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RESEARCH ARTICLE

INVESTIGATION OF SERUM LIPID CONCENTRATION IN MICEPLACED ON EXPERIMENTAL DIET

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ABSTRACT

Background: Lipid abnormalities such as low levels of high-density lipoprotein cholesterol (HDL-C), levels of low-density lipoprotein cholesterol (LDL-C) and elevated triglycerides are linked to an increased risk of chronic heart disease (CHD) and cardiovascular events which act as predictors of the disease process since excessive intake of cholesterol is likely to lead to hyperlipidaemia. **Method:** A total of forty (male and female) mice aged between 2 and 12 months were used. The animals were maintained in group cages, and fed with water and experimental food ad libitum six weeks prior to start of the study.Blood samples collected in EDTA-containing tubes following heart puncture, and allowed to cloth and then centrifuged at 12 000 rpm for 2 minutes for serum cholesterol and triglyceride analysis, using standard routine enzymatic method. **Result:** The serum triglyceride showed (no significant) increasein the 4 to 6 months old male mice (5.47 to 5.70mmol/L).However, there was a decrease in the 12 month old male mice.There was significant increase in the serum triglyceride level from 2 to 6 months female mice (2.23 – 6.83mmol/L), but recorded a slight decrease in its level (5.5mmol/L) in the 12 months old female mice. There was a steady increase in cholesterol in male and female mice with increasing age (2 to 12 months). **Conclusion:** Age of mice studied have direct influence on the blood serum levels of cholesterol and triglyceride. While the male mice appear to be at a slightly higher risk of raised cholesterol level than the female mice, there is a tendency for the female mice to risk a raised serum TAG level.

INTRODUCTION

Lipid abnormalities such as low levels of high-density lipoprotein cholesterol (HDL- C), levels of low-density lipoprotein cholesterol (LDL-C) and elevated triglycerides are linked with an increased risk of chronic heart disease (CHD) and cardiovascular events, hence acting as predictors of the disease process (Gordon *et al*., 1989; Sarwar*et al*., 2007; Bansal*et al*., 2007). Life style greatly people's wellbeing, and often time, people tend to eat foods with excessive fat contentbelieved to contain a high proportion of saturated fatty acids which subsequently raise blood cholesterol and low density lipoprotein (Grundy and Denke, 1990). Cholesterol (CHOL) and triglyceride (TAG) are essential structural lipids of the cell membrane which serve as solvents for fat-soluble vitamins. TAG is one of the energy sources of the body and could be used as fuel, however, ifthe build up of energy continue to rise without proper utilisation, the end result isalways increased adiposity which ultimately leads to obesity (John and Marek, 2009). Similarly, cholesterol synthesis occurs in the body by the liver following meal intake. Its concentration in the blood is determined by its synthesis (John and Marek, 2009). Cholesterol is transported via blood plasma, thus, it has been reported that thecombination of proteins and cholesterol (lipoprotein) could either be helpful or dangerous to our health (NHS, 2012). In both instances, the NHS argued that LDL tends to deposit cholesterol in the walls of the arteries when its plasma level is raised in the cells. However, HDLappears to be beneficial as excess cholesterol is returned to the liver where it is broken down and excreted as wastes in the body. The levels of CHOL and TAG in the blood serum have clinical significance which can be used to diagnose abnormality as excessive intake of cholesterol is likely to lead to hyperlipidaemia (NHS, 2012). Thus, the risk of developing chronic heart disease is relative to the type of fatty acid triglyceride contains (Wanpen*et al.,* 2001). The outcome of this study will help to bring to light vital findings that will be beneficial to policy makers in the local health institutions and further educate the general populace, in the language they will understand, on the dangers of excess fats and oil consumptions.

Aim: The aim of the experiment was to:

- Investigate serum lipid concentration in mice
- Compare the level of occurrence between male and female mice
- Classify the mice based on Fredrickson's (WHO) classification for hyperlipidaemia

METHODS AND MATERIALS

Animal model and sample extraction: The investigation conforms to the United Kingdom Animal procedures act 1986, and with the Guide for the Care and Use of Laboratory

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Animals published by the US National Institutes of Health (NIH publication, 8th Edition, 2011). Ethical approval was granted by the University Ethics Committee. In this study, a total of forty mice, 20 male and 20 female aged between 2 and 12 months were used. The animals were maintained in group cages, on saw dust bedding, and subjected to a 12 h–12 h light/–dark cycle with water and experimental food (table 1 and 2) as described byWanpen*et al* (2001) with few modification, provided *ad libitum*six weeks prior to start of the study. Animals were culled using intraperitoneal administration of pentobarbital sodium (60mg/kg i.p.; JM Loveridgeplc, Southampton, UK), and blood samples collected in EDTA-containing tubes following heart puncture. To prevent the activity of protease enzyme in the samples, phenymethysulfonyl fluoride (Sigma chemical Co., St. Louse, MO) was added to make up to 0.01 mmol/L. Blood was allowed to cloth and was centrifuged at 12 000 rpm for 2 minutes for serum cholesterol and triglyceride analysis. Serum samples were stored at 4 °C and analysis carried out within 12 hours.

Total CHOL and TAG were measured with routine enzymatic method. A standard curve was prepared in duplicate using a range ofconcentrationsfrom 10.0 mmol/L stock of cholesterol and triglyceride. Gilson pipette was used to transfer appropriate volumes of TAG infinity reagent into Eppendorf tubes with the required volume of water, and wasproperly mixed using a vortex mixer. 95µl of standardTAG was transferred into a 96-well microliter plate using P100 Gilson pipette. This was followed by transferring5µl each of themixture from the Eppendorf tubes into the awaiting 96-well plate for analysis. The plate was rotated in a 10-motion clockwise and anti-clockwise pattern to ensure samples were properly mixed as the infinity reagent was very viscous. Furthermore, 5µl of triglyceride wasalso transferred into empty wells in duplicates. The plate was then mixed by rotation in clockwise and anti-clockwise pattern as earlier described. The preparation for serum CHOL followed similar method, but this time,CHOL infinity reagent was used. The plate was left for 10 minutes at room temperature to allow for full colour development before absorbance readings were taken at Abs 500nm using Epoch 2 Microplate Spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA) (table 3 and 4). As can be expected, the plastic microliter plate had background absorbance as well as the reference blanks. Due to this systemic error, the readings were subtracted from the blank, hence correcting the other values. The absorbance readings ofTAG and CHOLwere mean values. Using Microsoft office Excel 2010 (Microsoft Corporation, Redmond, USA), the standard curve forTAG and CHOL concentrations were plotted against their mean absorbance at Abs 500nm.

RESULTS

Table 1. Content of experimental diet

Composition	g/100 g
Corn meal	25
Fish meal	20
Soybean extract	10
Wheat bran	16
Rice flour	20
Mineral mixture	3
Vitamin mixture	2
Sugar	2
Vegetable oil	2

Table 2. Chemical analysis of experimental diet

Composition	g/100 g	mg/g
Protein	22.86	
Fat	12.95	
Moisture	12.33	
Ash	9.07	
Dietary fibre	15.45	
Cholesterol		0.96

Table 3. Mean absorbance (Abs 500nm) at different concentrations of TAG

Duplicate	Volume of standard µl	Water µl	[TAG] mmol/l	Volumetransferred To TAG infinity reagent µl	Volume of TAG infinity reagent µl	Mean Absorbance (500nm)
1,2	0	50	0.00	5	95	0.000
3,4	3	47	0.60	5	95	0.080
5,6	5	45	1.00	5	95	0.175
7,8	10	40	2.00	5	95	0.250
9,10	20	30	4.00	5	95	0.520
11,12	30	20	6.00	5	95	0.840
13,14	40	10	8.00	5	95	1.097
15,16	50	0.0	10.0	5	95	1.200

The serum TAG andCHOL concentrations were obtained either through extrapolation of the graphs or by calculation using the equation of graphs y = 0.129x for TAG and y = 0.082x for CHOL. The serum [TAG] showed no significant increase from the 4 to 6 months old male mice (5.47 to 5.70mmol/L). However, there was a decrease in the 12 month old male mice. There was significant increase in the serum TAG level from 2 to 6 months female mice (2.23 – 6.83mmol/L), but recorded a slight decrease in its level (5.5mmol/L) in the 12 months old female mice.

Table 4. Mean absorbance (Abs 500nm) at different concentrations of CHOL

Duplicate	Volume of standard µl	Water µl	[CHOL] mmol/l	Volumetransferred To CHOL infinity reagent µl	Volume of CHOL infinity reagent µl	Mean Absorbance (500nm)
1,2	0	50	0.00	5	95	0.000
3,4	3	47	0.60	5	95	0.041
5,6	5	45	1.00	5	95	0.140
7,8	10	40	2.00	5	95	0.180
9,10	20	30	4.00	5	95	0.409
11,12	30	20	6.00	5	95	0.500
13,14	40	10	8.00	5	95	0.611
15,16	50	0.0	10.0	5	95	0.826

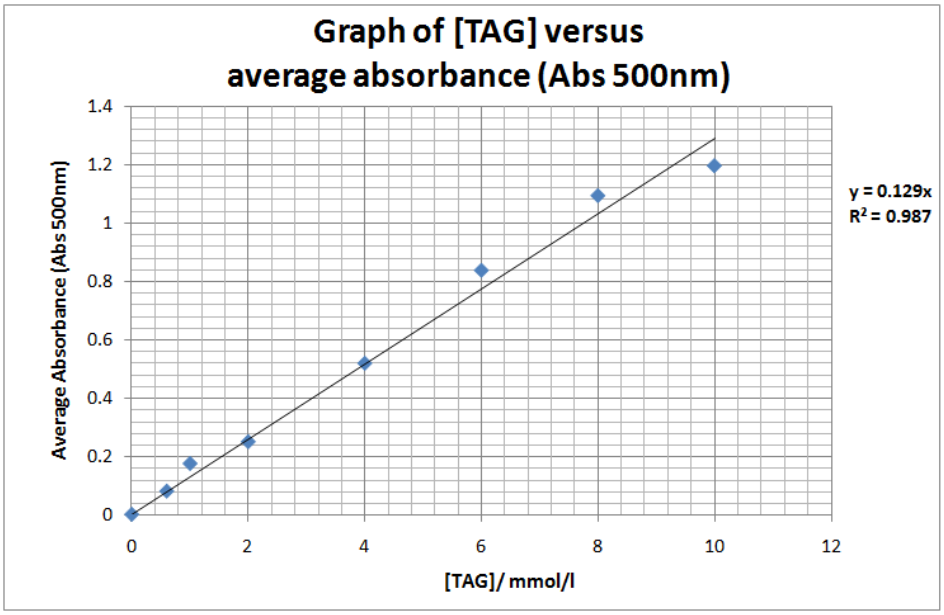

Figure 2. Graph of mean absorbance (Abs 500nm) plotted against [CHOL]

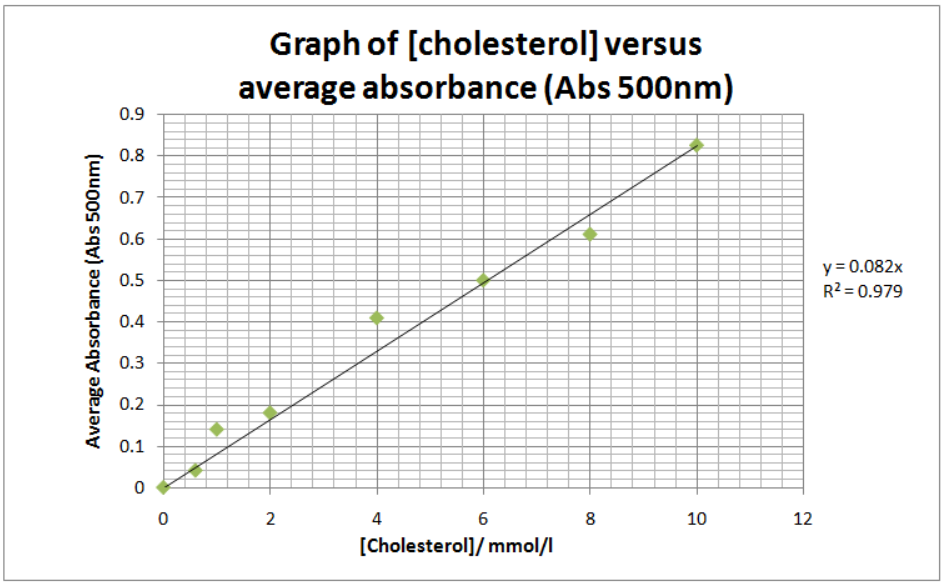

Figure 2. Graph of mean absorbance (Abs 500nm) plotted against [CHOL]

There was a steady increase in [CHOL] in male mice with increasing age (2 to 12 months), but with marked increase from the 2 to 6 months old mice. Similar trend was observed in the [CHOL] levels in female mice. There was also an increase in [CHOL] level as the female mice get older. In the 2 months old male and female mice, the [CHOL] level was 3.88mmol/L and 4.20mmol/L respectively, as well as the 2 months old female mice (2.23mmol/L of TAG).

These values were below the 5.2mmol/L and 2.23mmol/L benchmark for [CHOL] and [TAG], beyond which point the organism will be at risk.

Statistical analysis

Results are expressed as means ± standard error of mean (SEM), and n shows the number of mice used for analysis (n=20).

Table 5. Mean concentrations of mice serum TAG

MALE		
Age / months	Mean absorbance (500nm)	[TAG] /mmol/l
2 (6)	0.705	5.47
6 (6)	0.735	5.70
12 (8)	0.568	4.40
FEMALE		
Age / months	Mean absorbance (500nm)	[TAG] / mmol/l
2 (6)	0.288	2.23*
6 (6)	0.881	6.83
12 (8)	0.773	5.99

Table 6. Meanconcentrations of mice serum CHOL

MALE		
Age / months (number)	Mean absorbance (500nm)	[CHOL] /mmol/l
2 (6)	0.318	3.88*
6 (6)	0.675	8.23
12 (8)	0.687	8.38
FEMALE		
Age / months	Mean absorbance (500nm)	[CHOL] /mmol/l
2 (6)	0.344	4.20*
6 (6)	0.475	5.79
12 (8)	0.546	6.66

*Not/or at low risk of CHD; values in brackets are number of animals per group.

The variables were compared by t-test and calculations performed with two - way ANOVAto compare the levels of TAG and total CHOL and in both gender. A value for $p < 0.05$ was considered to be statistically significant.

DISCUSSION

The present study tested the serum levels of TAG and CHOL in both male and female mice aged between 4 and 12 months, with body weight between 300g and 500g. Serum TAG appeared to have an initial increase in its level in the male mice and then a sudden drop in level as the male mice gets older. Wanpen*et al* (2001) has earlier reported that a similar trend in the TAG level was noticed when male rats were studied. While the reason for this change still remains unclear, it could be suggested that as the mice grow older, there would be an optimum age, beyond which the serum level of TAG would drop.However, there was a different outcome in the serum cholesterol levels as it increased steadily with age in both male and female mice. This observation was supported by a similar report (Carlson *et al.,* 1968) in which there was an increased level of serum cholesterol in male and female rats as they get older. The Fredrickson's (WHO) classification for hyperlipidaemia stipulated a normal serum TAG at <5.2mmol/L and normal CHOL at <5.2mmol/L, beyond which an individual organism is considered to be at risk for hyperlipidaemia. Hence from the outcome of the study, it can be concluded that only the two months old female mice (for serum TAG) and the two months old male and female mice (for serum cholesterol) are thought to be at less or no risk for hyperlipidaemia. Finally, it can be concluded that the age of the mice studied may have direct influence on the blood serum levels of CHOL and TAG. While the male mice appear to be at a slightly higher risk of raised cholesterol level than the female mice, there is a tendency for the female mice to risk a raised serum TAG level. In as much as genetic can play a role in individual predisposition to hyperlipidaemia, it is beyond the scope of this research to suggest that the outcome of the study could not have been affected by the genetic variations of the individual mice. Hence, further research is encouraged.

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