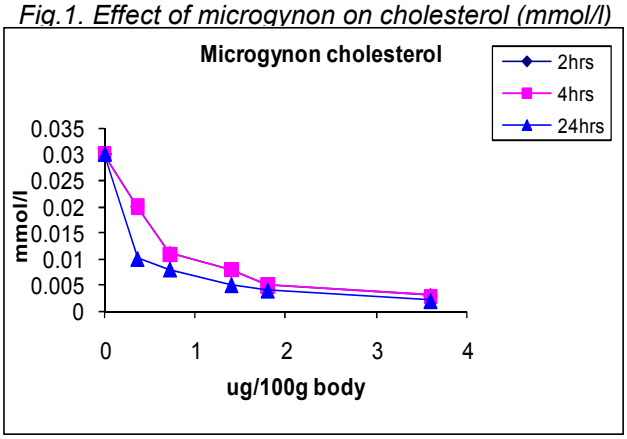


Fig.2. Effect of primolut - N on cholesterol (mmol/l)

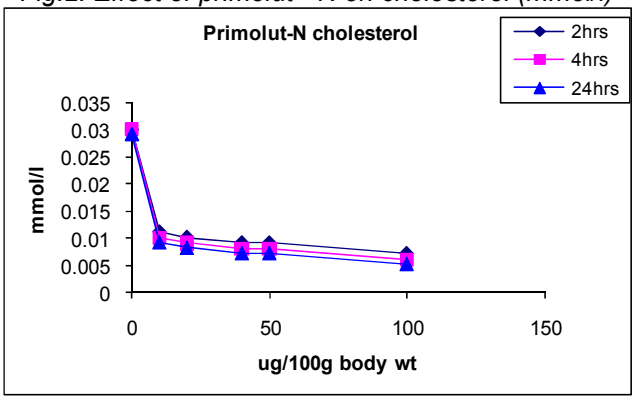


Fig. 3.Effect of microgynon on triglyceride (mEq/l)

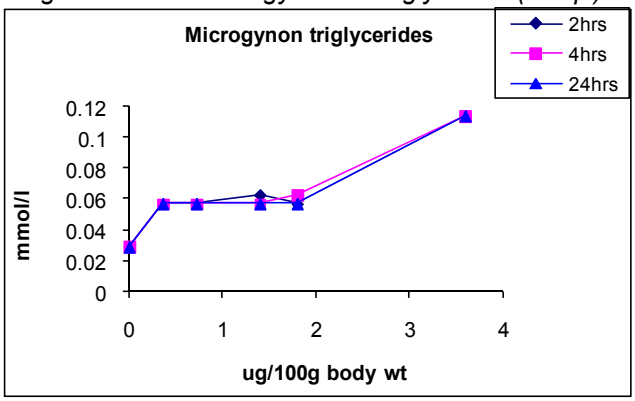
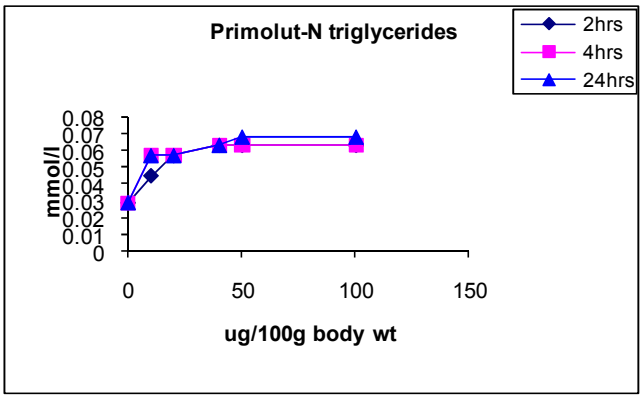


Fig.4.Effect of primolut on triglyceride (mEq/l)



an important role in the body; they serve as hormone precursors, aid in digestion, provide energy, storage and metabolic fuels, act as functional and structural components in biomembranes and form insulation to allow nerve conduction and prevent heat loss (Richmond, 1973; Roeschlau *et al.*, 1974). Low density lipoproteins (LDL) is the "bad cholesterol", which carries cholesterol for cell building needs, but leaves behind any excess on artery walls and in tissues. High density lipoproteins (HDL) is the "good cholesterol" which helps to prevent narrowing of the artery walls by removing the excess cholesterol and transporting it to the liver for excretion. High LDL and low HDL levels indicate diets high in refined carbohydrates and/or carbohydrate sensitivity (Simonson *et al.,* 2004). The results from this research agree with the studies in which lipid levels were measured before and after the ingestion of several low dose estrogen-progestin formulations, including the triphasic formulation containing levonorgestrel.

The authors found no adverse alterations in the levels of HDL cholesterol or LDL cholesterol or in the ratio of total cholesterol to HDL cholesterol (Kloosterboer *et al.,* 1986). However, in two separate studies, the triphasic formulation with norgestrel but not the triphasic formulation with norethindrone, still significantly lowered the level of the HDL2 subfraction that is believed to be the cardioprotective fraction of HDL cholesterol (Percival-Smith *et al.,* 1987). In a randomized study of the three triphasic formulations, two with norethindrone and one with levonorgestrel, each had similar effects on carbohydrate and lipid metabolism, including changes in HDL, HDL2, and LDL cholesterol (Patsch *et al.*, 1989). Another randomized study comparing the effects of a norethindrone and a levonorgestrel triphasic formulation on serum lipid levels also found no statistically significant difference between the two formulations (Notelovitz *et al.*, 1985).

Presented on fig.3-4 are the mean results ± SD of the triglyceride determinations. The drugs increased the levels of triglyceride with

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Microgynon showing the highest value (0.114 ± 0.006 vs contol 0.029 ± 0.006mmol/l) followed by Primolut (0.068 ± 0.060 vs control 0.029 ± 0.000 mmol/l). The results showed that the drug had an increasing effect on the triglyceride level. 293.10% was observed for the highest dose of 3.60µg/100g body wt**.** The differences in weight and hour were statistically significant on the effect of the drug on the plasma triglyceride levels at 95.0% confidence level ($P < 0.05$). These are fats used as fuel by the body, and as an energy source for metabolism. Increased levels are usually a sign of too much carbohydrate intake. Decreased levels are seen in hyperthyroidism, malnutrition and malabsorption. The changes in serum triglyceride concentration induced by OC intake must be considered by the clinician and are useful for taking a clinical and risk decision in an individual woman.

Conclusion

This study revealed that the intake of the Microgynon and Primolut-N decreased cholesterol levels while they increased triglyceride levels. It is advisable to obtain a lipoprotein profile before oral contraceptive use is started, as hypertriglyceridemia may be present and oral contraceptive use will further raise triglyceride levels.

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