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Full Length Research Paper

Lipid composition of microsomal fraction of female rat liver at different stages of growth

Chidozie, V. N.[1,3]*, Anekwe, G. E.[1], Chukwu, O. O. C.[2], Olabode, O. A.[3], Igweh, A. C.[4] and Adeyanju, O. N.[3]

[1]Department of Biochemistry, University of Jos, Nigeria.
[2]Molecular Biology Department, Federal College of Veterinary and Medical Laboratory Technology, Vom, Nigeria.
[3]Federal College of Veterinary and Medical Laboratory Technology, Vom, Nigeria.
[4]Biochemistry and Chemotherapy Division, Nigerian Institute for Trypanosomiasis Research, Vom, Nigeria.

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It has been reported that lipids are the only macromolecules which display consistency in their pattern of biosynthesis for all living system. In this study, lipid biosynthesis in the liver microsomes of female Sprague-Dawley rats as they aged (3, 7, 12 , 16 and 20 weeks) was studied. The microsomes were isolated by homogenization and centrifugation. The aliquot of the microsomes and soluble enzymes fraction were dried and separated into neutral and polar lipids. The separation of neutral and polar lipids fractions was by silicic acid column chromatography. The data obtained from the study showed that the total lipid content of the rat liver microsomes, the cholesterol, the triglycerides, and the polar (phosphoglycerides) lipids increased as the rats grew older. The results imply that similar pattern of lipid biosynthesis occurs in rats as in humans. This study has given a better understanding of the pattern of biosynthesis of lipids in female rats.

Key words: Liver microsomes, cholesterol, triglycerides, phosphoglycerides, lipidoses.

INTRODUCTION

Lipids are a heterogenous group of compounds, which are relatively insoluble in water but are soluble in non-polar solvents. A common feature of lipids is that they contain a non-polar group of aliphatic and/or aromatic character. In additon many of them have ampiphilic character, in that they possess polar groups some of which form anions and cations. The balance between lipophilic non-polar and hydrophilic polar properties determines the molecule's solubility in water and in fats and thus the behaviour in biological system (Krister, 1978). Lipids have many important functions in the mammalian body such as energy production, serving as major building blocks of the cells, biological membranes cellular and in-tracellular. They are involved in the solubilisation of non-polar substances in the body fluids and their transport. They also serve as thermal insulators in the subcuta-neous tissues and around some organs, and some have im-portant and specific physiological roles in the control of metabolic processes.

A lot of work has been done on lipid biosynthesis in mammals (Rao and Johnston, 1960; Reiser and Fu, 1960; Senior and Isselbacker, 1963; Lucas and Ridout, 1964). These works have sufficiently proven that lipid biosynthesis in mammalian systems and indeed all vertebrates takes place in the microsomes. Lipids are the only macromolecules which display consistencey in their pattern of biosynthesis for all living systems (Anekwe, 1972). Cholesterol biosynthesis in human females follows an ascending curve which plateaus at menopause (Anekwe, 1975; Salami, 2004).

The present work was ,therefore ,designed to ascertain if cholesterol biosynthesis in female rats follows the same pattern as in human females. The study goes futher to find out if there is a similar pattern in the biosynthesis of triglycerides during growth in female rats. This is with a view having of a better understanding of the pattern of biosynthesis of lipids in female rats and extending the frontiers of knowledge in this area of research.

MATERIALS AND METHODS

Experimental animals

Sprague-Drawley rats weighing from 30-200 g, obtained from the

*Corresponding author. E-mail: atoka2010@yahoo.com.

University of Jos animal house were used for the work. These animals were acclimatized for a period of one week. They were fed with ECWA Growers marsh and given water ad libitum.

Design

The rats were placed into five groups according to their ages as follows; 3 weeks (12 rats), 7 weeks (4 rats), 12 weeks (4 rats), 16 weeks (3 rats) and 20 weeks(3 rats).

Collection of liver samples

The animals were sacrificed by suffocation with chloroform in a desiccator. The removal of the rats' liver, homogenization and lipid extraction was a modified version of that used by Emokpae et al. (1982). The rats' liver were removed and submerged in 0.02M phosphate buffer of pH 7 in labeled containers. This was to remove excess blood and connective tissues. The liver samples were chopped into small pieces with a pair of scissors.

Homogenization of liver samples

The pieces of the liver samples were homogenized in a potter Elvehjen homogenizer at 4°C with 40ml of phosphate buffer pH 7.0 (that is, using a volume twice the weight of the liver). The resultant pulp was then centrifuged at 7000 revolutions per minute (r.p.m) for 30 minutes using MSE table clinical centrifuge. The supernatant containing the microsomes and soluble enzymes was collected and resuspended in phosphate buffer twice the volume of the supernatant and this mixture was then centrifuged at 7000 r.p.m for 60 minutes. This was done three times until a clear transparent supernatant was obtained. The supernatant was kept at 4°C while the sediment containing the mitochondria and nuclei, was discarded.

Isolation of liver microsomes from liver homogenate

The isolation of liver microsomes was carried out according to the method of Anekwe and Dubal (1971). The homogenization resulted in the deposit which contatined mitochondria and nuclei and the supernatant which contained microsomes and soluble enzymes. The purified supernatant was then dispersed in a round bottom flask and concentrated using a rotary vacuum evaporator. The concen-trated supernatant was collected with chloroform- methanol mixture (2:1 v/v) and transferred into a pre-weighed test tube and dried to complete dryness in an isotemp fisher oven. The test tube containing the dried supernatant was re-weighed and the weight of the total lipid calculated. The lipid extract was stored and protected from direct sunlight.

Fractionation of lipid by silic acid column chromatography

The total lipid in the test tube was re-suspended with the aid of a little quantity of chloroform. Using silicic acid column chromatography, the total lipid was fractionated into neutral and polar lipid according to the methods of Borgstrom (1952), Anekwe et al. (1973) and Beach et al. (1980). Prior to chromatography the silicic acid was activated at 120 degrees celcius for 24 h in the oven to remove moisture. 5 g of the activated silicic acid was suspended in 50 mls of pure chloroform. The slurry was poured into a chromatography column plugged at the base with Whatman No. 1 filter paper cut out to the precise diameter of the width of the column. The column was properly washed with one volume of chloroform and allowed to settle. Another Whatman No. l filter paper cut out to the diameter of the column was placed on top of silicic acid column. The length of the column was 15 cm.

The lipid sample, dissolved in pure chloroform was then carefully poured into the column. Elution of the column at the flow rate of 32 drops per minute was carried out with 100 mls of pure chloroform to separate the neutral lipid. The polar lipid was obtained using 100 mls of choroform methanol mixture (2:1 v/v). Finally, the very polar lipid was collected using 50 mls of pure methanol. The polar and the very polar lipid were pooled together and labelled as the polar lipid fraction. The separated volumes of the neutral and polar lipid fractions were respectively reduced in a rotary evaporator.

The two fractions were then transferred into pre-weighed test tubes, labelled neutral and polar lipid respectively. The test tubes were dried completely at 30 degrees celsius in an oven. The test tubes were re-weighed and the weights of the neutral and polar lipids determined.

Thin layer chromatography

The neutral lipid fraction was then separated into their different components using thin layer chromatography (TLC). The TLC was carried out on commercial sillica gel glass plates of 10 x 20 cm size. The glass plates were activated at 105 degees celcius for 1 h. The neutral lipid was re- suspended in a small amount of chloroform. Us-ing a micro-pipette, a spot of the neutral lipid was made on the ori-gin of the plate, 2 cm from the lower edge of the plate.

Development was carried out on a chromatography tank using ascending technique. Before development the solvent system (Benzene) was poured into the tank and Whatman No.1 filter paper placed in the tank. The environment within the closed tank was allowed to be saturated for a period of 1 h. Thereafter, the plate containing the sample was placed in the tank in an upright position, so that about 1 cm of the plate was in the solvent. The solvent level was below the spot. The tank was covered with its lid and the solvent allowed to migrate until it reached the upper edge of the plates. The plates were then removed and allowed to dry.

Sulphuric acid charring technique was used to detect the spots containing the separated lipid fractions. 55% and 0.06% by weight of sulpuric acid and potassium dicromate sollution mixture was used to spray on dried TLC plates. The plates were placed in an oven and allowed to clear at 180°C for 15 min, and then removed and allowed to cool. The separated fractions were observed.

The Retardation factor (R.F) values of the different neutral lipid fractoins were calculated. Using the RF values already published in the literature (Anekwe, 2002), the separated spots on the plates were identified.

Quantification of the neutral lipid fractions

Preparative thin layer chromatography plates were used for this procedure. The method is the same as earlier described for the commercial thin layer chromatography. After development, the prepartive TLC plates were dried and sprayed with bromthymol blue for identification. The area of the spot identified as cholesterol was scraped into a test tube and re-dissolved with chloroform methanol mixture (2:1 v/v). The absorbance of this mixture was read at 580 nm in a colorimeter (Lab Tech 6050, Jenway Ltd UK) using chloroform methanol mixture (2:1 v/v) as blank. The same procedure was applied for triglycerides.

The concentration of the test cholesterol and triglycerides was calculated using the formula, where material is either cholesterol or triglycerides.

RESULTS

There was significant increase ($p < 0.05$) in the profiles of

Table 1. Weights of the female rats' lipids at different ages.

Age (weeks)	Total lipid (mg/g liver)	Neutral lipid (mg/g liver)	Polar lipid (mg/g liver
3	31.34	6.95	23.27
7	39.61	8.99	30.11
12	42.76	9.81	32.86
16	46.73	10.73	35.94
20	60.20	13.80	46.20

Table 2. Retardation Factor (Rf) of the female rats' neutral lipids at different ages.

Age (Weeks)	Cholesterol	Triglycerides
3	0.247	0.680
7	0.250	0.710
12	0.244	0.630
16	0.240	0.640
20	0.237	0.640

Table 3. Optical density of cholesterol and triglyceride at different ages.

Age (weeks)	Cholesterol	Triglyceride
3	0.011	0.009
7	0.012	0.012
12	0.013	0.013
16	0.014	0.014
20	0.018	0.018
Cholesterol Std	0.020	-
Triglyceride Std	-	0.400

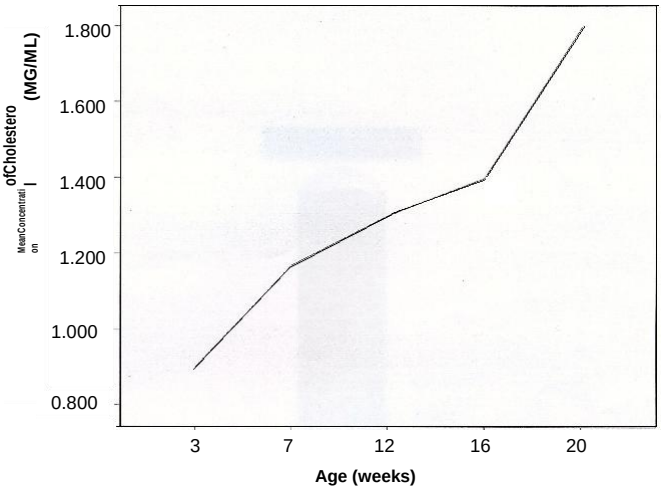

Figure 1. Cholesterol concentration in rats at various ages.

the lipids from 3 weeks of age to 20 weeks of age. Polar lipids are significantly ($p < 0.05$) higher than neutral lipids.

The weights of the lipids

Table 1 shows the weight of the lipids (Total lipids, neutral lipids and polar lipid) at the different stages of growth. The result shows significant increase ($p < 0.05$) in the profiles of the lipids from 3 weeks of age to 20 weeks of age in the experimental schedule. The polar lipids (phospholipids) are significantly higher ($p < 0.05$) than the neutral lipids (Cholesterol and triglycerides). Development, detection and identification of the separated lipid fractions using thin layer chromatography techniques are shown in Plate 1. The Retardation Factor (RF) values of the neutral lipids (cholesterol and
triglycerides) at different stages of growth are shown in Table 2.

Optical density of the neutral lipids

The optical density (OD) of the neutral lipids (cholesterol and triglycerides) at the different stages of growth are shown in Table 3.

Concentration of cholesterol and triglycerides

Figures 1 and 2 show the concentration of cholesterol and triglycerides at different stages of growth of the experimental animals. Figure 3 shows by regression analysis the association between time (weeks) and cholesterol concentration. The concentration of cholesterol and triglycerides significantly ($p < 0.05$) increased with age. With age being the independent variable and cholesterol change/level as the dependent variable. Figure 3 shows a linear upward association between time and choleste-rol. Plotting the line of best fit and calculating the regres-sion equation, the result shows that cholesterol can be estimated using this model at 93% confidence or certainty from the R statistics. This shows that there is a positive linear association between time and cholesterol level of rat, and this level can be estimated using the regression model:

$$y = 0.77 + 0.047x$$

Where:
y is the cholesterol level, x is the time in weeks.

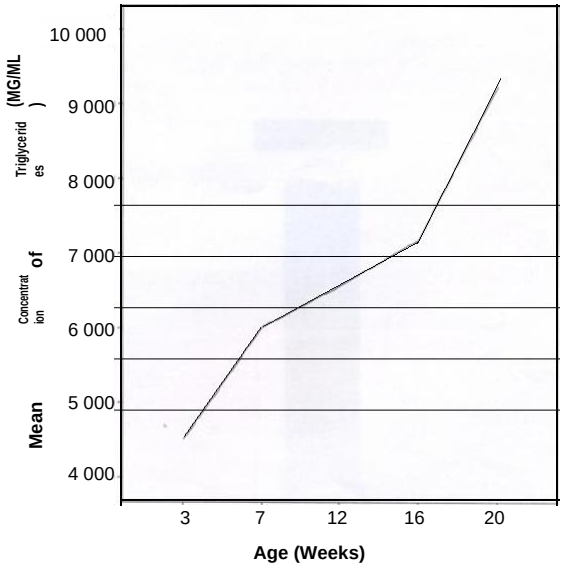

Figure 2. Triglyrides concentration in rats at various ages.

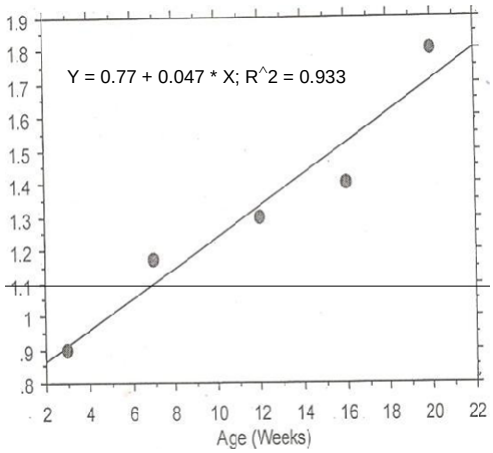

Figure 3. Graph showing association between time and cholesterol concentration (mg/g) in rats.

Plate 1. Picture of the separated lipids using TLC.

DISCUSSION

Concentration of cholesterol and triglycerides

The present study shows that the total lipid of female rats increased linearly with age. The polar lipids (phospholipids) were recorded to be significantly ($p < 0.05$) higher than the neutral lipids (cholesterol and triglycerides). This agrees with the work of Fex (1971) who found the phospholipid content of normal adult rats to be 74% of the total lipid content of the rats. Uchida et al. (2001) working on age related changes in cholesterol and bile acid metabolism in male rats reported that the serum and liver lipid levels increased with age. However, unlike the results obtained in the present work where female rats were used, the phospholipid level of the rat liver and serum in the work of Uchida et al. (2001) remained unchanged as they rats aged.

The results of the concentrations of cholesterol and triglycerides (Figures 1 and 2) show a linear relationship between age and concentrations of cholesterol and triglycerides. The triglycerides concentration was significantly ($p < 0.5$) higher than that of cholesterol. The fact that cholesterol increases with age has been established in human females, Caucasian females in the United States and in African females in Jos, Nigeria (Anekwe ,1977; Salami, 2004).

In this present work, triglyceride level was higher than the cholesterol level; and the triglyceride level increased with age in the rats. However, Fex (1971) working on normal adult rats observed that the triglyceride content of the rat liver was 67% of the neutral lipid of the rat liver while the total cholesterol content was 24%. This is in agreement with the result obtained in the present work.

According to Anekwe (1975) and Salami (2004) cholesterol biosynthesis in human female follows an ascending curve which plateau at menopause. However, the result in this aspect in the present work did not plateau. This could be due to the age of the rats used (20 weeks maximum) which could not tally with the age of menopause.

In conclusion, this study implies that a similar pattern of lipid biosynthesis occurs in rats as in humans hence, giving us a better understanding of the pattern of biosynthesis of lipids in female rats.

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