Blood concentrations of triglyceride, cholesterol and hdl-cholesterol

Determination of triglyceride, cholesterol and high-density lipoprotein cholesterol levels in serum using enzymatic reactions and spectrophotometry.

Key words: Exercise, Coronary heart disease, Lipoprotein lipase, LDL-cholesterol.

Abstract

The concentrations of triglyceride, total cholesterol, HDL-cholesterol and LDL-cholesterol in a human serum sample were determined using a standard sample of known concentration and an unknown sample to verify the accuracy of the method. A series of enzymatic reactions were used combined with spectrophotometry. The sample was found to have a ‘ desirable’ level of triglyceride, total cholesterol and LDL-cholesterol and a reasonably high HDL-cholesterol concentration. These results are healthy and indicate a low risk of coronary heart disease and atherosclerosis due to lipid factors. Diet and exercise in particular may explain the concentrations.

Introduction

Fat is needed as it provides a fuel source for energy and is important in the absorption of fat-soluble vitamins. Fats are also important for cell membrane structure, hormone synthesis, insulation and the protection of vital organs. Christian and Greger (1994). Dietary lipids include triglycerides, phospholipids and cholesterol. Triglycerides are produced internally from carbohydrates. The levels of triglycerides in human serum can be used to diagnose and treat diseases involving lipid metabolism or various endocrine disorders. Hypercholesterolemia in particular, has been associated with

coronary heart disease. McGill et al (2001). Therefore, it is important to check and monitor blood cholesterol levels. High levels of LDL-cholesterol and total cholesterol have been associated with atherosclerosis in a number of clinical trials. Ingelsson et al (2007). Whereas high levels of serum HDL-cholesterol levels offer protection from heart disease. (AHA Commitee Report 1982). The concentration of HDL-cholesterol is influenced by heredity, gender, exercise and diet. Saturated fats increase LDL-cholesterol and unsaturated fats increase HDL-cholesterol. Therefore, it is important not only to monitor the total cholesterol or triglyceride concentrations in the blood, but also to analyse the types of fats to see whether they are beneficial or detrimental to health. The recommendation is for adults over 20 to have their fasting lipoprotein profile measured every 5 years so that treatment can then take place if necessary.

Method

A fasting venous sample was collected and centrifuged to give a serum sample. Three tests were conducted to measure the triglyceride concentration, the total cholesterol and the HDL-cholesterol concentration in the serum sample. A series of enzymatic reactions (appendix A) were used to prepare the serum for testing both the triglyceride and total cholesterol levels. A pipette was used to add the different solutions to the cuvettes, see appendix B for the solutions and quantities. A red colour was produced from the reactions and a spectrophotometer was then used to analyse the absorbance of the samples. These were measured against a standard of known concentration, used as a control from which the sample concentration could be calculated and an unknown sample which was used to ensure the

method was conducted accurately. The HDL-cholesterol concentration was found by firstly precipitating the serum sample (appendix B) to remove the LDL, VLDL and chylomicrons and then centrifuging. Spectrophotometry was also used to analyse the sample, an unknown and a standard so that the concentration of HDL-cholesterol could be calculated. The LDL-cholesterol concentration was calculated from the results. For reliability the absorbencies for all the experiments were repeated three times. See appendix B for a full method of all techniques used.

Results

See appendix C for the raw data.

Triglyceride Concentration

Average Absorbency (arbitrary units)

Concentration of triglyceride (mg/dl)

Sample

0. 132

88. 24

Unknown

0. 121

90. 06

Total Cholesterol Concentration

Average Absorbency (arbitrary units)

Concentration of total cholesterol (mg/dl)

Sample

0. 187

125. 21

Unknown

0. 230

154. 01

HDL-cholesterol concentration

Average Absorbency (arbitrary units)

Concentration of HDL-cholesterol (mg/dl)

Sample

0. 236

57. 64

Unknown

-0. 007*

-1. 64*

LDL-cholesterol concentration of sample: 49. 92mg/dl

*After repeat, please see appendix C for original data.

Appendix B details the equations used to calculate the concentrations from the average absorbencies.

The graph shows the concentrations of triglyceride in the sample and unknown solutions. The line at 150mg/dl represents the threshold for ‘ borderline high’ and the line at 200mg/dl for ‘ high’ on the recommendations for lipid profile measurements. This shows more clearly how both results are significantly below this point and therefore suggest good health.

The above graph demonstrates the recommendations for the total cholesterol in the serum samples. The ‘ borderline high’ is the line at 200mg/dl and the ‘ high’ at 240mg/dl. Both the sample and unknown are significantly below 200mg/dl.

The result for the HDL-cholesterol for the sample was between the recommendations for ‘ low’ and ‘ high’ concentrations. However as the graph shows, the result is nearer to the ‘ high’ level which is healthy. The unknown sample produced a negative result after being repeated which is an impossible result. This is examined further in the discussion.

The LDL-cholesterol concentration is calculated from the HDL-cholesterol subtracted from the total cholesterol. The recommendation for this was above 130mg/dl for ‘ borderline high’ and above 160mg/dl for ‘ high’. The sample concentration was considerably below these values.

Discussion

There are lipid profile recommendations for good health regarding the concentrations of triglyceride, total cholesterol, HDL-cholesterol and LDL-cholesterol in human serum samples. (Appendix D)

The result for the sample triglyceride concentration was 88. 24mg/dl. This is significantly below the levels that would be considered potentially threatening to health. The participant was a young healthy female who exercised regularly and had a balanced diet. Research has found that exercise can decrease serum triglyceride levels. Couillard et al (2001)

If the levels of triglyceride in the blood are too high it is detected by the liver. This can be due to either a high intake of dietary fat or carbohydrates, which increase the blood glucose concentration. If either of these are too high the liver will release insulin from beta cells. This promotes the formation of triglycerides from carbohydrates, increasing the concentration of triglyceride in the blood and the storage of triglycerides as adipose tissue. Large quantities of adipose tissue are detrimental to health which is why it is particularly important to monitor triglyceride concentration. (Martini, 2001)

The total cholesterol in the sample was 125. 21mg/dl and the HDL-cholesterol was 57. 64mg/dl. Evidence has shown that exercise decreases total cholesterol whilst increasing the HDL-cholesterol concentration. This is not completely due to weight loss from exercising, as lowered total cholesterol and triglycerides with increased levels of HDL-cholesterol have also been found in participants whose weight remained constant in studies. Huttunen et al (1979). Therefore other reasons for these results have been

suggested. It is possible that the contracting, skeletal muscle takes up triglycerides. Also, exercise has been shown to influence the activity of the enzyme lipoprotein lipase. A part of HDL-cholesterol is derived from the catabolism of VLDL-cholesterol by this enzyme. This could account for the decrease in triglyceride and increase in HDL-cholesterol due to exercise. Huttunen et al (1979)

The result for the total cholesterol was ‘ desirable’. The HDL-cholesterol concentration was reasonably high which is favourable. Finally, the LDL-concentration was also ‘ desirable’ at only 49. 92mg/dl as the recommendations for ‘ desirable’ are less than 130mg/dl. This is demonstrated more clearly by the graph in the results section that shows that the sample concentration was significantly below the risk level.

All these results were consistent with the participant being a healthy individual with a low risk of coronary heart disease and atherosclerosis from lipid factors. They show that the participant had a low total cholesterol and triglyceride serum concentration. This was likely to be because the participant was a young, healthy individual with a balanced diet and regular participation in sport. As shown above, exercise can decrease cholesterol and triglyceride concentrations. The participant also had a high level of HDL-cholesterol which is beneficial to the body.

The unknown sample was used to indicate whether the method was conducted accurately and producing precise results. The results for the unknown samples should have been 95. 6mg/dl for the triglyceride concentration, 158mg/dl for the concentration of total cholesterol and 2.

37mg/dl for the HDL-cholesterol concentration. This means that there was a percentage error of 5. 79% for the triglyceride result and of 2. 53% for the total cholesterol result. The result for the HDL-cholesterol came out as a negative value, which is an impossible value. However the actual concentration of the unknown sample was a very low number, therefore taking into account a percentage error of approximately 10% this may explain the negative result. This means that all the results for the sample serum concentrations may also be incorrect by approximately 10%.

There are many possible reasons for the slight inaccuracy of the results. It is possible the solutions in the cuvettes were not mixed thoroughly enough so the reaction did not fully occur. Another reason could be that although care was taken the cuvettes may have been contaminated, affecting the amount of light being absorbed. Also most of the enzymatic reactions had to have a period of incubation for the reaction to occur. It is possible when the absorbance values were recorded that the reactions were not fully completed, which would have affected the results.

Conclusion

In conclusion, the concentrations of triglyceride, total cholesterol and HDL-cholesterol in the blood are important factors in indicating high risk of coronary heart disease and atherosclerosis. Therefore, it is important to monitor these levels and compare them to the recommended concentrations. Diet and exercise can both be used to decrease levels of triglyceride, total cholesterol and LDL-cholesterol, whilst increasing HDL-cholesterol. Therefore, it is important to have a healthy balanced diet and to

regularly exercise to minimise the risk factor, from lipid levels, of heart disease.