

Full Length Research Paper

## Effects of dietary tyrosine on serum cholesterol fractions in rats

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The present study was undertaken to measure the effects of dietary tyrosine added to rat diet on serum cholesterol levels in the rat. A total of twenty Wistar strain albino rats were fed with different doses of tyrosine enriched diets containing 0.8 g/100 g, 1.0 g/100 g and 1.2 g/100 g. After 3 weeks of experimental feeding, there was significant increase ( $p < 0.05$ ) in total postprandial serum cholesterol of rats fed with graded of tyrosine when compared with the normal control. Same trend was followed in the week 2 of the same feeding pattern. The effects of dietary tyrosine supplementation on cholesterol levels of the high density lipoprotein (HDL) fraction were comparable, but not all significant on the week 3 treatment. However, there was significant decrease ( $p < 0.05$ ) in week 2 of rats fed with the different graded doses of the tyrosine meal when compared with the normal control group. In addition, significant increase was also observed in the low density lipoprotein (LDL) as compared to the control after week 2 and 3 of tyrosine meal treatment. These results revealed that tyrosine supplementation in a physiological amount may increase cholesterol levels in the rat when added to diet, with a slow release of tyrosine during digestion.

**Key words:** Dietary tyrosine, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL).

### INTRODUCTION

Food helps humans maintain good health by providing all essential nutrients. Consuming a variety of foods in balanced proportions will prevent deficiency diseases and chronic diet-related disorders. Amino acids have many functions in the body. They are the building blocks for all body protein - structural proteins that build muscle, connective tissues, bones and other

structures, and functional proteins in the form of thousands of metabolically active enzymes (Elwes et al., 1989; Fernstrom, 2000). Amino acids provide the body with the nitrogen that is essential for growth and maintenance of all tissues and structures. Proteins and amino acids also serve as a source of energy, providing about 4 calories per gram. Aside from these general

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**Table 1.** Composition of basal diet (Growers feed, Grand Cereals LTD, Enugu).

Diet composition	Amount (%)
Crude protein	15
Fat	7
Crude fibre	19
Calcium	1.0
Phosphorus	0.35
Metabolisable energy	2550 kcal/kg

functions, individual amino acids also have specific functions in many aspects of human physiology and biochemistry (Salter, 1989).

Tyrosine is a non essential amino acid the body makes from another amino acid called phenylalanine. It is a building block for several important brain chemicals called neurotransmitters, including epinephrine, norepinephrine and dopamine (Moller et al., 1995). Neurotransmitters help nerve cells communicate and influence mood (Thomas et al., 1999). Tyrosine also helps produce melanin, the pigment responsible for hair and skin colour. It helps in the function of organs responsible for making and regulating hormones, including the adrenal, thyroid and pituitary glands (Shurtleff et al., 1994). It is involved in the structure of almost every protein in the body. It is rare to be deficient in tyrosine. Low levels have been associated with low blood pressure, low body temperature and an underactive thyroid (Sole et al., 1985; Deijen and Orlebeke, 1994; Deijen et al., 1999).

Cholesterol is insoluble in the blood; it must be attached to certain protein complexes called lipoproteins in order to be transported through the bloodstream. Low-density lipoproteins (LDLs) transport cholesterol from its site of synthesis in the liver to the various tissues and body cells, where it is separated from the lipoprotein and is used by the cell (Gordon et al., 1989). Cholesterol attached to LDLs is primarily that which builds up in atherosclerotic deposits in the blood vessels hence LDLs are termed 'bad' cholesterol (Olson, 1998). High-density lipoproteins (HDLs) may possibly transport excess or unused cholesterol from the tissues back to the liver, where it is broken down to bile acids and is then excreted thereby serving to retard or reduce atherosclerotic buildup, thus, it is termed 'good' cholesterol (Lewis and Rader, 2005).

When we take a close look at the diet of depressed people, an interesting observation is that their nutrition is far from adequate. They make poor food choices and select foods that might actually contribute to depression. Salter (1989) has reported that dietary tyrosine aids to reduce stress among troops. Recent evidence by Tumilty et al. (2011) suggests that oral tyrosine supplementation improves exercise capacity in athletes. A lot of research

has been carried out on tyrosine. This study is aimed at determining the effects of dietary tyrosine added to rat diet on serum cholesterol levels in rats.

## MATERIALS AND METHODS

### Reagents

L-tyrosine used in this study was sourced from Sigma Aldrich USA (Lot# SI bb7526V). All other chemicals and reagents used are of analytical grade.

### Animals

A total of twenty (20) Wistar strain albino rats weighing between 134 -180 g bred in the animal house of the Department of Zoology, University of Nigeria Nsukka, were used in the experiment. The animals were kept under room temperature and were acclimatized in the new environment for a period of 7 days and fed non purified diet with the following diet composition as shown in Table 1 before the addition of the dietary tyrosine. The use of animal for research studies was ethically approved by the authorized committee of animal ethics, Department of Biochemistry, University of Nigeria, Nsukka.

### Experimental design

After the acclimatization period, a total of 20 rats were used for the experiment and was divided into four groups consisting of four rats in each group as follows: Group 1: Control were fed basal diet; Group 2: Rat fed with 0.8 g/100 g of tyrosine diet; Group 3: Rat fed with 1 g/100 g of tyrosine diet; Group 4: Rat fed with 1.2 g/100 g of tyrosine diet.

The treatment lasted for twenty one (21) days in which blood samples of the rats were analysed on day 0, 14, and 21. The animals were anaesthetised and blood sample were collected through ocular puncture for biochemical analysis. Blood samples were received into clean dry centrifuge tube and left to clot at room temperature, then centrifuged at 33.5 g for 15 min to obtain the serum. The serum was carefully separated into dry clean Wassermann tubes, using a Pasteur pipette and kept frozen at (-20°C) until estimation of some biochemical parameters.

### Cholesterol determination

Total cholesterol was determined according the method of Abell et al. (1952) as outlined in commercially available kits. Determination of the concentration of the serum total HDL and LDL was determined as described by Kameswara et al. (1999).

### Cholesterol determination

The method of Abell et al. (1952) was followed.

### Principle

Cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxidase and 4-aminoantipyrine in the presence of phenol and peroxidase.

### Test procedure

Three (3) test tubes were set up in a test tube rack and labeled blank, standard and sample, respectively. To the blank, was added (10  $\mu$ l) distilled H<sub>2</sub>O, 10  $\mu$ l standard specimen was added to the standard test tube and 10  $\mu$ l sample (serum) was added to the sample test tube. To each of these test tubes was added 1000  $\mu$ l of the cholesterol reagent. It was thoroughly mixed and incubated for 10 min at room temperature (20-25°C). The absorbance of the sample ( $A_{\text{sample}}$ ) against the blank was taken within 60 min at 500 nm.

### Low density lipoprotein (LDL)

#### Principle

LDL-C can be determined as the difference between total cholesterol and the cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulphate (PVS) in the presence of polyethyleneglycol monomethyl ether.

#### Procedure

The serum samples were kept at 2-8°C. The precipitant solution (0.1 ml) was added to 0.2 ml of the serum sample and mixed thoroughly and allowed to stand for 15 min. This was centrifuged at 2,000 xg for 15 min. The cholesterol concentration in the supernatant was determined. The concentration of the serum total cholesterol as described by Kameswara et al. (1999) was used.

#### Calculation

LDL-C (mmol/L) = Total cholesterol (mmol/L) – 1.5 x supernatant cholesterol (mmol/L).

### High density lipoprotein (HDL)

#### Principle

LDL and VLDL are precipitated from serum by the action of a polysaccharide in the presence of divalent cations. Then, HDL present in the supernatant is determined.

#### Procedure

The precipitant solution, 0.1 ml was added to 0.3 ml of the serum sample and mixed thoroughly and allowed to stand for 15 min. This was centrifuged at 2,000 xg for 15 min. The cholesterol concentration in the supernatant was determined. Determination of the concentration of the serum total HDL as described by Kameswara et al. (1999) was used.

### Triacylglycerol

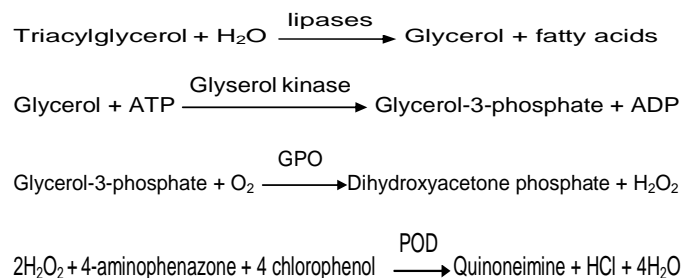
#### Clinical significance

Triacylglycerols measurements are used in the diagnosis and treatment of diseases involving lipid metabolism and various endocrine disorders e.g diabetes mellitus, nephrosis and liver obstruction.

#### Principle

The triacylglycerols are determined after enzymatic hydrolysis with

lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.



#### Method

A quantity of the sample (0.1 ml) was pipetted into a clean labeled tube and 1.0 ml of trichloroacetic acid (TCA) was added to it, mixed and then centrifuged at 250 rpm for 10 min. The supernatant was decanted and reserved for use.

The mixtures were allowed to stand for 20 min at 25°C and the absorbance of the sample and standards read against the blank was taken at 540 nm.

#### Calculation

The concentration of triacylglycerol in serum was calculated as follows:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Standard concentration (mmol/l)} = \text{mmol/l}$$

#### Statistical analysis

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 16.0. One way analysis of variance was adopted for comparison, and the results were subject to post hoc test using least square deviation (LSD). The data were expressed as mean  $\pm$  standard deviation.  $P < 0.05$  was considered significant.

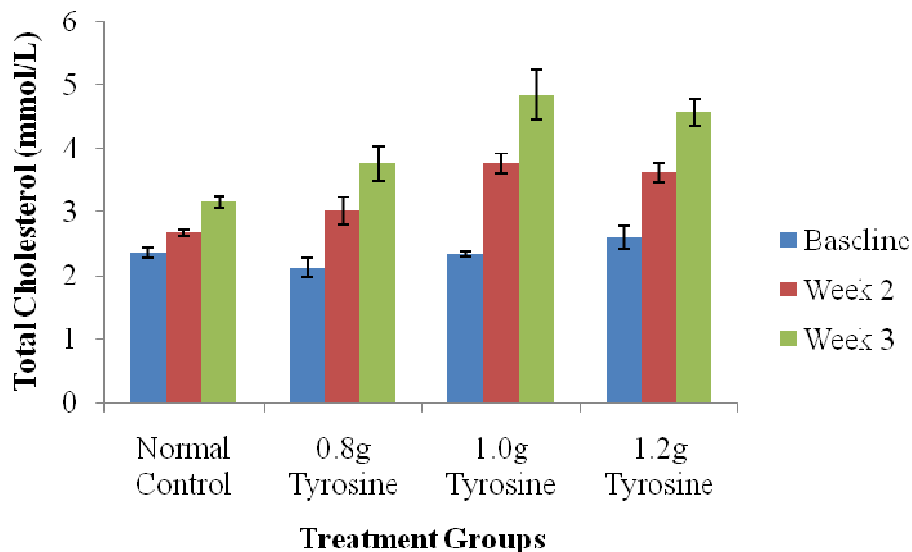
## RESULTS

### The effects of tyrosine supplemented diet on the total cholesterol levels of rats

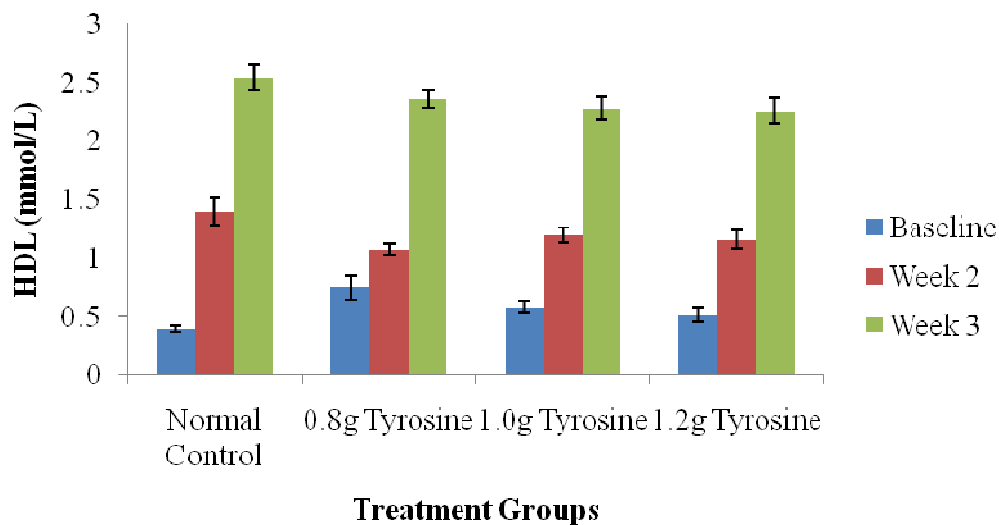
There was significant increases ( $p < 0.05$ ) in total cholesterol of week 2 of rats fed with 0.8 g/100 g of tyrosine as compared to that fed with 1.0 g/100 g and 1.2 g/100 g of the tyrosine supplemented diet. There was also a significant increase ( $p < 0.05$ ) of rats fed with 1.0 g/100 g and 1.2 g/100 g of tyrosine meal diet. The same trend was also observed in week 3 (Figure 1). This increases were not dose dependent.

### The effects of tyrosine supplemented diet on the HDL levels of rats

The bar shows the result of the HDL of rats fed with tyrosine supplemented diet. There was significant



**Figure 1.** Bar chart representing the effects of tyrosine meal diet on the total cholesterol levels of rats. Values indicates mean  $\pm$  SEM (n=4). Significance is at  $p < 0.05$ .



**Figure 2.** Bar chart representing the effects of tyrosine meal diet on the HDL levels of rats. Values indicates mean  $\pm$  SEM (n=4). Significance is at  $p < 0.05$ .

decrease ( $p < 0.05$ ) in HDL when rats fed with 0.8 g/100 g of tyrosine meal diet was compared to the normal control of week 2. There was no significant difference ( $p > 0.05$ ) in weeks 2 and 3 rats fed with 0.08 g/100 g tyrosine supplemented diet when compared with 1.0 g/100 g and 1.2 g/100 g meal diet (Figure 2).

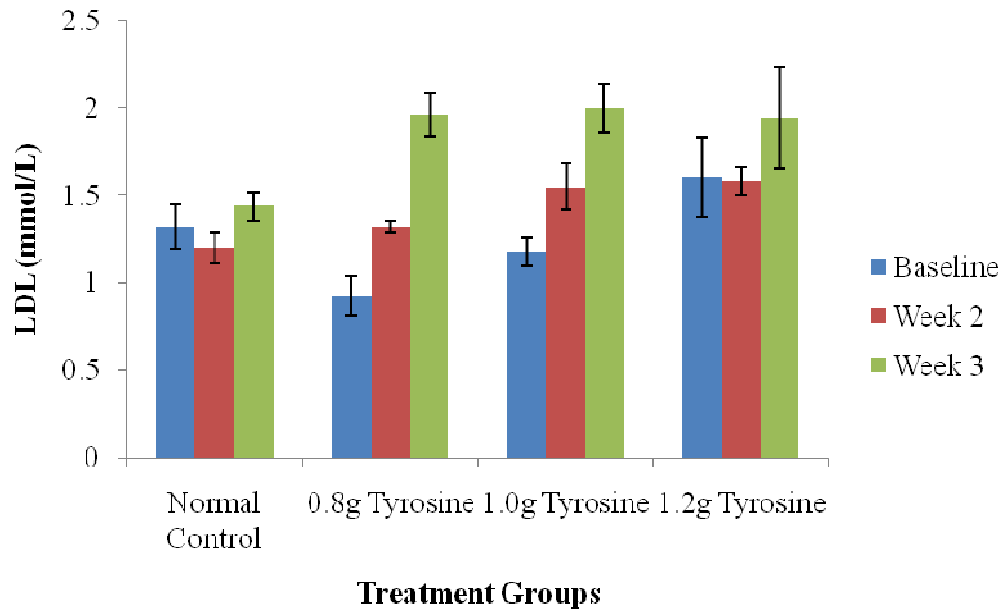
#### The effects of tyrosine supplemented diet on the LDL levels of rats

The bar shows the result of the LDL of rats fed with

tyrosine supplemented diet. There was significant increases ( $p < 0.05$ ) in week 2 of the LDL of rats fed with 1.0 g/100 g and 1.2 g/100 g tyrosine supplemented diet as compared to the control. Significant increases ( $p < 0.05$ ) was also observed in of rats fed with 0.8 g/100 g, 1.0 g/100 g and 1.2 g/100 g tyrosine meal diet at week 3 when compared with the control (Figure 3).

#### DISCUSSION

The effects of dietary tyrosine on serum lipid profile were



**Figure 3.** Bar chart representing the effects of tyrosine meal diet on the LDL levels of rats. Values indicadates mean  $\pm$  SEM (n=4). Significance is at  $p < 0.05$ .

analyzed in this study. The animals fed with 0.8 g/100 g, 1.0 g/100 g and 1.2 g/100 g of tyrosine supplemented diet showed significant increases ( $p < 0.05$ ) in total cholesterol of week 2 of rats as compared to that fed with 1.0 g/100 g and 1.2 g/100 g of the tyrosine supplemented diet. There was also a significant increase ( $p < 0.05$ ) in rats fed with 1.0 g/100 g and 1.2 g/100 g of tyrosine meal diet. The same trend was also observed in week 3 as reflected in this work. Increased total cholesterol concentration when fed with high tyrosine meal diet allows one to investigate the effects of treatment in dietary protein source (tyrosine), on this fraction. These result followed similar trend in the rise of total cholesterol levels using other sources of proteins (soy protein and casein) as reported by other researchers (Forsythe et al., 1980; Nagata et al., 1982c; Van der Meer, 1983; Van der Meer et al., 1985). Our result showed significant elevation of cholesterol in the serum of experimental rats as compared to the control. Cholesterol is synthesized in the liver, this result demonstrate the ability of tyrosine to influence liver metabolism towards increased synthesis of lipids. The high levels of cholesterol may be due to a number of factors such as the increased availability of fatty acids for esterification (Bopama et al., 1997), reduced catabolism of LDL, inhibition of tissues, activation of acetyl-CoA caboxylase (McCarthy, 2001) and production of triglycerides precursors such as acetyl-Coa and glycerol phosphate (Fatiha et al., 2014). The elevation of cholesterol in the liver might suggest that the dietary tyrosine supplement contain ingredients capable of enhancing the activities of hepatic lipogenic and cholesterologenic enzymes, such as malic enzyme, fatty

acid synthase, glucose 6-phosphate dehydrogenase and HMG-CoA reductase (Vega et al., 2003) which are required for cholesterol synthesis.

Atherosclerosis is characterized by liver disease without alcohol which is manifested by the significant lipid deposition in hepatocytes of liver parenchyma as a single macro-vesicular steatohepatitis and can develop fibrosis in cirrhosis which is increasingly recognized as an important cause of mortality (Angulo and Lindor, 2002).

There was significant decrease ( $p < 0.05$ ) in HDL in rats fed with 0.8 g/100 g of tyrosine supplemented diet as compared to the control of week 2. There was no significant ( $p > 0.05$ ) increase in weeks 2 and 3 of rats fed with 0.8 g/100 g tyrosine meal diet when compared with 1.0 g/100 g and 1.2 g/100g supplemented diet. In the present study, in the rats fed higher doses of tyrosine serum HDL continued to have higher amount as in the case of total cholesterol as compared to the normal rats fed supplemented tyrosine diet during the 21 days study. These observations was in constrast with the work of Nagaoka et al. (1990a) who reported that prolong administrations of dietary tyrosine caused hypercholesterolemia in male Wistar rats. There was significant increases ( $p < 0.05$ ) in week 2 of the LDL of rats fed with 1.0 g/100 g and 1.2 g/100 g tyrosine supplemented diet when compared with the control. Significant increases ( $p < 0.05$ ) was also observed in week 3 of rat fed with 0.8 g/100 g, 1.0 g/100 g and 1.2 g/100 g tyrosine supplemented diet when compared with the control. Excess dietary tyrosine or certain xenobiotics increases HDL and LDL cholesterol (Kato and Yoshida, 1981; Nagaoka et al., 1985b). In the hypercholesterolemia

seen following feeding of cholesterol, serum LDL cholesterol was relatively increased (Quazi et al., 1983). It seems likely that cholesterol metabolism is quite different in rats fed higher doses of tyrosine from those of rats fed cholesterol-containing diet. Many investigators have already suggested that the hypercholesterolemia seen in feeding dietary tyrosine (Qureshi et al., 1978; Solomon and Geison, 1978) was mediated by enhancement of of cholesterol synthesis in the liver.

## Conclusion

The alterations in the serum cholesterol reveals that long term feeding of dietary tyrosine may escalate cholesterol accumulation in the adipose tissues.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

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