STUDIES ON THE RELATIONSHIP BETWEEN BLOOD CHOLESTEROL AND THE BASAL METABOLIC RATE OF COLLEGE WOMEN

by

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INTRODUCTION

This research was done in conjunction with the Department of Foods and Nutrition which was making a survey of the basal metabolic rates of Kansas College women. The purpose of the survey was to determine whether there was a correlation between the blood cholesterol level and the basal metabolic rate. If an inverse correlation existed between blood cholesterol levels and basal metabolic rates, as indicated in the literature, blood cholesterol determinations could be used as an index of thyroid activity.

REVIEW OF THE LITERATURE

Thomas (31) reports normal blood cholesterol levels as follows: total cholesterol, 130-275 mg per 100 cc (mean of 210), cholesterol ester, 75-255 mg per 100 cc (mean of 140), free cholesterol, 40-140 mg per 100 cc (mean 70) and that the ratio of cholesterol ester to the total cholesterol is 0.7. Thomas has found that in cases of hyperthyroidism the blood cholesterol is normal or in the low normal range. In cases of hypothyroidism the blood cholesterol (total, free and esterified) was found to rise. The cholesterol level was reduced on treatment with thyroxine and rose again when treatment was discontinued. Under normal and abnormal conditions, wide individual variations in cholesterol levels were found.

In cases of hyperthyroidism, potassium iodide therapy did not reduce lipid levels.

Peters and Mann (21, 22) report that even though blood cholesterol rises when the thyroid is removed and falls when an active thyroid preparation is given, a normal concentration of cholesterol may be found in either hypo or hyper thyroid patients, since the rise or fall of cholesterol in these disorders is roughly related to the patient's normal level of cholesterol. These investigators found that the ratio of free cholesterol to total cholesterol was unaffected by the thyroid gland. This ratio was found to be constant, .24-.32 mg per 100 cc (mean of .28).

Rourke et al. (27) from case records of the Massachusetts General Hospital report the normal blood cholesterol values for total cholesterol to be 150 to 230 mg per 100 cc of plasma with the ester making up 65 per cent of the total.

Foldes and Murphy (6, 7) found the ratio of cholesterol ester to the total cholesterol to be increased in cases of hypothyroidism. The ratio shifts toward normal with treatment. Normal values for the ratio were determined to be .68±.06, hyperthyroid values .62±.33 with a rise to .69±.05 on treatment. The basal metabolic rates were from +33 per cent to +52 per cent before treatment and +18 per cent to -22 per cent after treatment. In hypothyroid cases the ratio of cholesterol ester to total cholesterol was found to be .73±.06 before treatment and .72±.06 with a significant increase in total lipid value. The basal metabolic rate ranged from -40 per cent to +3 per cent before treatment and -13 per cent to +11 per cent after treatment.

Peters and Mann, and Foldes and Murphy report the ratio of total cholesterol to phospholipid phosphorus is the most constant of the lipid ratios.

Forbes et al. (8) report the elevation of the readily extractable fraction of the blood cholesterol in cases of hypothyroidism. This is a small, but constant fraction amounting to about 40 per cent of the free cholesterol. It appears to be loosely bound, if bound at all, to a protein. The normal value for this fraction is from 12-37 mg per 100 cc. In four cases of hypothyroidism, diagnosed by a slightly low basal metabolic rate and slightly high total cholesterol, values were 40, 43, 49, and 58 mg per 100 cc, respectively.

Jennings et al. (12) suggest establishing a range of blood cholesterol levels corresponding to normal basal metabolic rate for each patient. This chart would help in controlling the dosage of the material used for treatment and eliminate the necessity for so many basal metabolic rates.

Beutel (2) reports a low blood cholesterol in cases of hyperthyroidism. Irradiation increased the blood cholesterol and upon further irradiation it became nearly normal. In rare cases of hypercholesteremia in hyperthyroidism the cholesterol was lowered after irradiation. The changes in blood cholesterol were reported a delicate indicator of the action of irradiation in hyperthyroidism.

Abel-Proto and Estefan (1) report that in thyroid deficiency accompanied by a low basal metabolic rate, the blood cholesterol is high (180-460 mg per 100 cc). Administration of thyroid, in sufficient quantities to raise the basal metabolic rate, decreased the cholesterol levels to nearly normal.

Pillar (24) reports cholesterol values of 190-210 mg per 100 cc in cases of hyperthyroidism and an Iodo-Lipermic Index of .0121 to .0156. The Iodo Lipermic index is the ratio of total cholesterol multiplied by the iodine absorbed to the fatty acids present.

Tanhauser (29) gives the following values for normal blood cholesterols: total 150-260 mg per 100 cc; free cholesterol, 40-70 mg per 100 cc; and cholesterol ester, 70-75 mg per 100 cc. In cases of hypothyroidism, the serum cholesterol is elevated though the reason for this is not known. Thyroid medication reduces cholesterol levels of hypothyroid patients. In cases of hyperthyroidism, blood cholesterol is low normal or subnormal. In both hypo and hyper thyroidism, the ratio of cholesterol ester to total cholesterol is normal.

Muller (19) cites the work of Epstein and Lande in 11 cases of hyperthyroidism. The basal metabolic rate ranged from +10 to +15. Six cases had normal cholesterol levels, four showed increased blood cholesterol and one was low. In

Muller's review, most all cases of myxedema had high cholesterol and low basal metabolic rate. The basal metabolic rate in some cases of thyroid diseases does not always correspond to the severity of the condition as observed clinically since the damage outlasts the basal metabolic rate elevation. Several workers at the Mayo Clinic disagree with this inverse ratio, but the number of cases they studied was quite small.

In large scale studies of hyper thyroid cases, support was given to the inverse ratio between basal metabolic rate and blood cholesterol. The decreased cholesterol was not due to undernutrition which is common in cases of hyperthyroidism. In 47 cases of hyperthyroidism, Mason and co-workers found cholesterol from 71-183 mg per 100 cc with an average basal metabolic rate of +57. In cases of thyrotoxicosis, no definite correlation was found, though some workers claim it exists.

Variations in blood cholesterol in thyroid diseases may be associated with different amounts of cholesterol secreted in the bile and the secretion of this substance in tho intestine. In hyperthyroidism cholesterol excretion in the bile has been shown to be increased, while it is decreased in myxedema (hypothyroidism). Another theory is that cholesterol is a part of a general lipopenia. Still another viewpoint is that of faulty fat transportation and interference with esterification. This is supported by the number of times hypatitis appears with hyperthyroidism.

Muller also found that, though there are wide variations in blood cholesterol levels among individuals, the value for the individual remains constant. The value is not affected by diet or menstrual cycle. Some of the work of others cited in Muller's review does not agree with this. Mann and Gildea found that diet does affect cholesterol levels and that cyclic changes in males exceed those in females. Okey and Boyden's work is cited in which they found a slight premenstrual rise in cholesterol followed by a distinct fall in combined cholesterol at the onset and during menstruation. A sudden rise in cholesterol is found during or shortly after cessation of bleeding. Muhlbock and Kaufmann have verified Okey and Boyden's work. All workers have agreed that pregnancy increased blood cholesterol about 25 per cent. Basal metabolic rates are difficult to do accurately with children. The standards used for calculations of the rate are unsatisfactory. The low O_2 consumption found in obese children make these quite inaccurate. The standards used in calculating rates for normal children do not apply to dwarf or giants.

Cookson (4) reports that blood cholesterol levels and basal metabolic rates show a fairly close inverse relationship.

Lundbaek (16) found that serum cholesterol is a sensitive indicator of thyroid function. The rise of cholesterol follows a lowering of basal metabolic rate in treatment of hyperthyroidism.

McGavac and Drekter (18) observed a more or less straight line relationship between per cent elevation of blood cholesterol and decreasing basal metabolic rate in treatment of thyrotoxic patients. Pretreatment cholesterol values were above 200 mg per 100 cc plasma and the basal metabolic rate ranged from +37 to +64 (mean +48.5). Some cholesterol values still remained high, but the basal metabolic rate dropped to a range of 0 to +15. This shows that thyrotoxicosis appears in the presence of hypercholesteremia.

EXPERIMENTAL METHOD

For the experimental procedure, two methods were tried. The first method used was the Reinhold-Sheils modification of the Myers-Wardell method. This is a reflux extraction procedure and did not prove satisfactory.

The method used was Sackett's modification of the Bloor Method for determination of cholesterol in whole blood or serum (28). The procedure is as follows: A mixture of 9 cc 95 per cent ethyl alcohol and 3 cc of ether was placed in a graduated centrifuge tube (enough alcohol and ether was mixed for each day's use). To this tube was added 0.4 cc of plasma. The tube was corked, shaken vigorously for 1 minute, and allowed to lay on its side for 30 minutes. The mixture was centrifuged rapidly for 3 minutes and the supernatant liquid poured into a small beaker. The residue in the centrifuge tube was reextracted with the alcohol-ether mixture, recentrifuged and the supernatant liquid was added to the beaker containing the first liquid decanted. The supernatant liquid was then evaporated just to dryness and the residue extracted with two 5 cc portions of chloroform. The extract wás placed in a 15 cc centrifuge tube and made up to 10 cc. To this was added a mixture of 2 cc acetic anhydride and 0.1 cc concentrated sulfuric acid. The content of the tube was mixed by inversion and kept in the dark at 25° for 15 minutes. The color developed was compared in a Klett colorimeter with a commercial standard equivalent to 0.4 mg cholesterol in 100 cc plasma. Using this standard, the cholesterol content was determined by the following formula:

$$\frac{15}{u} \times 200 = \text{mg cholesterol per 100 cc plasma}$$

u = reading of the unknown solution

The esterified cholesterol was determined by Arthur E. Terri's method (30). The extraction, centrifuging, and evaporation were carried out as outlined above for total cholesterol. After the alcohol-ether is evaporated, the cholesterol is transferred to a 15 cc centrifuge tube by extracting the residue with three 3 cc portions of petroleum ether. A 0.1 ml of digitonin reagent (1 gram of digitonin dissolved in 10 cc methanol and 10 cc water) was added to precipirate the free cholesterol and contents of the tube thoroughly mixed by occasional inversion over a period of 10 minutes. The mixture was centrifuged and the supernatant liquid was poured through a cotton plug. The cotton plug was washed with small portions of petroleum ether. The supernatant liquid and washings were evaporated to dryness. The residue was extracted with chloroform, the color produced with acetic anhydride and sulfuric acid, read in the colorimeter against the standard, and calculated as stated above for the total cholesterol.

Terri (30) found that the esterified cholesterol gave 1/5 more color than the total cholesterol.

The true cholesterol values were calculated by the following equations:

(1) True esterified cholesterol equals $\frac{4}{5}$ the apparent ester.

(2) True total cholesterol equals the apparent total $-\frac{1}{5}$ the apparent ester.

(3) True free cholesterol equals (2) - (1).

The basal metabolic rates (expressed as per cent of deviation from the Boothby and Sandiford Standards) were determined by the Department of Foods and Nutrition using the Benedict-Roth metabolism apparatus. The determinations were made just prior to the collection of the blood samples.

Each student was given a uniform breakfast (low in cholesterol) just after the basal metabolic rate was determined.

Two groups of girls (from 18 to 22 years of age), one containing 5 and another containing 7 girls were studied. Three basal metabolic rate determinations, which were part of a series of nine, were considered here because they were taken on the same day as the blood sample for the cholesterol determination. The first at the beginning of the experiment was assumed to be the normal basal metabolic rate for the individual. Two mg of sodium or potassium iodide were then administered daily for 28 days as Pittman and co-workers (25) had suggested that iodide salts by increasing the activity of the thyroid gland increases the basal metabolic rate. After iodide therapy, the basal metabolic rate was again determined. A month later the third basal metabolic rate determination was mado and a return to normal was expected. After the experiment had been started, it was decided to determine the esterified and free cholesterol as well as the total cholesterol.

RESULTS

The results of the individual cholesterol and basal metabolic rate determinations are shown in Tables 1 and 2 and graphically in Figs. 1 and 2.

Table 1. Relation of total cholesterol, cholesterol ester, and free cholesterol of the blood with the basal metabolic rate, Group I.

		Student I	II	III	IV	V
Series I (normal)	Total cholesterol	262.24	216.67	182.78	144.11	105.7
	B. M. R.	-9.1	-2.7	-8.9	-15.0	-21.0
	Cholesterol ester	104.89	144.6	105.5	105.4	90.45
	Free cholesterol	157.35	73.07	72.28	38.97	15.25
Series II on iodide	Total cholesterol	122.35	127.89	137.56	256.56	134.82
	B. M. R.	-11.3	-4.9	-13.5	-7.0	-24.5
	Cholesterol ester	91.73	74.94	71.3	79.08	99.38
	Free cholesterol	30.62	52.93	66.26	177.48	34.44
Series III 1 month after iodide	Total cholesterol	212.8	163.27	198.35	161.3	192.06
	B. M. R.	-3.3	-4.4	-2.4	-15.1	-24.1
	Cholesterol ester					
	Free cholesterol					

Cholesterol values in mg per 100 cc plasma

B. M. R. values in per cent deviation from standard

Table 2. Relation of total cholesterol, cholesterol ester, and free the blood with the basal metabolic rate, Group II.

		Student						
		I	II	III	IV	V	VI	VII
Series I (normal)	Total cholesterol	239.52	203.57	257.74	405.56	185.81	96.74[1]	117.06
	B. M. R.	-17.2	-2.7	-9.5	-13.5	-8.4	-.8	-20.3
	Cholesterol ester	163.4	115.96	157.6	115.68	158.92	70.16	79.72
	Free cholesterol	76.12	87.61	100.14	289.98	26.89	26.59	37.34
Series II on iodide	Total cholesterol	198.71	144.01	352.07[1]	191.07	104.73	179.84	272.94
	B. M. R.	-18.1	-7.7	-10.7	-13.7	-13.0	-2.2	-13.6
	Cholesterol ester	128.36	111.64	130.12	86.8	79.08	125.64	96.36
	Free cholesterol	70.35	32.37	221.95	104.27	25.65	54.2	176.58
Series III 1 month after iodide	Total cholesterol	151.68	219.06	226.63[1]	223.27	299.24	216.8	222.83[2]
	B. M. R.	-16.9	-5.7	-5.3	-14.3	-6.1	-7.0	-16.0
	Cholesterol ester	134.0	95.66	128.0	135.88	198.4	162.8	161.8
	Free cholesterol	17.68	123.39	98.63	87.39	100.84	54.0	61.75

Cholesterol values in mg per 100 cc plasma
B. M. R. values in per cent deviation from standard

[1] Sample showed hemolysis.
[2] A low fat diet (essentially no butter) for 3 weeks preceding Series III.

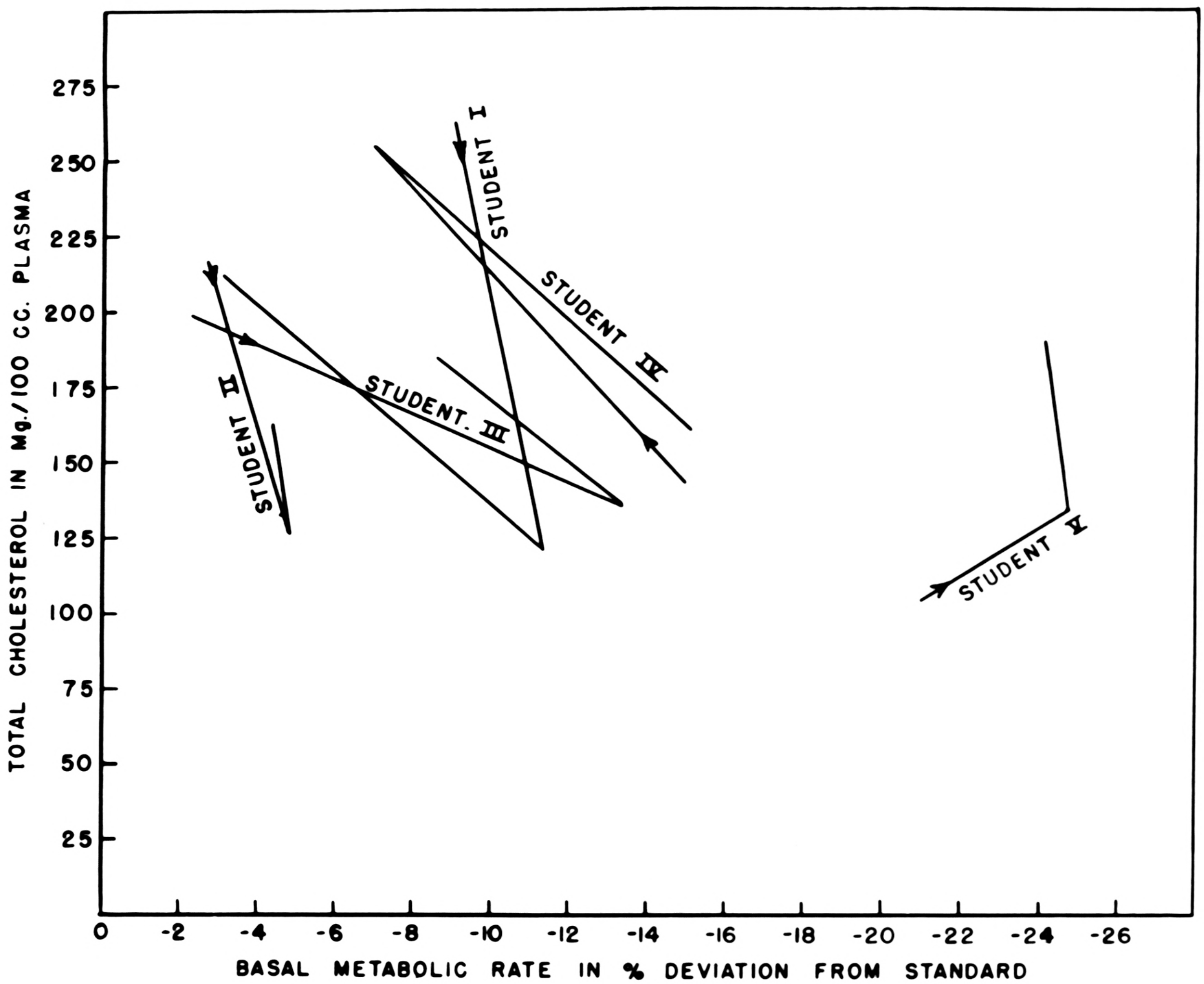

FIG. I THE RELATION OF TOTAL BLOOD CHOLESTEROL AND THE BASAL METABOLIC RATE, GROUP I

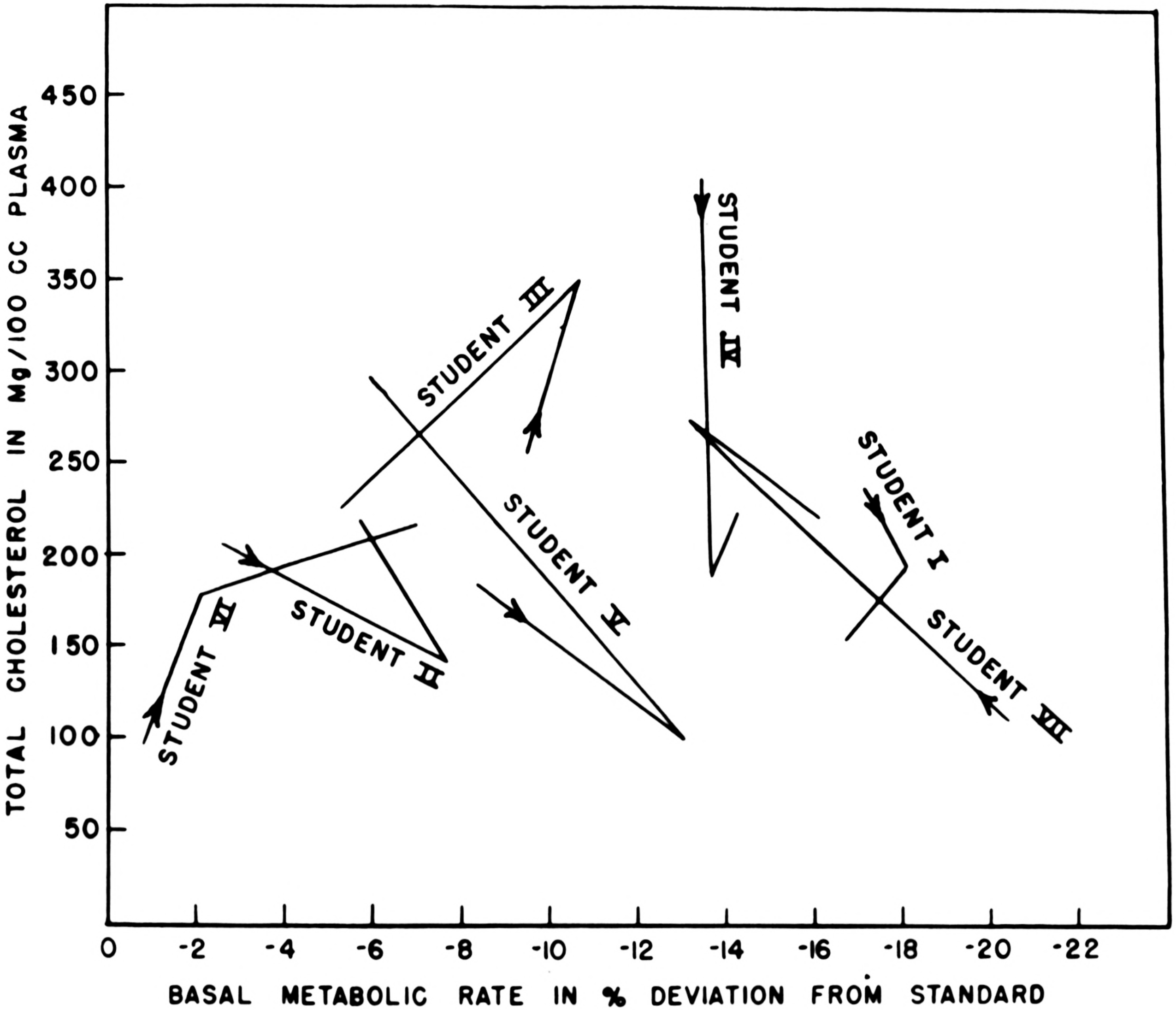

FIG. 2 THE RELATION OF TOTAL BLOOD CHOLESTEROL AND THE BASAL METABOLIC RATE, GROUP II

DISCUSSION

Little correlation can be seen in the experimental data as far as an inverse relationship between total blood cholesterol levels or the free or esterified fraction and the basal metabolic rate. This correlation is even less than that found by Cookson (4) who states that the ratio of the basal metabolic rate and blood cholesterol is not so close that cholesterol determinations should be used to replace basal metabolic rate determinations.

The change in total cholesterol values may be due to a change in either the free or the esterified fraction; i.e., the change in total cholesterol is not due to a consistent change in one fraction. This is shown in Tables 3 and 4.

The fact that the subjects were students, and less easily controlled than laboratory animals will explain, to some degree, the wide variation of results. This is in agreement with Muller (19) who found there were wide variations in blood cholesterol levels among individuals.

The literature cites several factors other than thyroid conditions which might cause a variation in cholesterol levels. These factors are: (1) the adrenocorticotropic hormone, (2) insulin, (3) sex, (4) age, (5) diet, especially one high in lipids.

Mason (17) found a sharp decrease in free plasma cholesterol, but no significant change in the ester upon adminis-

Table 3. Analysis of Table 1.

Student	Series I	Series II	Series III	Correlation of total cholesterol & B.M.R.
I	Free cholesterol high	Free cholesterol low		Direct
II		Free cholesterol high		Direct
III	Free cholesterol high	Free cholesterol high	Free cholesterol high	Good Direct
IV	Ester high	Free cholesterol high		Good Direct
V	Ester high	Ester high	Ester high	Inverse to Direct

Table 4. Analysis of Table 2.

Student	Seriss I	Series II	Series III	Correlation of total cholesterol & B.M.R.
I		Ester low	Ester high	Inverse to Direct
II	Ester low	Ester high	Ester low	Direct
III		Ester low	Ester high	Fair Inverse
IV	Free cholesterol high	Free cholesterol high		Neither
V	Free cholesterol low	Free cholesterol low		Good Direct
VI	Free cholesterol low	Free cholesterol low	Free cholesterol low	Good Inverse
VII	Free cholesterol low	Free cholesterol high	Free cholesterol low	Good Direct

tration of adrenocorticotrophic hormone.

Villaverde (33) found that cholesterol levels varied in all possible ways after injection of insulin into normal patients. The average values were 186.8 mg per 100 cc before injection, 195.8 one half hour after injection and 187.4 one hour after injection.

Kountz et al. (13) found that in patients from 40 to 85 years of age the females had a higher blood cholesterol (237 mg per 100 cc) than men (196 mg per 100 cc). Hypercholesteremia was accompanied by a low basal metabolic rate in women but not in men.

Gram and Leverton (11) found a significant rise in blood cholesterol with increasing age. No significant variation in basal metabolic rate with age was observed. As the students were all of approximately the same age, this factor could not account for the variations in these results.

Gough (10) found no correlation between cholesterol intake and blood levels of cholesterol.

Leenson (15) reports that diet does affect blood cholesterol levels. After heavy feeding of carbohydrate, a significant rise in blood cholesterol was found. A significant drop was observed after heavy protein feeding. The very high cholesterol value of student IV, Group II, Series I apparently had nothing to do with diet. Her diet was checked and no food with high content of free cholesterol had been eaten. This student as well as student III of Group II, Series II and Student V of Group II, Series III, who also showed abnormally high cholesterol values, were near the end of their menstrual cycle which may partially explain these high values. This is in agreement with Muller's (19) review of the work of Okey and Boyden. This might also affect some of the values for other students in Group II who were near the end of their menstrual cycle.

Bose et al. (3) report that in starvation the blood cholesterol is quite low.

Favarger (5) found that high fat diets decrease esterification of cholesterol thus leading to a low ester fraction. It was concluded that cholesterol esterification plays a part in the transportation of fatty acids.

Villaverde and Vidal (34) found that after injection of 100,000 units of vitamin A, the blood cholesterol did not indicate hypercholesteremia. The fact that the blood cholesterol level varied less 30 minutes after A injections and salt less 60 minutes later is accepted as evidence that vitamin A somehow regulates cholesteremia.

Van Bruggen and Straumfjord (32) gave daily supplements of 100,000 units of vitamin A and found that in 24 months the supplemented group had cholesterol values 11 per cent higher than controls and at the end of 36 months the values were 22 per cent above the controls. Both the free and ester fractions increased leaving the ratio of free cholesterol to total cholesterol the same. The values returned to normal six months after supplements were discontinued.

Pantaleon (21) reports that in the normal individual the ratio of free cholesterol to cholesterol ester is quite constant at about 0.5. The results shown in Tables 3 and 4 did not substantiate this statement, particularly students I, III, IV, and V in Group I and students I, II, III, IV, V, VI and VII in Group II.

Gigon and Noveraz (9) found that in the normal person the ratio of cholesterol ester to total cholesterol is about 0.76. There are numerous instances in which the results do not verify this statement but the relationship is closer than between the free and esterified fractions; i.e., student II, Group I and all of Group I in Series III, in Group II, student III, Series I and Students IV and V, Series III.

Nedjvetskii (20) finds that the stability of cholesterol varies with the species.

Labarde et al. (14) report that the Libermann Burchard color is more stable at 18° C. and fades rapidly at higher temperatures.

Students I, II and III of Group I and students I, II, IV, and V of Group II showed a significant decrease in cholesterol levels on iodide therapy. Students IV and V of Group I and students III, VI and VII of Group II showed a significant increase in cholesterol levels on iodide therapy. Only student

IV of Group I and student VI of Group II confirmed the suggestion of Pittman et al. (25) that iodide therapy might increase the basal metabolic rate.

Muller (19) states that the postulated inverse relationship between blood cholesterol and basal metabolic rate is not invariable. This is certainly shown by the results of this study.

CONCLUSIONS

The results of this research do not bear out the consensus from the literature that the basal metabolic rate will show a fairly close inverse relationship to the blood cholesterol level for the normal individual or one with uncomplicated thyroid disorders. Thus, it is concluded that blood cholesterol determinations cannot be used to replace basal metabolic rate determinations as an index of thyroid activity. This would be especially true for women before the time of the menopause because of the effect of the menstrual cycle on cholesterol levels.

In this research, iodide therapy produced a more consistent decrease in cholesterol levels than increase in the basal metabolic rate.

The major factor in the variations of the results may be the inability to carefully control the student subjects under the conditions of this experiment.

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