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THE EFFECTS OF JOGGING THERAPY ON FASTING PLASMA TOTAL CHOLESTEROL, HDL-CHOLESTEROL, GLUCOSE, RATIO OF HDLC TO TOTAL CHOLESTEROL, AND BODY WEIGHT IN OBESE MALES AND FEMALES

The University of North Carolina at Greensboro PH.D. 1982

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THE EFFECTS OF JOGGING THERAPY ON FASTING PLASMA TOTAL CHOLESTEROL, HDL-CHOLESTEROL, GLUCOSE, RATIO OF HDLC TO TOTAL CHOLESTEROL, AND BODY WEIGHT IN OBESE MALES AND FEMALES

by

Joan A. Yuhas

A Dissertation submitted to
the Faculty of the Graduate School at
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Approved by

Dissertation Adviser

YUHAS, JOAN ANDREA. The Effects of Jogging Therapy on Fasting Plasma Total Cholesterol, HDL-Cholesterol, Glucose, Ratio of HDLC to Total Cholesterol, and Body Weight in Obese Males and Females, Directed by: Dr. Terry L. Bazzarre. Pp. 113.

This dissertation research examined the influence of a 24-week walking/jogging program on fasting plasma total cholesterol, HDL-cholesterol, glucose, the ratio of HDLC to total cholesterol, and body weight. Participants were 11 obese females and 10 obese males, 18-30 years of age and 15-40 percent over ideal weight.

The first 12 weeks consisted of increasing time periods of walking and jogging at 65-75 percent maximum effort three times per week with a jogging therapist. In the second 12 weeks, subjects ran on their own four times per week. The data collected at weeks 0, 4, 8, 12, and 24 included stress test maximum heart rate, aerobic capacity, skinfold measurements, height, weight, and fasting plasma blood samples (before, after, 20 minutes after test). Data from week 24 and the 20-minutes-after test were eliminated due to incompleteness.

Mean body weight loss was nonsignificant for both females and males by ANOVA analysis. ANOVA procedures indicated a significant ($p=0.0367$) change in aerobic capacity for females only. Inconsistent significant ($p < 0.05$) associations between blood parameter changes and aerobic capacity changes were observed from week to week using simple regression. Posttest glucose was significantly ($p < 0.05$) higher than pretest levels for all time periods for both females and males in ANOVA analyses.

Significant results were limited due to several factors. The 12-week period was not significant for appropriate physiological

changes to occur. The subjects' intensity of effort varied in the jogging sessions. Also, small sample sizes reduced the power of the statistical tests.

APPROVAL PAGE

This dissertation has been approved by the following committee of the Faculty of the Graduate School at the University of North Carolina at Greensboro.

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HAPTER 1

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CHAPTER 1

INTRODUCTION

Regular aerobic physical activity provides many benefits to the health of the individual. Improved cardiovascular fitness, favorable changes in the fasting lipids profile, decreased fasting glucose, and weight control are primary goals in the reduction of the risk of coronary heart disease (CHD). CHD is a leading cause of death in the United States (Gordon, Castelli, Hjortland, Kannel, & Dawber, 1977). Methods of prevention and control of CHD, including physical activity, are under intensive investigation.

Murray, Murray, Murray, and Megan (1978) reported that the lack of regular physical activity is a risk factor of CHD. Cooper, Pollock, Martin, White, Linnerud, and Jackson (1976) found improved levels of physical fitness were associated with reduced CHD risk in a cross-sectional study of 3000 men. One means by which physical activity may reduce CHD risk is by lowering plasma total cholesterol and raising high density lipoprotein cholesterol (HDL-C). These blood components are postulated to play roles in the development of CHD (Kannel, Castelli, & Gordon, 1979).

Clinical work demonstrates that physical activity influences blood lipid and lipoprotein levels, i.e., total cholesterol and HDL-C, as well as glucose (Milesis, 1974; Joseph & Bena, 1977; Wood &

Haskel, 1979). Favorable alterations in these blood components may ultimately decrease the chance of CHD development.

The practical implications of using physical activity as a means of lowering CHD risk through alterations in blood profiles is a pertinent and timely topic of study. Also, the effect of physical activity on weight and/or fat loss in obese individuals in relation to blood profiles deserves consideration since obesity has also been reported to increase one's risk of developing CHD (Gordon et al., 1977).

This dissertation research examined the influence of the 24-week jogging program on fasting plasma blood samples' total cholesterol, HDL-C, glucose and body weight/fat in a sample of obese males and females, 18-30 years of age and 15-40 percent over their ideal weight. This ancillary study was part of a larger research project, RESHAPE, which evaluated the effects of jogging therapy on weight reduction of obese individuals.

CHAPTER 2

REVIEW OF LITERATURE

The practical implications of using physical activity as a means of lowering CHD risk through alterations of blood profiles and body weight/fat is a current concern of preventive medicine. The literature review summarizes research concerning the influence of exercise on the blood parameters, body weight, and body composition. Total cholesterol is discussed first, followed by HDL-cholesterol, the ratio of HDL-C to total cholesterol, and glucose. The last sections review body weight and composition changes with exercise and the effects of these changes on the blood parameters.

Total Cholesterol

Clinical studies have indicated physical activity alters blood lipid and lipoprotein levels. Milesis (1974) determined the effects of an 11-week physical training program (four weekly, 30-minute, walking/running/calisthenics sessions) on serum lipids in 11 male subjects compared to 11 male controls 28-54 years of age. A trend towards a reduction in serum total cholesterol was seen after 11 weeks. The experimental group exhibited a 29.6 mg% greater decrease in serum total cholesterol than did the controls. Subjects whose total cholesterol levels were relatively high tended to show the greatest overall decrease. A significant ($p < 0.05$) body fat loss was observed in the experimental group while body weight was not appreciably altered.

The sum of five skinfolds for the experimental group decreased from a mean and standard deviation of 102.3 ± 28 mm to 88.8 ± 27 mm while the control group values only changed from 94.4 ± 27 mm to 90 ± 26 mm. Fat loss was not significantly correlated with serum total cholesterol change, suggesting that the cholesterol reduction was affected by other factors. Physical activity itself may play an independent role.

Lopez-s, Vial, and Arroyave (1974) examined serum lipids and lipoprotein changes in 13 medical students (mean age of 22) after seven weeks of an exercise regime (four weekly, 30-minute, jogging/biking/calisthenics sessions, 7 METS intensity). A moderate decrease, significant at $p = 0.03$, in serum total cholesterol (mean ± SD, 169 ± 22 to 162 ° 25 mg%) was observed. An earlier study by Vial, Vial, Balart, and Lopez-s (1971) with four adult males also observed a significant decrease (values not given) in serum total cholesterol at the conclusion of a seven-week exercise program (daily, 30-minute, running/biking/calisthenics sessions).

Joseph and Bena (1977) studied an adult active control group (already in an exercise class), an experimental group (beginning an exercise class for 30 weeks), and an inactive control group. The subjects totaled 57 males with an age range of 33 to 62. Training heart rates (determined using the Karvonen equation) were maintained during the sessions. A significant ($p < 0.01$) 18% decrease in serum total cholesterol (mean ± SD, 229 ± 54 to 188 ± 36 mg%) was observed in the experimental group but no significant changes in the control groups were reported (active control : 178± 33 to 180 ± 40 mg%;

inactive control: 205± 38 to 208 ± 46 mg%). A significant ($p < 0.025$) reduction in skinfold thickness in the experimental group was observed (mean ± SD, 26 ± 11 to 21 ± 9%) but not in the active control group (20 ± 8 to 19 ± 7%). Body weight was not changed which was probably due to an increase in muscle tissue concomitant with the decrease in fat. The authors noted that the active control group might not have changed because the study acted as a maintenance program, resulting in stable measurements.

Interpretation and comparison of the results of these studies are limited because of variations in the experiments' designs. The length of time per exercise period, the number of exercise periods per week, the intensity of exercise, and program duration differ among studies. These variations in designs exacerbate efforts to compare results. Thea mount of exercise necessary to favorably alter the risk factors for CHD is currently under investigation.

Dressendorfer and Gahagen (1979) addressed the issue of how much activity is necessary to influence blood lipid levels. Their cross-sectional study of 80 male recreational joggers and 64 nonrunner controls (ages 22-59) observed lower cholesterol levels (7-26 mg%) in the runners than in the nonrunners ($p < 0.05$). No significant differences were observed in weight and body fat between groups. When compared on the bsais of distance run per week, the high-mileage group (mean ± SD, 36.2 ± 10 miles per week) was significantly ($p < 0.05$) less fat (13.6 ± 4%) than the low mileage group (8.6 ± 1 miles per week, 17.7 ± 7%) but exhibited no significant differences

in total cholesterol from the low-mileage group. The authors suggested that as little as three miles of jogging per session, three times per week, was sufficient to lower serum total cholesterol in a long-term exercise program.

Study designs have not consistently evaluated weight change, percent fat change, and diet in relation to the level of physical activity. As already indicated, some investigators observed that the reductions in serum total cholesterol associated with physical activity were independent of body weight and/or percent fat change. However, there are contradictory data which indicate that a decrease in fat is associated with a decrease in total cholesterol. A ten-week exercise study by Campbell (1968) evaluated the effect of exercise (three weekly treadmill runs consisting of six alternating five-minute bouts with five-minute rests between bouts and a gradual increase from 5 mph at 0° grade to 7.5 mph at 10° grade on serum total cholesterol in 130 college-age males in relation to the morphological constitutions. Obese, muscular and slim classifications were used in the experimental and control groups. Serum total cholesterol concentrations were reduced significantly ($p < 0.05$) in the obese active subjects only (207 mg% to 195 mg%), in comparison to the muscular (184 to 181 mg%) and slim (177 to 177 mg%) categories. Although a mean weight loss of 33 pounds occurred in the obese active students, the decrease in serum total cholesterol was independent of alterations in weight and diet. Campbell concluded that the serum total cholesterol concentration

of individuals of various morphological configurations was influenced differently by the exercise program and by changes in configuration.

Campbell (1965) previously examined the effect of the type of exercise upon serum total cholesterol in the 133 college-age males. Ten-week programs of various aerobic (i.e., cross-country running) and anaerobic (i.e., weight training) activities were compared. Based on the results, total cholesterol concentration was affected differently by different types of exercise. Activity of a vigorous and dynamic nature resulted in a significant ($p < 0.05$) serum total cholesterol reduction (180 to 160 mg%), while activity of a vigorous but static nature did not affect serum total cholesterol significantly (changes in cholesterol ranged from a 7 mg% decrease to a 3.5 mg% increase for various activities). The nature of the physical activity is an important consideration in the development of a therapeutic exercise regime.

The examination of total cholesterol level changes with exercise includes observations of alterations over time periods ranging from weeks to years. It may also be of interest to assess the changes in total cholesterol levels observed from immediately before to immediately after an exercise bout. Since serum total cholesterol is not considered a direct energy substrate for physical activity, this dimension has been reported in relatively few studies.

Jirka and Dolezel (1969) reported the influence of one exercise session on serum total choolesterol levels in 93 twelve-year-old children.

Serum total cholesterol increased after exercise; the rise was significantly higher ($p < 0.05$) in boys (mean ± SD, 140 ± 15 to 167 ± 23 mg%) than in girls (146 ± 18 to 157 ± 12 mg%) although at rest cholesterol levels were significantly higher ($p < 0.05$) in girls. There was no relationship between total cholesterol level changes and hematocrit, maximal working capacity, working capacity at the pulse rate of 170, and maximal oxygen consumption. The serum total cholesterol level increase was greater in boys with greater amounts of fat; this relationship was not observed in girls. The authors queried whether increased estrogen activity in females might be the cause of the smaller increase in serum cholesterol after effort.

Rochelle (1961) compared the mean total cholesterol levels of six adults, ages 20 to 38, immediately before and after exercise. Preexercise total cholesterol was 192 mg% and postexercise was 209 mg%. The difference was significant at $p < 0.001$. The author noted that the increased cholesterol concentrations may have been indicative of fat mobilization and use as an ultimate energy source in activity. The change in cholesterol level is an interesting observation which could stimulate further research.

HDL-cholesterol

The contribution of serum cholesterol to CHD risk can be determined by the cholesterol concentration in various lipoprotein fractions. The five major lipoproteins are the chylomicrons, the

very-low-density, intermediate-density, low-density, and high-density lipoproteins. A very large percentage of the total cholesterol is carried in the low-density fraction and is considered atherogenic. The presence of relatively high cholesterol levels in the high-density portion appears protective against CHD (Kannel, Castelli, & Gordon, 1979). Researchers have concluded that plasma high-density lipoprotein cholesterol (HDLC) levels are associated with less risk of CHD. The plasma concentraiton of HDLC is presently under study as an "inverse" risk factor for CHD.

HDLC is divided into two categories by density range: HDL2 and HDL3. HDL2 ranges from 1.063 to 1.125 g/ml and HDL3 from 1.125 to 1.210 g/ml. HDL2 is larger than HDL3, less dense and has a higher concentration of cholesterol. Total HDLC is higher in women than in men primarily because of a higher concentration of HDL2; HDL3 levels are similar (Wood, 1980). Higher HDLC levels are also found in long-distance runners and are the consequence of higher concentrations of HDL2 (Wood, Haskell, Stern, Lewis, & Perry, 1977).

Controversy exists about the role of HDLC in CHD risk (Forde, Thelle, Miller, & Mjos, 1978); however, mounting epidemiological evidence supports the association between high HDLC and lowered CHD risk (Wood, 1980). HDLC is independent of other coronary risk factors in its effects. That is, without changing any other variable known to affect this disease state, increasing

HDLC appears to lower occurrence rates for CHD in both men and women (Gordon, Castelli, Hjortland, Kannel, & Dawber, 1977).

Many longitudinal and cross-sectional studies have indicated that regular physical activity increases HDLC. Investigation across various age groups of males and females demonstrates lower plasma total and LDLC concentrations, and higher HDLC concentrations with daily physical activity, i.e., running (Wood & Haskell, 1979; Wood et al., 1977; Wood, Haskell, Klein, Lewis, Stern, & Farquhar, 1976; Enger, Herbjornsen, Erikssen, & Fretland, 1977; Roundy, Fisher, & Anderson, 1978; Webster, Smith, LaRosa, Meusing, & Wilson, 1978). On the basis of analyses of HDL subfractions in seven male runners (ages 42-58) and six female runners (ages 34-46) in comparison to sedentary controls, the increased HDL level was due to a significantly higher HDL2 concentration (male runners vs controls: mean ± SD, 115 ± 47 mg% vs 53 ± 44 mg%, $p < 0.005$; female runners vs controls: 218 ± 79 mg% vs 122 ± 85 mg%, $p < 0.05$. Unchanged HDL3 levels were observed (male runners vs controls: 259 ± 22 mg% vs 227 ± 45 mg%, $p < 0.05$; female runners vs controls; 220 ± 38 mg% vs 220 ± 28 mg%, $p < 0.05$ (Krauss, Lingren, Wood, Haskell, Albers, and Cheung, 1977).

Hartung, Foreyt, Mitchell, Vlasek, and Gotto (1978) observed significantly higher (no level given) plasma HDLC in 59 male marathon runners (65 mg%), and in 85 male joggers averaging 11 miles per week (58 mg% compared to HDLC levels in 74 sedentary males

44 mg%). It appeared that even males running 11 miles per week had significantly higher HDL levels than sedentary men. Continuation of this higher activity level maintained this increased HDLC level.

Increases in HDLC concentration and decreases in LDLC concentrations have been observed in many longitudinal studies. Carlson and Froberg (1967) observed a drop in LDLC from 129 mg% to 61 mg% at the end of a ten-day 500-km walk in 12 males. HDLC increased from 62 mg% to 70 mg% by day six. Altekreuse and Wilmore (1973) observed a significant redistribution of plasma lipoproteins in 39 sedentary males in ten weeks of a running program (averaging five miles per week at 7.5 mph). A study of 22 obese women (40% fat) engaged in a 17-week jogging program (20 minutes twice weekly, 80% maximum heart rate intensity) and, following a self-determined calorically restricted diet, demonstrated a significant ($p < 0.05$) increase in the HDL to LDL-cholesterol ratio (mean ± SD, 0.38 ± 0.14 to 0.43 ± 0.16) (Lewis, Haskell, Wood, Manoogian, Bailey, & Pereira, 1976).

Ratliffe, Elliot, and Rubenstein (1978) observed a significant ($p < 0.05$) increase in HDLC in 14 subjects who jogged three times per week for 20 weeks at 76% maximum heart rate intensity (mean ± SD, 42 ± 10 mg% to 50 ± 10 mg%) as compared to no change in a control group (43 ± 12 to 41 ± 9 mg%). The exercise group experienced a significant loss ($p < 0.05$) in body fat (amount

not stated). An inverse relationship was seen between the change in HDLC and the change in percent fat.

In a study of the effects of ten weeks of exercise versus ten weeks of exercise plus a weight-loss diet in 35 men (mean age 47) and 37 women (mean age 44), a significant decrease ($p < 0.05$) in total cholesterol and LDLC occurred with or without diet (Weltman, Stamford, Levy, Matter, Short, & Fulco, 1978). In the male exercise group total cholesterol decreased from 205 mg% to 188 mg%. In the male exercise plus diet group, total cholesterol decreased from 192 mg% to 178 mg%. This total cholesterol decrease was due to a decrease in LDLC with a slight increase in HDLC. The LDLC to HDLC ratio was significantly reduced ($p < 0.05$) in both the male exercise group (2.61 to 2.08) and the male exercise plus diet group (2.38 to 2.08). Similar results were observed in the females, whose HDLC was significantly higher and LDLC to HDLC ratio was significantly lower than for the males both pre- and poststudy.

Erkelens, Albers, Hazzard, Frederick, and Bierman (1978) found HDLC increased significantly (4.9 mg%, $p < .001$) after one week of a three-month exercise program in 18 male myocardial infarction survivors. HDLC remained high (total rise of 6.1 mg%, $p < 0.001$) after three months of training. Lopez-s et al. (1974) studied the effects of a seven-week exercise program (four 30-minute sessions weekly of jogging, biking, and calisthenics, 7 METS) on serum lipoproteins in 13 medical students (mean age 22). A significant ($p < 0.01$) rise in HDL was observed (mean ± SD,

286 ± 86 to 332 ± 75 mg%), along with significant decreases in VLDL (93 ± 57 to 43 ± 41 mg%, $p < 0.01$) and LDL (185 ± 57 to 158 ± 39 mg%, $p = 0.02$).

The direct association between exercise and an increased HDLC was demonstrated in a study of 12 marathon runners (mean age 40) who ran ten days (average 28 km/day), rested three days, and then ran another eight days (Dressendorfer, Wade, Hornick, & Timmis, 1982). A significant ($p < 0.05$) 18% rise in HDLC occurred after one week of running. This change was reversed with the three-day rest period. HDLC then again rose significantly ($p < 0.05$) when running resumed (increased 19% on day 17 and 22% on day 20). The training distance appeared to be a determinant of HDLC level. Results suggested HDLC concentration was related to the amount and regularity of exercise training.

Rotkis, Cote, Coyle, and Wilmore (1980) also observed a relationship between HDLC and weekly running mileage. Their cross-sectional examination of 109 male subjects indicated HDLC was significantly correlated ($p < 0.05$) with miles run per week ($r=.50$) and percent body fat ($r=-.36$). There was a significant increase ($p < 0.05$) in HDLC across the groups from nonrunners (mean ± SD, 34 ± 7 mg%), low-mileage runners (10-19 miles per week, 47 ± 7 mg%), intermediate mileage runners (20-39 miles per week, 53 ± 9 mg%), to high mileage runners (40 miles per week, 60 ± 9 mg%). This association was found independent of body composition, age, and diet.

Farrell and Barboriak (1980) determined HDLC every two weeks in an eight-week running program (70% VO_2 max, 30-minutes a day, 3-4 days a week) in 17 formerly sedentary subjects. Participants were seven males (mean age 22) and 9 females (mean age 23). An insignificant decline in HDLC occurred at two weeks (53.8 to 51.1 mg%). HDLC increased significantly ($p < 0.05$) only at eight weeks (51.1 to 57.4 mg%). This occurred in both males and females. This change occurred later than the increase in VO_2max, indicating a lag in HDLC alteration in the exercise program in previously sedentary individuals.

HDLC:Total Cholesterol

In reviewing past training studies and cross-sectional studies of various age groups and sexes, a common pattern of change in plasma lipoprotein concentrations is apparent. Work indicates that changes which may be desirable can occur in sedentary individuals who engage in a regular exercise regime. VLDLC and LDLC concentrations can be decreased and HDLC concentrations increased. The risk of CHD may be reduced with these changes.

Contradictions exist in the literature concerning the effect of exercise on total cholesterol (Holloszy, Skinner, Toro, & Cureton, 1964). The inconsistencies in total cholesterol change may be explained by the fact that this value represents the sum of the cholesterol in all the lipoprotein fractions. Alterations occur in these fractions in such ways that total cholesterol may or may not

reflect them. The HDLC increase and VLDLC and LDLC reductions often observed with exercise may cause an increase, decrease, or no change in total cholesterol, depending on the extent of these changes.

The ratio of HDLC to total cholesterol may be the most informative parameter to examine rather than total cholesterol or HDLC alone (Kannel, Castelli, & Gordon, 1979). This ratio may provide a better indication of CHD risk status because the ratio allows for a relative comparison of the variables which seem to have a contrary influence upon CHD incidence. The higher the HDLC value, the better the ratio. It is of current research interest to recognize and evaluate this parameter in conjunction with the use of physical activity for weight control and CHD risk therapy.

Glucose

Hyperglycemia and impaired glucose tolerance are often observed among the obese (Jourdan, Goldbloom, Margen, & Bradfield, 1974). An increased risk of developing vascular abnormalities may occur (O'Sullivan, Cosgrave, & McCaughan, 1968). Such changes may lead to the development of CHD. Individuals with diabetes have a greater risk of CHD. Methods which attempt to improve glucose tolerance and decrease fasting blood glucose levels are currently receiving attention.

Weight loss appears to improve glucose tolerance in obese individuals. This effect occurs independently of dietary manipulations (Jourdan et al., 1974), although some controversy exists concerning the effects of dietary restrictions on insulin levels (Farrant, Neville, & Stewart, 1969). Physical training decreases insulin response to

blood glucose (Quigley, 1979; Lohman, Liebold, Heilman, Senger, & Pohl, 1978). The Lohman et al. study of six runners (mean age 22, sex not given) demonstrated that while glucose appearance and disappearance rates were similar between the athletes and 115 controls, insulin release was different. The basal insulin level of the athletes was 50% lower than that of the controls. This level increased 100% after glucose injection in both groups and remained constant for three hours in the athletes. However, in the controls the initial insulin increase was followed by a further continuous increase for an hour (after a 30-minute lag). The authors suggested glucose disposal by insulin is more efficient in trained individuals. The low insulin response could be due to muscle conditioning, and therefore this response could not be generally attributed to a diabetic or prediabetic state. Both regular physical activity and weight loss may positively affect both glucose tolerance and fasting blood glucose levels over time in an exercise program.

The acute effects of exercise on fasting blood glucose levels have also been studied. Serum glucose level rose significantly ($p < 0.05$) from 96 ± 11 mg% to 170 ± 48 mg% during a single extended exercise bout (10 km run) in 13 male athletes, ages 18 to 44 (Lavine, Lowenthal, Gellman, Klein, Woedman, & Rose, 1977). This increase was inversely related to exercise time; that is, the more exertion (the shorter the time also), the greater the glucose rise. Some evidence has indicated that in short exercise bouts (i.e., 1-20 minutes), blood glucose level remains unchanged while muscle glycogen plays a major role in energy supply (Keppler, Keul, & Doll, 1969).

The Keppler et al. study of 56 male athletes and 14 students observed that a decrease in blood glucose was noted during exercise of longer duration. Observations of glucose levels before and after a bout of maximum effort (i.e., stress test) would be of interest particularly in relation to training effects over time.

Body Weight and Composition

References have been made to changes in body fat and body weight in relation to blood lipid profiles through the effects of exercise programs. Significant changes in body composition can result from regular physical activity, and can occur with or without dietary changes (Pollock, Wilmore, & Fox, 1978). A large amount of weight loss by diet alone may result from lean tissue and water loss, rather than fat tissue (Pollock et al., 1978). Exercise can induce hypertrophy of muscle tissue and thus cause a gain in lean body weight. Body fat loss can occur simultaneously. Pollock et al. (1978) state that little body weight change usually occurs in the first six to eight weeks of exercise since fat loss and lean weight gain are occurring simultaneously. However, body composition is changing, which is a more important indicator of the physical effects of regular activity.

Several studies have demonstrated changes in body composition with the adoption of an exercise regime. Carter and Phillips (1969) observed a 3-kg decrease in body weight and a 3.8% decrease in fat percentage in seven males (mean age 47) who ran 30 minute per session two or more times weekly for 26 weeks. Pollock, Cureton, and

Greninger (1969) demonstrated a 2.9-kg weight loss and a 1% fat loss in 11 males (mean age 32) in a 20-week running program (30 minutes per session, 4 days per week). Oscai, Williams, and Hertig (1968) observed a 2.4 kg weight loss and a 2.2% fat loss in a 20-week running program, 30 minutes per session, 3 times per week, in 14 males (mean age 37).

Pollock et al. (1978) noted that comparisons of body composition changes in exercise studies are difficult to make because of considerable variability in experimental designs. The lack of dietary control and quantification of training data exacerbates comparisons. While many studies have shown body composition changes with exercise as noted earlier, several others have not reported significant weight or fat changes (Pollock et al., 1978).

In their analysis of several studies, Pollock et al. (1978) noted that those programs with greater combinations of frequency, duration, or intensity demonstrated greater change in body composition. Eight-to-ten week regimens tended to show only small changes. The small changes reflected the lack of sufficient time for full adaptation to the exercise to occur, since the first few weeks of training are developmental in nature. Carter and Phillips (1969) observed a continuous body weight and fat loss over a year from the beginning of an exercise regime.

Gwinup (1975) found no weight loss in female subjects until walking time exceeded 30 minutes daily. Frequency also appears to be important. Pollock et al. (1978) concluded that two-day-a-week

programs may not be adequate to cause a significant body fat loss. From their review of research, they suggested that for weight and fat loss to occur by endurance exercise the following are required: (1) 20- to 30-minutes of continuous physical activity; (2) sufficient exercise intensity to use approximately 30 kilocalories per session; (3) three days per week frequency. Increased frequency, intensity, and duration of training should cause greater reductions, total energy expenditure being highly related to weight and fat loss.

Body Composition and Lipids Profile

The assessment of the relationship between extremes in percentage of body fat and serum lipid levels can give an indication of the combined risks of a high percentage of body fat and an unfavorable lipids profile for CHD. Weltman (1981) examined 69 females for body composition (hydrostatic weighing), serum total cholesterol, and HDLC. Two groups were formed: 48 females with 40% or more body fat were defined as extremely overfat for the study (mean age = 43, mean % fat = 48); 21 females with 35% or lower body fat constituted the normal controls (mean age = 40, mean % fat = 30). Serum total cholesterol levels were significantly ($p < 0.05$) higher in the extremely overfat (208 mg%) versus the control females (189 mg%). Significantly ($p < 0.05$) lower HDLC levels were also observed in the overfat (57 mg%) versus the control females (67 mg%). A significantly ($p < 0.05$) higher total cholesterol to HDLC ratio was seen in the overfat (3.6) as compared to the controls (2.8).

Extremely overfat females had unfavorable serum lipid profiles. An increased CHD risk in overfat females may be due in part to a lower HDLC level. Favorable changes in body composition and lipids profile through physical activity would be advantageous.

Favorable changes in lipid levels and body composition were demonstrated in a study by Hicks, Morton, Brammel, Johnson, Keller, and Mathias (1980) in 19 males, ages 28-64. A 12-week exercise program (20 minutes to an hour per session, three times per week) resulted in significant decreases in body weight (3.85 kg, $\underline{p} = 0.003$), body fat (4.6%, $\underline{p} < 0.01$), and total cholesterol (24 mg%, $\underline{p} < 0.01$). HDLC increased by 5.6 mg%. The blood values were most affected by the change in body fat. Exercise was significantly related to body fat loss which, in turn, had a significant effect on blood lipid changes.

The studies described in this review have provided data concerning specific segments of the population using various designs. However, this past research suffers from limitations in design. Investigations of the effects of regular aerobic exercise in obese females is lacking. The paucity of data available pertaining to exercise in obese females makes this an opportune group to examine.

The influence of exercise on the acute changes in blood values as well as the chronic alterations merits investigation. A more comprehensive evaluation of physiological changes is appropriate. Most past research has not included both the acute and chronic

blood value variations. Also, the frequent monitoring of physiological changes occurring with exercise allows time-controlled evaluation of the effects of a program. Most results reported have been only pre- and postdata, with no reference to alterations in the course of the program. The combination of the positive points from past designs with those factors described above would provide more comprehensive information for the field.

CHAPTER 3

EXPERIMENTAL DESIGN

Statement of Purpose

The research project RESHAPE was designed to evaluate the effects of jogging therapy on physiological and psychological parameters of obese males and females. This dissertation research examined the influence of the 24-week training program upon fasting plasma total cholesterol and high density lipoprotein cholesterol, as well as fasting plasma glucose levels. Concurrently, changes in body weight and skinfold thickness were also measured. Frequent evaluations of both the acute and chronic changes in the blood values were made.

Objectives

The obejctives of this research were to measure changes in obese males and females during a 24-week jogging program in

a) fasting plasma total cholesterol,

b) fasting plasma high density lipoprotein cholesterol,

c) the ratio of fasting plasma high density lipoprotein to total cholesterol, and

d) fasting plasma glucose.

Further objectives were to measure the relative association of fasting plasma total cholesterol, high density lipoprotein cholesterol, and

glucose with a) relative body weight and b) changes in body weight and the sum of four skinfold thickness measures throughout a 24-week jogging program in obese males and females. A final objective was to measure changes in fasting plasma levels of total cholesterol, high density lipoprotein cholesterol, and glucose directly before, after, and twenty minutes after a maximum effort exercise bout.

Hypotheses

1) A 24-week jogging program has a variable effect on fasting plasma total cholesterol in obese males and females.
2) A 24-week jogging program will increase fasting plasma high-density lipoprotein cholesterol in obese males and females.
3) A 24-week jogging program will decrease fasting glucose in obese males and females.
4) The higher the relative body weight in comparison to ideal body weight,
   a) the higher the fasting plasma total cholesterol,
   b) the higher the fasting plasma glucose, and
   c) the lower the fasting high-density lipoprotein cholesterol in obese males and females.
5) A decrease in body weight or the sum of four skinfold thickness measures will be accompanied by
   a) a decrease in fasting plasma total cholesterol,
   b) a decrease in fasting plasma glucose, and

c) an increase in fasting plasma high-density lipoprotein cholesterol in obese males and females.

6) A 24-week jogging program will increase the ratio between fasting plasma high-density lipoprotein cholesterol and total cholesterol in obese males and females.

7) Fasting plasma levels of total cholesterol will not vary in measurements before, after, and twenty minutes after a maximum effort exercise bout in obese males and females.

8) Fasting plasma levels of high-density lipoprotein cholesterol will not vary in measurements before, after, and twenty minutes after a maximum effort exercise bout in obese males and females.

9) Fasting plasma levels of glucose will increase from before to after measurements and decrease from after to twenty-minutes after measurements in a maximum effort exercise bout in obese males and females.

Sample Selection and Data Collection

Through campus advertisements and personal contacts, 39 volunteers were recruited from the UNC-G student-faculty-staff population. Two replicate studies were undertaken, with 20 subjects in the first group and 19 in the second group. The first replication began in August 1980 and ended in February 1981. The second replication began in January 1981 and finished in July 1981. Each replication consisted of a one-on-one contact

between each subject and one particular jogging therapist. In the second 12 weeks, each participant was expected to run on his or her own. Table 1 presents the training program used in the study.

Table 1

Jogging Therapy Program

Week	Schedule	Frequency	Time
1-4	walking-jogging with therapist	3x/week	20 minutes
5-8	walking-jogging with therapist	3x/week	40 minutes
9-12	jogging with therapist	3x/week	60 minutes
13-24	jogging independently	4x/week	60 minutes

Each volunteer was interviewed for screening purposes. The objectives and design of the study were discussed, a medical history was reviewed, consent was obtained, and initial questionnaires were administered (discussed below). Requirements for participation were as follows:

1) 18-30 years of age
2) 15-40% above ideal weight for height, age, and sex according to standards set by the Metropolitan Life Insurance Comapny (1960)
3) No known history of angina, high blood pressure, hyperlipidemia (above 95th percentile of the Lipid Research Clinics Program for triglycerides and blood pressure*) and/or overt diabetes

*(Williams, Heiss, Beaglehole, Dennis, Bazarre, and Tyroler, 1980)

The trained jogging therapists consisted of graduate students and faculty (see Appendix A for training instructions). Each jogging session was preceded by stretching and warm-up activities for five to ten minutes. Appropriate running techniques were discussed by the therapists and subjects. Running style was evaluated by the therapists and adjusted according to the subjects' individual needs. The first jogging session consisted of 20 minutes of alternate walking and jogging at the pace and duration commensurate with the participant's level of conditioning. A target training heart rate was determined by a maximal effort treadmill test (discussed below). Heart rate was monitored by palpation to insure no over- or underworking. During the program the participants gradually increased the jogging time with fewer walking intervals until each was able to run continuously during the entire activity period. Five to ten minutes of cool-down walking and stretching completed the sessions.

Data were collected at five points in the study: Weeks 0, 4, 8, 12, and 24. The data obtained were grouped in the following categories:

a) anthropometrics including height (cm), weight (kg), and four skinfold measurements (tricep, bicep, subscapular, suprailеac),

b) blood pressure,

c) blood chemistries,

d) three-day food records,

e) treadmill tests, and

g) psychological questionnaires.

Blood pressure was determined in the supine position. Three blood samples, taken at the time of the treadmill test (before, after, and 20 minutes after), were analyzed for total cholesterol, HDL-cholesterol, and glucose. Other blood analyses were completed by other investigators in the Nutrition Department. Three-day food records were obtained to approximate appetite change. The psychological questionnaires included ratings of factors such as stress, body image, and depression.

The treadmill test consisted of a continuous, grade-speed incremented exercise bout using the Bruce Protocol (Appendix B). The participant walked or ran until reaching maximum fatigue. Measurements of heart rate, ventilation, temperature of the expired gas, and percentage of oxygen and carbon dioxide in the expired gas were obtained at one-minute intervals during the last several minutes of the test. Heart rate was determined from electrocardiograph recordings obtained by direct leads using a Beckman Type R411 Dynograph Recorder during the last ten seconds of each minute throughout the test. The highest heart rate measured during the test was considered the maximal heart rate. Training heart rate range was 65-75% of the maximum heart rate during most of the training period. This control of heart rate ensured adequate levels of conditioning while not overstressing the participant.

Oxygen consumption and carbon dioxide production were measured by open-circuit spirometry using the Douglas Bag Method. The volume of expired air was measured using a Parkinson-Cowan CD-4 dry gas meter previously calibrated using a Tissot gasometer. Concentrations of oxygen and carbon dioxide in the expired air were determined using the Beckman OM-11 and LB-2 electronic gas analyzers, respectively. Gas temperature was obtained from a thermistor inserted in the inlet port of the CD-4 gas meter. The method described by Consolzaio, Johnson, and Pecora (1963) was used to compute O2 uptake and CO2 production. The highest rate of O2 consumption obtained during the test was considered the maximal O2 uptake provided the subject reached an age-estimated maximum heart rate and R value greater than 1.0.

Before the treadmill test, 25 ml of blood were drawn from the antecubital vein using the vacutainer system (1½", 20 gauge needle). Blood samples were taken again immediately after the test and twenty minutes later. Hematocrit samples were obtained by micropipettes. The blood samples were kept on ice one to two hours and then centrifuged (3000 rpm, 20 minutes) and the plasma removed. Aliquots for glucose analysis were taken. The remaining samples were labeled and frozen for later analysis. Determination of glucose, total cholesterol, and HDL-cholesterol (heparin-manganese isolation) were made using the Technicon AA II System (Appendix C).

Statistical Analyses

Simple regression was used to assess the effects of changes in weight and aerobic capacity (VO_{2max}ml/kg.min) over time on the changes in the blood data. The regression procedure was also used to examine the relationship of body composition with the relative concentrations of the blood parameters. Sex differences and the acute responses to exercise were evaluated using the ANOVA procedure. The ANOVA procedure was also used in the analysis of the response versus the nonresponse group comparisons as discussed in Chapter 4.

The inclusion of both sexes in this research will add to the limited data concerning the effects of physical activity in obese females. The consideration of the acute and chronic blood value changes in exercise conditioning will provide a more comprehensive evaluation of physiological alterations than reported in the past. The periodic four-week assessments will allow closer examination of the influence of conditioning. The combination of these factors in the design will yield valuable information for the field.

CHAPTER 4

RESULTS AND DISCUSSION

Results

The results of the first 12 weeks of the jogging therapy program for both replications combined are presented in Tables 2 through 7. The means and standard deviations by sex are presented for weeks 0, 4, 8, and 12. Data for subjects who dropped out of the study before 12 weeks have been eliminated. For each variable, subjects were eliminated if data from two or more periods were missing. Reasons for missing data included absenteeism, missing blood samples, and inadequate volume of blood for al analyses. The data from week 24 and the postexercise data were eliminated because the number of subjects having complete data was too small to consider in these analyses. All statistically significant data are noted as such; other data described are nonsignificant.

Participation

A total of 23 females and 16 males were recruited into the pilot study; the study consisted of two replications (11 females and 9 males in the first replication; 12 females and 7 males in the second replication). Four of the females and one male were black. All of the subjects were between 18-30 years of age and 13-40% over their desirable weight according to Metropolitan Life Insurance Standards.

None of the subjects had a history of CHD, hypertension, or diabetes. The target recruitment goal was a total of 32 subjects (16 males and 16 females). Blood data were "complete" as described earlier for 11 females and 10 males.

Table 2

Drop-Out Rates

Dropouts:	n	Week	0	4	8	12	24	%	Total Dropouts
Replication I	(11)	females	2	3	-	-	1	55%	4
	(9)	males	-	-	1	1	2	44%	4
Replication II	(12)	females	2	1	-	-	1	33%	4
	(7)	males	-	-	1	2	-	43%	3

The dropout rates were similar for both sexes; however, females were more likely to drop out early in the study (Table 2). The two female dropouts in the first study (week 1) dropped out because of peripheral reasons--e.g. one female dropped out because she left school following the heart attack of her father, while the other caught the flu and subsequently left school after a two-week illness. The male who dropped out of the study at eight weeks during the first replication had lost 15 pounds and found it was easier to run at home by himself (he commuted 40 miles to and from school every day).

Analysis of the Data

Weight changes and aerobic capacity changes were analyzed using ANOVA procedures for comparisons of weeks 0, 4, 8, and 12.

The data was also analyzed using ANOVA by the grouping of subjects into categories according to weight and $\dot{V}O_{2max}$ml/kg.min changes (aerobic capacity). Two categories were used. A "responder" group consisted of those who lost a minimum of 1.4 kg and exhibited an increase in $\dot{V}O_{2max}$ml/kg.min from week 0 to week 12. The "non-responder" group consisted of all those who lost less than 1.4 kg, whether their aerobic capacity increased or not. All subjects fell into one or the other of the categories.

These group comparisons were also used to ascertain if any changes occurred in the blood parameters in relation to "response" to the jogging program in terms of weight loss and increased aerobic capacity. Comparisons of the responders versus the nonresponders for blood parameter changes were made. Also, effects of the jogging program in terms of weight loss and improved cardiovascular fitness via aerobic capacity were ascertained using the relative changes in the plasma concentrations of total cholesterol, HDL-cholesterol, the ratio of HDL-cholesterol to total cholesterol, and glocuse between weeks (weeks 0-4, 4-8, 8-12, and 0-12). Simple regression procedures were used with the blood data changes as dependent variables and weight and $\dot{V}O_{2max}$ml/kg.min changes as separate independent variables. ANOVA analysis were performed to assess sex differences and pre- and postexercise differences for the blood data. However, these differences could not be corrected for initial sex differences in physical condition due to lack of information concerning initial fitness. The individual blood data are presented in Appendix D.

Body Weight

The mean body weight (kg) values of both females and males at weeks 0, 4, 8, and 12 are presented in Table 3. There was no significant change in body weight across time.

When the females were divided into the responder versus the nonresponder groups, the average weight loss of the responder group was 6.3 ± 2.7 pounds as compared to 0.97 ± 3.9 pounds for the nonresponder group from weeks 0 to 12 (Table 4). The mean weight changes of the two groups were significantly different ($p=0.0093$). The average weight loss over the twelve weeks for the male responder group (8.3 ± 4 pounds) was significantly greater ($p=0.006$) than the weight changes of the nonresponder group (plus 1.8 ± 4 pounds (Table 5).

Aerobic Capacity

Aerobic capacity values for both females and males are presented in Table 3. Mean aerobic capacity $\dot{V}O_{2max}$ ml/kg.min increased from 33.9 ± 4.1 to 40.2 ± 7.1 for females and from 39.2 ± 4.7 to 44 ± 4.7 for males. ANOVA for the effect of week was statistically significant for females ($p=0.0367$) but not for males. Scheffé's conservative post hoc analysis test showed no significant differences between specific weeks.

The responder group females increased their aerobic capacity by a mean of 8.6 ± 0.2 mg/kg.min as compared to 4.6 ± 6.4 ml/kg.min for the nonresponder group. The responder group males had a

Table 3

Body Weight and Maximum
Oxygen Consumption
(Mean ± standard deviation)

Females

Week	0	4	8	12
Weight, kg [a]	70±8	70±8	68±7	70±8
(n)	(11)	(11)	(8)	(11)
$\dot{V}O_{2max}$ ml/kg. min [b]	33.9±4.1	35.9±4.6	32.5±2.5	40.2±7.1
(n)	(9)	(9)	(6)	(7)

Males

Week	0	4	8	12
Weight, kg	87±12	86±12	85±11	85±12
(n)	(10)	(10)	(10)	(10)
$\dot{V}O_{2max}$ ml/kg. min	39.2±4.7	40.9±2.2	42.3±3.3	44.0±4.7
(n)	(9)	(8)	(8)	(8)

[a] ANOVA not statistically significant for weight.

[b] ANOVA statistically significant at $p = 0.0367$

Table 4

Changes in Weight, Maximum Oxygen Consumption, and the Blood Parameters for the Female Responder and Nonresponder Groups for Weeks 0 and 12 (Mean± standard deviation)[a]

	Groups	
	Nonresponder (n)	Responder (n)
Weight,[b] kg	0.44±1.8 (7)	-2.9±1.2 (4)
$\dot{V}O_{2max}$ ml/kg. min	4.6±6.4 (5)	8.6±0.2 (2)
Total Cholesterol, mg%	-1.3±39 (7)	25.8±45 (4)
HDL-Cholesterol, mg%	0.6±13 (7)	13.3±8 (3)
HDL-C:TC	-0.02±0.13 (7)	0.05±0.01 (3)
Glucose, mg%	-8.6±10 (7)	-5.3±17 (4)

[a]Values are expressed as week 12 minus week 0.

[b]Significantly different at $p = 0.0093$ in ANOVA analysis.

Table 5

Changes in Weight, Maximum Oxygen Consumption and the Blood Parameters for the Male Responder and Nonresponder Groups for Weeks 0 and 12 (Mean ± standard deviation)[a]

	Groups	
	Nonresponder (n)	Responder (n)
Weight,[b] kg	0.82±1.8 (4)	-38±1.8 (6)
$\dot{V}O_{2max}$ ml/kg. min	3.5±4 (4)	6.4±3 (4)
Total Cholesterol, mg%	-1.8±45 (4)	32.5±59 (6)
HDL-Cholesterol, mg%	8.5±9.1 (4)	-0.6±13.1 (5)
HDL-C:TC	0.04±0.06 (4)	-0.01±0.09 (5)
Glucose, mg%	1.3±17 (4)	-3.5±7 (6)

[a]Values are expressed as week 12 minus week 0.

[b]Significantly different at $p = 0.006$ in ANOVA analysis.

greater increase in aerobic capacity of 6.4 ± 3 ml/kg.min as compared to the nonresponder group (3.5 ± 4 ml/kg·min).

Percentage of Fat and the Sum of Skinfold Changes

Adequate data were not available for analysis of the percentage of fat and the sum of skinfold changes for the subjects under consideration. Questionable validity and reliability of testers and relatively large variations in measurements limited the usability of the data.

Quetelet and the Blood Parameters

In order to ascertain if a relationship existed between body composition and relative blood parameter concentrations, a regression analysis was done using Quetelet values. Quetelet (wt/ht^2) gives an estimate of body composition and is frequently used in surveys (Beal, 1980). No associations were observed between the Quetelet index and the relative concentrations of fasting plasma total cholesterol, HDL-cholesterol, glucose, or the ratio HDL-cholesterol: total cholesterol ($p > 0.05$).

Fasting Plasma Total Cholesterol

Fasting plasma total cholesterol (TC) concentrations are presented in Table 6. No significant sex differences in TC were observed, nor were there any significant differences in pre- and postexercise TC concentrations for either sex at any time period.

Table 6

Fasting Plasma Total Cholesterol (mg%)
(Mean ± standard deviation)

Females

Week	0	4	8	12
Test				
Pre-exercise	192±53	183±51	186±33	201±42
(n)	(11)	(11)	(8)	(11)
Post-exercise	218±38	192±43	217±53	208±40
(n)	(8)	(9)	(6)	(9)

Males

Week	0	4	8	12
Test				
Pre-exercise	179±43	186±40	196±39	198±47
(n)	(10)	(10)	(10)	(10)
Post-exercise	206±31	196±32	218±50	205±41
(n)	(9)	(9)	(10)	(10)

However, it should be noted fasting plasma preexercise cholesterol levels were consistently lower than TC concentrations immediately following the termination of each stress test for both males and females at all time periods.

According to a simple regression analysis, a significant ($p=0.0028$) inverse relationship between the change in preexercise fasting plasma total cholesterol and the change in $\dot{V}O_{2max}$ml/kg·min for weeks 4 to 8 for females was observed. The regression equation stated that total cholesterol = -14.78-12.27 $\dot{V}O_{2max}$ml/kg·min. Preexercise fasting plasma total cholesterol levels for males exhibited a significant ($p=0.0218$) direct relationship with $\dot{V}O_{2max}$ml/kg·min for weeks 4 to 8. The regression equation stated that total cholesterol = -5.17+5.5 $\dot{V}O_{2max}$ml/kg·min. There were no other significant changes among weeks for fasting plasma TC levels.

The mean fasting plasma total cholesterol level decreased 1.3 ± 3.9 mg% and increased 25.8 ± 45 mg% for the female nonresponder and responder groups, respectively, over the 12 weeks (see Table 4). In males, the mean fasting plasma total cholesterol level increased 32.5 ± 59 mg% for the responder group and decreased 1.8 ± 45 mg% for the nonresponder group (Table 5).

Fasting Plasma High-Density Lipoprotein Cholesterol

Mean fasting HDLC concentrations for females and males for weeks 0, 4, 8, and 12 are presented in Table 7. Mean HDLC concentrations were significantly different by sex at week 0, preexercise

Table 7

Fasting Plasma High Density Lipoprotein Cholesterol (mg%)
(Mean ± standard deviation)

Females

Week	0	4	8	12
Test				
Pre-exercise	48±17	48±15	55±16	52±10
(n)	(10)	(10)	(7)	(10)
Post-exercise	50±14	47±18	57±21	47±8
(n)	(6)	(8)	(5)	(7)

Males

Week	0	4	8	12
Test				
Pre-exercise	34±8	38±9	42±12	37±10
(n)	(9)	(8)	(7)	(9)
Post-exercise	37±11	39±10	37±9	40±9
(n)	(8)	(7)	(7)	(9)

(females: 48 ± 17 mg%, males: 34 ± 8 mg%, p=0.0436). Week 12, preexercise values were also significantly different (p=0.0052) between females (52 ± 10 mg%) and males (37 ± 10 mg%). Post-exercise concentrations of HDLC were significantly different (p=.0256) at week 8 for females (27 ± 21 mg%) and males (37 ± 9 mg%). Pre- and postexercise differences were not significant for either sex.

A significant (p=0.0492) direct relationship between the change in HDLC from week 8 to 12 and the change in weight for males was noted by simple regression analysis. The regression equation stated that HDLC = 3.22 + 2.14 weight. No significant relationships between changes in HDLC concentrations and changes in weight were observed for females. There were no other significant changes among weeks for fasting plasma HDLC levels.

Fasting plasma HDLC levels increased only 0.6 ± 13 mg% for the female nonresponder group, but increased 13.3 ± 8 mg% for the responder group (Table 4). HDLC concentrations decreased 0.6 ± 3.1 mg% for the male responder group while the nonresponder group's HDLC increased 8.5 ± 9.1 mg% over the 12 weeks (Table 5).

The Ratio of HDLC to Total Cholesterol

The means and standard deviations for the HDLC to TC ratio are presented in Table 8. Sex differences for the ratio were significant at week 12, preexercise (females: 0.27 ± 0.07, males: 0.20 ± 0.07, p=0.0495) and at week 8, postexercise (females: 0.29 ± 0.11, males: 0.18 ± 0.07, p=0.0464).

Table 8

HDL-Cholesterol: Total Cholesterol
(Mean ± standard deviation)

Females

Week	0	4	8	12
Test				
Pre-exercise	0.27±0.13	0.27±0.09	0.31±0.10	0.27±0.07
(n)	(10)	(10)	(7)	(10)
Post-exercise	0.25±0.09	0.28±0.07	0.29±0.11	0.27±0.05
(n)	(5)	(7)	(5)	(6)

Males

Week	0	4	8	12
Test				
Pre-exercise	0.19±0.06	0.22±0.09	0.22±0.07	0.20±0.07
(n)	(9)	(8)	(7)	(9)
Post-exercise	0.18±0.04	0.21±0.06	0.18±0.07	0.21±0.06
(n)	(8)	(7)	(9)	(9)

No significant pre- or postexercise differences were observed for either sex within each week. There was no consistent pattern of change between mean preexercise HDLC to TC ratios and mean postexercise HDLC to TC ratios. The change in the ratio postexercise from week 0 to 4 was significant ($p=0.0494$) in direct relation to change in $\dot{V}O_{2mas}$ml/kg·min for females. The regression equation stated that ratio = -0.05 ± 0.027 $\dot{V}O_{2max}$ml/kg·min. A significant ($p=0.0204$) direct relationship was also observed for females for postexercise ratio changes from week 4 to 8. The regression equation stated that ratio = $0.037 + 0.037$ $\dot{V}O_{2max}$ml/kg·min. No other significant changes among weeks for the ratio were observed.

The mean ratio of HDLC to TC decreased 0.02 ± 0.13 for the female nonresponder group while it increased 0.05 ± 0.01 for the responder group (Table 4). The male nonresponder group's HDLC to TC ratio decreased a mean of 0.04 ± 0.06 while the responder group's value decreased a mean of 0.01 ± 0.09.

Fasting Plasma Glucose

Fasting plasma glucose concentrations are presented in Table 9. Significant sex differences in fasting plasma glucose concentrations were observed at week 0, preexercise (females: 93 ± 17 mg%, males: 100 ± 6 mg%, $p=0.0294$). Significant differences were also observed at week 8, preexercise (females: 90 ± 10 mg%, males: 99 ± 8 mg%, $p=0.0464$) and week 12, preexercise (females: 86 ± 10 mg%, males: 99 ± 11 mg%, $p=0.0117$).

Table 9

Fasting Plasma Glucose (mg%)
(Mean ± standard deviation)

Females

Week	0	4	8	12
Test				
Pre-exercise	93±17	90±6	90±10	86±10
(n)	(11)	(11)	(8)	(11)
Post-exercise	121±13	124±14	127±±2	124±19
(n)	(8)	(9)	(6)	(9)

Males

Week	0	4	8	12
Test				
Pre-exercise	100±6	96±8	99±8	99±11
(n)	(10)	(10)	(10)	(10)
Post-exercise	120±34	124±20	125±21	128±25
(n)	(9)	(9)	(10)	(10)

Significant pre- and postexercise fasting plasma glucose differences were observed for both females and males. For females, significant differences ($p=0.0001$) were noted at weeks 0, 4, 8, and 12. Postexercise fasting plasma glucose concentrations for females for weeks 0 (93 ± 17 mg%), 4 (90 ± 6 mg%), 8 (90 ± 10 mg%), and 12 (86 ± 10 mg%) were significantly higher than preexercise levels for weeks 0, 4, 8, and 12, respectively (121 ± 13 mg%, 124 ± 14 mg%, 127 ± 13 mg%, 124 ± 19 mg%, respectively). For males, significant pre- and postexercise differences for fasting plasma glucose levels were observed for week 4 (pre:96 ± 8 mg%, post:124 ± 20 mg%, $p=0.001$), week 8 (pre:99 ± 11 mg%, post:128 ± 25 mg%, $p=0.0033$).

A significant ($p=0.0075$) inverse relationship between the change in preexercise fasting plasma glucose from weeks 0 to 4 with the change in $\dot{V}O_{2max}$ml/kg.min was observed in the females. The regression equation stated that glucose = 2.44 -2.7 $\dot{V}O_{2max}$ ml/kg.min. Females also exhibited a significant ($p=0.0456$) postexercise fasting plasma glucose change in direct relation to $\dot{V}O_{2max}$ ml/kg·min change from week 8 to 12. The regression equation stated that glucose = -9.1 + 1.66 $\dot{V}O_{2max}$ml/kg.min. There were no other significant changes among weeks for fasting plasma glucose.

Average fasting plasma glucose values decreased 8.6 ± 10.6 mg% for the female nonresponder group and 5.3 ± 17 mg% for the responder group (Table 4). For males, mean fasting plasma glucose levels increased for the nonresponder group by 1.3 ± 17 mg% and decreased 3.5 ± 7 mg% for the responder group (Table 5).

Discussion

The results of this study are significant in isolated instances. No generally consistent pattern of significant changes in the data were observed with the jogging program. The inconsistency of the fidings makes interpretations difficult.

The inconsistent significant results of this study were due to several factors. The level of conditioning observed in 12 weeks may not be enough to invoke changes in body weight, aerobic capacity, and the blood parameters examined. Many subjects in this study did not run over 30 minutes continuously until weeks 6 to 8. Also, the intensity of effort by the subjects in the running sessions varied considerably. Six weeks of minimal intensity and distance could not be expected to provide large physical changes.

Studies conducted for longer periods of time (i.e., 24 weeks up to 24 months) have observed larger decreases in body weight and increases in aerobic capacity than were found in this research (Dressendorfer & Gahagen, 1979; Pollock et al., 1978). The significant ANOVA for aerobic capacity changes in females during the program indicated improvement in physical condition with exercise from an initially low level. The nonsignificant association between Quetelet and the blood parameters merits further investigation. The use of hydrostatic weighing would be advisable as a more valid indicator of body composition.

The jogging program did not result in consistent significant changes in the blood parameters for the obese males and females. In addition to those factors already described which affected the results, the statistical analyses of the blood parameters were limited in power due to small sample sizes. When the effects of the program were defined by weight loss and increased aerobic capacity, the female responder group exhibited some appropriate changes: an increase in fasting plasma HDLC and the ratio of HDLC to TC from weeks 0 to 12 as compared to virtually no change in fasting plasma HDLC and a decrease in the ratio of HDLC to TC for the nonresponder females. The male responder group exhibited a decrease in fasting plasma glucose as compared to an increase for the responder group.

The significant associations between changes in aerobic capacity and some of the blood parameters in week comparisons using regression analysis also suggested that those who responded exhibited some appropriate changes. For example, the significant inverse association between preexercise fasting plasma glucose changes and aerobic capacity changes from weeks 0 to 4 indicates a training effect on fasting glucose levels in obese females. The significant positive relationship between postexercise fasting plasma glucose changes and changes in aerobic capacity from weeks 8 to 12 for females may reflect a conditioning effect. The direct relationship between mean preexercise HDLC change and weight change from weeks 8 to 12 for the males may have been due to increased muscle mass with longer running times. This direct association

between weight and HDLC is in disagreement with past work (Wood & Haskell, 1979). Examination of endocrinological changes would have been of interest. The lack of consistent significant associations across time between aerobic capacity changes and blood parameter changes limits conclusions.

The sex differences observed for aerobic capacity, the blood parameters, and the responder versus nonresponder groups are difficult to interpret. These differences may be due to the original differences in the level of conditioning and physical fitness between the sexes at the beginning of the study, rather than due to sex per se. If adjustments had been possible at the beginning of the study to compare the sexes on an equal basis, i.e., correcting for the level of fitness, sex comparisons of the blood data would be more meaningful.

The significant pre- and postexercise differences for fasting plasma glucose for both sexes illustrate the use of glucose as an energy source in the maximum effort stress test. The lack of significant changes for either males or females from pre- to postexercise for TC and HDLC may suggest that cholesterol is not directly involved in energy metabolism during acute periods of maximum exercise. The alterations observed from pre- to postexercise may have occurred at random. Consideration of changes in free fatty acid availability and triglycerides in response to acute bouts of maximum exercise stress would be of interest.

Future research with larger sample sizes, a longer time period, and with better control of intensity of effort in the subjects may result in more significant findings than observed here. The data currently available support the contention that exercise has positive effects on physical condition and obesity. Wood & Haskell (1979), Lopez-s et al. (1974), Dressendorfer & Gahagen (1979) among others cited previously have found significant physiological changes with regular aerobic activity. Most of this past work has been conducted with males.

Recommendations for future research include the repetition of this study with both males and females with greater care and control with data collection. A longer time period is essential to observe significant physical changes. Careful monitoring of the subjects' intensity of effort and progress by the jogging therapists is necessary. The use of hydrostatic weighing for body composition estimates would be preferable over skinfold measurements. This is true particularly with very obese individuals, where the use of skinfold calipers may not be possible due to the extreme thickness of the fat layer. If possible, more complete blood sampling from subjects and thus, increased sample size, would improve the power of statistical tests to detect significant differences. A major limitation was the lack of a control group for comparisons with the exercise group. The use of controls would help delineate those changes occurring due to the exercise conditioning versus random occurrences.

While the results of this research indicated inconsistent significant physical changes with regular aerobic activity, as a pilot study it has provided valuable information concerning design and data collection. A repetition of this research, with the changes suggested, is necessary to more thoroughly evaluate the nature and number of the positive results of exercise on improving cardiovascular function and thereby decreasing CHD risks associated with obesity in males and females.

CHAPTER 5

SUMMARY

This dissertation research examined the influence of a 24-week jogging program on fasting plasma total cholesterol, fasting plasma high density lipoprotein cholesterol, the ratio of high density lipoprotein cholesterol to total cholesterol, fasting plasma glucose, and body weight/fat in a sample of obese males and females, 18-30 years of age and 15-40 percent over their ideal weight. The goal was to determine if a jogging program could improve cardiovascular function (as measured by maximal volume of oxygen consumed in millileters per kilogram in body weight) and favorably alter body weight and composition. Changes in these physiological factors were examined in relation to changes in the blood parameters. Appropriate alterations in mean TC, HDL-C and fasting glucose in obese females and males would improve the risk profile of the subjects regarding the development of coronary heart disease.

Thirty-nine volunteers were recruited from the UNC-G student-faculty-staff population. The first 12-weeks of the program consisted of one-on-one contact between each subject and one particular trained jogging therapist. In the second twelve weeks, each participant was expected to run on his or her own at least 60 minutes, four times per week.

The first four weeks of the program consisted of walking and jogging with a therapist for 20 minutes, three times per week. Heart rate was monitored and maintained in the range of 65-75% of the maximum heart rate as determined in a maximum stress test. Weeks 5 through 8 consisted of walking and jogging with the therapist for 40 minutes, three times per week. Weeks 9 through 12 consisted of 60 minutes of jogging with the therapist three times per week. During the second 12 weeks the subject was expected to run 60 minutes, four times per week, on his or her own.

Each volunteer was interviewed for screening purposes. All subjects had no known history of angina, high blood pressure, hyperlipidemia and/or overt diabetes. An initial treadmill stress test was administered at week 0. Fasting blood samples were obtained before, after, and 20 minutes after exercise. Oxygen consumption was determined. Heart rate during the test and the maximum heart rate were measured. Prior to the test, supine blood pressure readings were obtained. Height, weight, and four skinfold measurements (tricep, bicep, subscapular, suprailiac) were determined. These measurements were collected again at weeks 4, 8, 12, and 24.

Fasting blood samples were centrifuged and the plasma removed. Glucose analysis was carried out on the fresh plasma. Fasting plasma total cholesterol and HDL-cholesterol measurements were determined later from frozen samples. The Technicon AA II system was used for the plasma analyses.

Statistical analyses of the effect of changes in weight and aerobic capacity over time on the changes in the blood data consisted of simple regression procedures. The regression procedure was also used to examine the relationship of body composition (as estimated by the Quetelet index) with the relative concentrations of the blood parameters. Sex differences and the acute responses to exercise were evaluated using ANOVA.

Data from week 24 and the 20-minutes-after exercise data were eliminated because the number of subjects having complete data was too small to consider in these analyses. Seven out of 16 males and eight out of the 23 females dropped out during the course of the study.

Mean body weight losses from weeks 0 through 12 for both males and females were nonsignificant. Mean aerobic capacity increased for both males and females over the 12 weeks. The changes were significant ($p=0.0367$) for females but not for males.

The data for sum of skinfold changes and percentage of fat estimations were eliminated due to incompleteness, measurement problems, and questionable reliability and validity. No significant relationships for the association of body composition using the Quetelet index (weight/height^2) with the blood parameters were noted.

Regression analysis of the relationships between weight and $\dot{V}O_{2max}$ml/kg·min changes with blood value changes over time indicated some significant changes for males and females. For weeks 0 to 4, females exhibited a significant ($p=0.0075$) inverse relationship

between changes in fasting plasma glucose and aerobic capacity pre-exercise. There was also a significant (p=0.0494) postexercise direct association between the ratio of HDL-cholesterol to total cholesterol and aerobic capacity for the females.

Between weeks 4 and 8 females exhibited a significant (p=0.0228) association between mean fasting preexercise plasma total cholesterol and aerobic capacity. A significant (p=0.0204) positive correlation between the ratio of HDLC to TC and aerobic capacity, postexercise was also observed. In this time comparison, males exhibited a significant (p=0.0218) positive relationship between fasting plasma total cholesterol and aerobic capacity.

For weeks 8 to 12, a significant (P=0.0456) positive association was observed for females between fasting postexercise plasma glucose and aerobic capacity. A significant (p=0.0492) positive relationship was noted preexercise between fasting plasma HDL-cholesterol and weight for males.

Significant pre- and postexercise differences in mean fasting plasma glucose occurred for both males and females. For females, post-exercise fasting plasma glucose concentrations were significantly (p=0.0001) higher than pre exercise values for weeks 0 through 12. For males, post exercise levels were significantly higher than pre-exercise for weeks 4 (p=0.0001), 8(p=0.0014), and 12 (p=0.0033).

Males and females differed significantly in preexercise fasting glucose levels at week 0 (p=0.0294), 8 (p=0.0464), and 12 (p=0.0117). The sexes differed significantly in fasting plasma HDL-cholesterol at

week 0 (p=0.0436) and week 12 (p=0.0052) pre-exercise and week 8 (p=0.0256) postexercise. The HDLC to total cholesterol ratio values were significantly different between the sexes at week 8, post-exercise (p=0.0464) and week 12, preexercise (p=0.0495). However, the comparisons of the males and females were exacerbated due to their unequal starting level of fitness. If adjustments could have been made to account for the differences in levels of conditioning, the sex comparisons would have been more meaningful. However, adjustments were not possible due to the lack of appropriate data (fat free weight, etc.).

Subjects were compared according to a minimum 1.4 kg increase in aerobic capacity. The groups were designated as responders versus nonresponders. For the females, the responders exhibited a greater mean weight change (loss) and higher mean $\dot{V}O_{2max}$ml/kg·min than that seen in the nonresponder group. Over the 12 weeks the responder group exhibited an increase in fasting plasma total cholesterol along with rises in fasting plasma HDL-cholesterol and the ratio of HDLC to total cholesterol. In contrast, the non-responder females showed a decrease in fasting plasma total cholesterol, a very small increase in fasting plasma HDL-cholesterol, and a decrease in the ratio of HDLC to total cholesterol. Mean fasting plasma glucose levels decreased more for the nonresponder females than the responder females over the 12 weeks.

The male responder group had a greater mean weight loss and a greater increase in aerobic capacity than the

nonresponder group. Fasting plasma total cholesterol increased for the responder group and decreased for the nonresponder group. The responder group's fasting plasma HDLC levels and ratio decreased while those for the nonresponder group increased. For fasting plasma glucose, the responder group exhibited a decrease while the nonresponder group's concentrations rose over the 12 weeks. ANOVA analyses indicated that none of the blood data comparisons for these groups were significantly different.

The significant findings in this study occurred in isolated instances. No generally consistent pattern of significant changes in the data were observed. The inconsistency of the findings make interpretations difficult.

Several factors affected the results of this investigation. The-12 week time period was not sufficient to invoke changes in body weight, aerobic capacity, and the blood parameters examined. The intensity of effort by the subjects in the running sessions varied considerably. Large changes in physical condition could not be expected with these time and degree of intensity limitations. In addition to these limiting factors, the statistical analyses of the blood parameters were limited in power due to small sample sizes.

Recommendations for future research include the repetition of this study with both females and males, with greater care and control with data collection. A longer time period is essential for the observation of significant physical changes. Careful monitoring of the subjects' intensity of effort and progress by the jogging

therapists is necessary. The use of hydrostatic weighing is preferable over skinfold measurements for validity and reliability. Accounting for different levels of fitness at the onset of the program between the sexes would allow for examination of true sex differences in the blood parameters.

A major limitation of the study was the lack of a control group for comparisons with the exercise group. The use of controls would help delineate those changes which occur due to the exercise conditioning versus random occurrence. The problem of obtaining blood samples needs further consideration; maintaining an adequate sample size is a necessity.

While the results of this research indicated inconsistent significant physical changes with regular aerobic activity, as a pilot study it has provided valuable information concerning design and data collection. A repetition of this research, with the changes suggested, is necessary to more thoroughly evaluate the nature and number of positive effects of physical conditioning on cardiovascular function and CHD risk in obese males and females.

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APPENDIX A

Jogging Therapist Training Instructions

GUIDELINES FOR EXERCISE PRESCRIPTION[1]

I. Introduction

This section deals with the prescription of exercise for participants entering the beginner's walk/jog program.

II. Principles of Training

Certain basic principles of training should be reviewed before discussing the actual method of exercise prescription for the obese participant. The principles are followed in the design of programs and should be understood by the therapists facilitating the exercise phases of the rehabilitation program.

A. Starting Level

The initial exercise level for the obese client should be kept at a low level due to the long deconditioning period that usually preceds entrance into the program. This level should be kept low for two reasons: 1) The muscles, ligaments, and tendons usually require a brief period of adjustment in the early stages of a training program to prevent undue soreness and minimize orthopedic problems; 2) The cardiovascular system of each client is compromised by virtue of the fact that they are diagnosed as obese and a low-level starter program will allow a safe adaptation.

B. Warm-up and Cool-down

Every participant should warm up slowly and gradually before each exercise session to allow adequate circulatory and metabolic adjustment to the stimulus phase of the exercise. With warm-up the body temperature increases, vasodilation of skeletal heart muscle occurs, and the metabolic demands are met in this manner.

After the stimulus phase each participant should cool down to allow a gradual return of the metabolic and circulatory demand to a safe level. During this phase the symptoms of exertion (dyspena, tachycardia) should return to normal before the individual leaves the program.

C. Overload and Progression

In order to produce a training effect the overload principle must be applied; ie., in order for a

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[1]Adapted from the guidelines of Bowman-Gray walk-jog program, Winston-Salem, NC.

physiological adaptation to occur the system must be subjected to a workload that is above the level to which it is accustomed. The overload, however, must be applied in the proper dosage so that it is high enough to exceed the threshold, yet low enough to be under the safe upper limit of the prescribed range. The overload is controlled by the intensity, duration and frequency of training. The overload must also be applied in a gradually increasing manner, and the rate of progression is important. The starter program allows for a slow adaptation until the overload can be applied at a faster rate. The rate of progression is determined by the experience of the individual. If the participant is not experiencing any sign of over-exertion during the program (extreme fatigue, dyspena) or overuse (joint, tendon or muscle injury) then the rate of progression is safe. The jogging therapist should be aware of these signs and symptoms during the program.

D. Specificity of Training

The change that occurs with specific types of training are specific to that training, and specific for the target muscle groups. Training for strength will not improve endurance, and training for endurance will not improve flexibility. Participants are being trained for general cardiovascular fitness which will be achieved by a program of walking/jogging and alternative rainy day methods of aerobic dance, etc. Weight loss should occur from an increase energy expenditure in conjunction with a modified eating plan.

III. Exercise Prescription

The exercise prescription is developed according to set criteria that establish the initial load and adjust it for overload. These criteria are identified below and the manner in which they are used in the prescription is explained.

A. Type of Activity

The type of activity prescribed for the participant is divided into warm-up activity, aerobics and cool-down activity. The warm-up activity consists of stretching exercises designed to increase flexibility.

Stretching (The Runner, September, 1980)

The therapist should keep in mind that the stretching phase be implemented according to the client's individual muscular structure, flexibility and varying tension levels. The key is regularity and relaxation. The object is to reduce muscular tension,

thereby promoting freer movements--not to concentrate on attaining extreme flexibility which leads to over-exertion.

A regular stretching program will help to:

- Reduce muscle tension
- Improve coordination
- Increase range of motion
- Prevent injuries such as muscle strains
- Decrease perceived exertion in response to stress
- Develop body awareness
- Promote circulation

The easy stretch

When you begin the stretch spend 10-30 seconds in it. Do not bounce. You want a static rather than ballistic movement. Go to the point where you feel a mild tension, and try to relax as you hold the stretch. The feeling of tension should subside as you hold the position. If it does not, ease off until you find a level of comfort. The easy stretch reduces muscular tightness and readies the tissue for the developmental stretch.

The developmental stretch

Move further into your stretch until you feel a mild tension and hold for 10-30 seconds. NO BOUNCING. Be in control. The tension should diminish. If not, ease off to the point of comfort. The developmental stretch fine tunes the muscles and increases flexibility.

1. Pelvic thrust. Stand erect with hands at sides and feet spread comfortably apart. Slowly bend the body back, while thrusting the pelvic area forward and raising hands over head.

2. Stride. To stretch the muscles at the top of the leg and in front of the hips, move one leg forward as shown so that the knee of the forward leg is above the ankle of that leg. The other knee should be resting on the floor. Lower the front of your hip downward to create an easy stretch.

3. Floor Squat. To stretch the groin area, put the soles of your feet together and hold onto your toes. Gently pull yourself forward, bending from the hips until you feel the stretch in your groin.

4. Modified V. Extend your legs in a V on the floor. Take your right hand down the inside of your right leg, grasp the bottom of your foot and extend the right leg into the air. Repeat with the left leg.

5. Backover. Lie on the floor with arms extended behind your head. While exhaling, lift your legs over your head keeping them straight until you feel the stretch in your back. DO NOT ATTEMPT THIS STRETCH if you have any history of back trouble.

6. Leg Hold. Sit with legs in the extended V position on the floor, grasping the lower part of the extended leg with both hands. Lean from the hips toward the leg you are holding. Repeat with the other leg.

7. Dance. Stand erect with feet spread wider than shoulder width, arm out to the side, the other straight ahead. Bend one leg to the side where the arm is straight ahead, keeping the other leg extended. Hold and repeat with the reverse position.

8. Leg Pull. Press against a wall with one hand. To stretch the quadricops and knee, hold the top of one foot with the opposite hand and gently pull your heel toward your buttocks. Hold and repeat with the other leg.

9. Right Angles. Find a suitable platform about waist high and place your leg on it, forming about a 90° angle with your other leg. (Lessen the angle if you have difficulty). Face straight ahead and slowly bend sideways with your shoulder toward the raised knee. Hold. Stand erect and lower body toward the knee on the ground. Hold and reverse the position.

10. Wall Push. Lean against a flat surface. One foot should be about 18" from wall and the knee bent with the heel flat on the ground. The other leg should be extended with the heel also being flat. Hold, then switch legs.

The aerobic activity consists of the walk/jog program. It is this phase of the program that is the most important since it develops the heart circulatory and muscular systems. Each subject will begin jogging therapy by completing a series of mini-walks and mini-runs. The purpose of these "mini cycles" is to promote enjoyment and a sense of accomplishment, rather than pain or muscle soreness.

Jogging Therapy Program

Week		Frequency	Time
1-4	Walking-Jogging with therapist	3 x / wk	20 min.
5-8	Walking-Jogging with therapist	3 x / wk	40 min.
9-12	Jogging with therapist	3 x / wk	60 min.
13-24	Jogging independently	4 x / wk	60 min.

The cool-down activity consists of selected stretching and relaxation sessions.

B. Intensity

The intensity of effort is the most important criterion to control since it represents the level of stress upon the subjects cardiovascular system. It is important from both a safety and a training viewpoint. Exercise at too high an intensity is not only ineffective but it is unsafe, while exercise at too low an intensity will fail to elicit a training effect.

The intensity of effort is prescribed after the graded exercise test and is based upon the heart rate and ECG response during the test. The upper safe limit of the heart rate training range and the exercise prescription is based upon this value. This upper safe limit can be in the form of specific heart rate or a specific percent of the functional capacity.

Functional Capacity

The range of heart rate is used to determine the various percentages of functional capacity rather than the maximal heart rate. The percentages of heart rate range correlate more closely with the actual percentages of the metabolic capacity (percent $\dot{V}O_2$ max or maximal oxygen uptake) than the percent of maximal heart rate, i.e., it is more representative of the actual work being done.

Percentages of functional capacity are determined as follows:

HR max	=	100%	=	180 beats/min
		90%	=	169
Upper limit	=	85%	=	163.5
		80%	=	158
		70%	=	147
Threshold	=	60%	=	136
		50%	=	125

		40%	=	114
		30%	=	103
		20%	=	92
		10%	=	81
HR rest	=	0%	=	70 beats/min

This computation method is used to determine the recommended heart rate range for training for each participant after each graded exercise test. Since each person differs in HR max and HR rest, each individual will have his own heart rate range for training.

In the early stages of training, the clients are instructed to stay within the 30 to 60 percent range as a starter program. After he/she has progressed with no complications; has learned to correctly count pulse rate; and recognizes his/her limitations, the 60 percent level can be exceeded. The participant should then operate within the training zone of 60-85%.

Recommended Readings

American College of Sports Medicine, Guidelines for Graded Exercise Testing and Exercise Prescription: Lea and Felinger, 1975.

American Heart Association, Exercise Testing and Training of Individuals with Heart Disease or at High Risk for its Development: A Handbook for physicians, New York: American Heart Association, 1975.

Amsterdam, E. A., J. H. Wilmore and A. N. DeMaria (eds.) Exercise in Cardiovascular Health and Disease, New York: Yorke Medical Books, 1977.

Cooper, Ken H. Aerobics. New York: Bantam Books, 1977.

Katch, Krank I., McArdley, William D. Nutrition, Weight Control and Exercise. Boston: Houghton Mifflin Co., 1977.

Tips: To be Included

- Breathing
- Clothing
- Creative Visualization
- Relaxation
- How to monitor Heart Rate
- Aerobics

When the obese lose weight they may be disillusioned when they find that most of their difficulties--mainly mental--still persist.

Run, before eating: With the body at rest, the energy needs are primarily in the internal organs, the heart, the digestive system, etc. About 80% of the blood supply goes to these organs, and 20% goes to the inactive skeletal muscles. But as you move into action the blood starts shifting to service these muscles with energy until, at maximum exertion, a complete shift has occurred with about 80% of the blood (and O_2) going to the muscles.

After exercise, it takes some time (an hour or more) for the blood to shift back again. Thus, the internal organs during this period are not being serviced maximally. In layman's language, the digestive system is then in no mood to take on a lot of food.

APPENDIX B

Treadmill Testing Procedures

Outline of Test Procedure: Rosenthal Laboratory, University of North Carolina at Greensboro.

I. Pre-Test Procedure
   A. Subject Preparation
      1. anthropometric measures, fasting blood sample
      2. apply electrodes
      3. 5-min rest -- record RHR and BP
   B. Warm-up

II. Test Procedure
   A. Administer Bruce T_m Protocol
   B. Record HR each minute
   C. Collect expired air at 2-minute intervals
   D. Collect blood sample at end of test

III. Post-Test Procedures
   A. Analyze expired air for FEO_2, $FECO_2$, volume, temperature
   B. Record BP
   C. Take blood sample after 20 minutes' rest

Position (5) Needed for Each Test--Review of Responsibilities

1. EKG (1 person)
   a. apply electrodes carefully
   b. check transmission
   c. record HR, transfer data
   d. remove electrodes
   e. clean-up

2. Treadmill (1 person)
   a. set clock and treatmill
   b. review procedures with subject
   c. run test, help record data for gas analysis

3. Spotter (1 person)
   a. administer any anthropometric tests, 24 hr history
   b. stand behind subject throughout warm-up and test
   c. put on headgear and noseclip and remove them.
   d. help subject on and off Tm
   e. clean headgear

4. Gas Collection and Analysis (2 people)
   a. check bags, analyzer, data sheet (record collection times)
   b. prepare headgear, mouthpiece, noseclip
   c. analyze content of bags

Treadmill Operator

The following responsibilities are assigned to the treadmill operator:

1. Upon arrival in lab, check T_m setting. Be sure both speed and grade are functioning properly. If not, check connection with Tm.

2. Briefly remind subject of procedure for getting on and off Tm.

3. Begin warm-up by saying something like: "_____(name)_____," we'll begin your warm-up. You'll be walking at ....." Then after the subject is comfortable go over stress test procedure. Remind subject of hand signals.

4. After warm-up, set beginning test speed and grade.

5. Watch subject for any signs of distress.

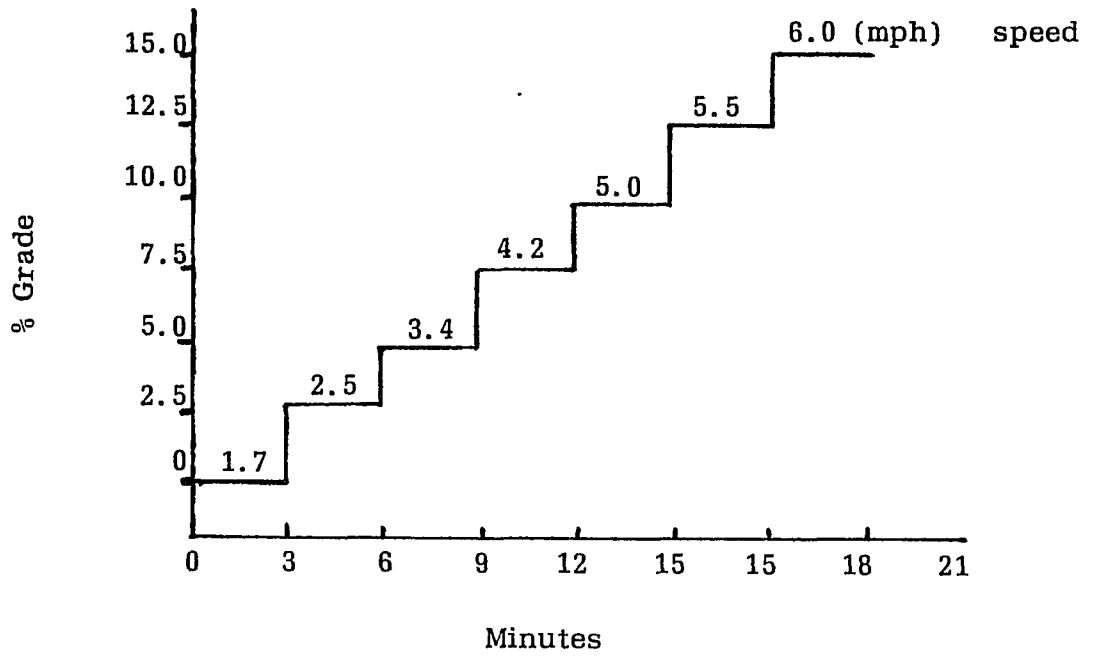
6. Be alert to time framework for changing speed and grade.

7. Stop test immediately if difficulties arise. Otherwise, ask subject for hand signal again to be sure of his/her fatigue level.

8. After test, reset Tm to warm-up speed and grade.

Treadmill Protocol: Bruce Protocol

After 5-minute warm-up at _____ mph and _____% grade, subject will rest briefly while headgear and mouthpiece are adjusted. The test will begin at 1.7 mph and 0% grade. The speed and grade will increase after each 3 minute period until maximal volitional fatigue.



EKG Recording:

Heart rate will be recorded by direct lead during the last 10-second period of each minute. Only record 10-12 "peaks." Label each recording with the workload and time (i.e., min 4, 2.5 mph, 2½% grade). Immediately upon completion of the test mark 7 R waves and distance between these in mm. Look up corresponding HR (b/min) from chart and circle. This procedure allows for a check system. After having a "check," record data on data sheet.

Gas Collection and Analysis

1. Upon arrival in lab, evacuate all bags and close valves.
2. Set up headgear, mouthpiece, noseclip, towels.
3. Check analyzers, drierite, telethermometer.
4. Record subject's name, weight, height along with date, T° lab, barometric pressure, name of test.
5. Rotate valves connected to bags at the exact time indicated. will involve either 1-min or 2-min sampling procedures.

6. After test completion, analyze contents of all bags according to procedures described in "dry" run
   a) take 30 sec. sample for O_2 and CO_2
   b) push expired air through CD-4 and record volume
   c) record temperature of the gas

*Be sure time of gas collection is written in space provided above results.

APPENDIX C

Determination Methods for Total Cholesterol, High Density Lipoprotein Cholesterol, and Glucose

Technicon™ AutoAnalyzer™ II

MICRO

Industrial Method #339-26

4/19/77

A/IV/a/3

CHOLESTEROL (DIRECT)
(Range: 10-500 mg/dl)

HISTORY

The Technicon cholesterol (direct) method is based on the work of Levine, Morgenstern, and Vlastelica[1] who automated the manual cholesterol method of Huang, Chen, Wefler, and Raftery.[2]

The reaction of cholesterol with strong acids was first described by Liebermann[3] in 1885 and developed by Burchard[4] in 1890. In the classical Liebermann-Burchard reaction, concentrated sulfuric acid is added to a solution of cholesterol and acetic anhydride. During the ensuing reaction, several color changes are observed in the reaction mixture. The initial red color changes to violet and finally to green at the completion of the reaction.

The main problems in developing a practical cholesterol method based on the Liebermann-Burchard reaction were the instability of the acid reagent and the instability of the final product. In 1961, Huang et al formulated a stable Liebermann-Burchard reagent. This formultion consisted of the following:

Glacial acetic acid	300 ml
Acetic anhydride	600 ml
Sulfuric acid	100 ml
Sodium sulfate, anhydrous	20 g

It was recommended that this stable reagent be mixed with the unknown cholesterol sample and that the mixture be allowed to stand at room temperature for 20 minutes; after which time, the absorbance of the reaction mixture was determined spectrophotometrically at either 550 nm or 610 nm.

After evaluating the cholesterol method of Huang et al, Ness, Pastewka, and Peacock[5] suggested that the cholesterol be added to a chilled (0°C) color reagent and then be allowed to slowly reach room temperature. It was reported that by controlling the reaction in this manner, broad and nonspecific absorption curves were prevented. Subsequent automation of the method by Levine et al introduced several other modifications: The formulation of the cholesterol color reagent was modified to prolong pump tube life; the cholesterol color reagent was chilled as suggested by Ness et al; and the absorbance of the analytical stream was measured at 630 nm, to minimize the effect of serum pigments.

SPECIAL MERITS AND LIMITATIONS

The automated cholesterol method allows rapid and precise determination of cholesterol in serum. It is limited, however, by the large number of substances known to interfere with it. In this regard, refer to the paragraph in this method titled "Interfering Substances."

EXPLANATION OF METHOD

As illustrated in the flow diagram, the prediluted serum sample is mixed with an air-segmented stream of cholesterol color reagent. During the ensuing reaction of cholesterol with Liebermann-Burchard reagent, the color of the reaction mixture changes from red, to violet, and finally to green. The following mechanism has been proposed[6] for this reaction:

(1) Cholesterol + H_2SO_4 ⟶ bis-cholestadienyl monosulfonic acid
(2) bis-cholestadienyl monosulfonic acid + H_2SO_4 ⟶ bis-cholestadienyl disulfonic acid

The absorbance of the analytical stream is measured at 630 nm in a flowcell that has a 15-mm light path and an inside diameter of 1.5 mm.

REAGENTS

GENERAL INSTRUCTIONS

1. The technologist must adhere strictly to all warning and precaution statements specified within this Section. Refer to the specific reagent container label for the product expiration date and for pertinent storage information.
2. All reagents, whether supplied by Technicon in a ready-for-use form or prepared by the technologist must be free of particulates and other foreign matter, and must not exhibit any obvious color changes. EACH REAGENT SHOULD BE EXAMINED THOROUGHLY BEFORE USE. Reagents displaying any signs of instability should be replaced.

CHOLESTEROL COLOR REAGENT (Technicon Product No. T21-0901)*(Must be prepared by the user)

Composition:

Part A (Technicon Product No. T01-0883)	1 bottle
Part B (Technicon Product No. T01-0890)	1 bottle

Preparation Instructions:

The two-part Cholesterol Color Reagent must be prepared for use as follows:

1. Obtain one bottle of Part A and one bottle of Part B.

-WARNING!-

THE FOLLOWING WARNING APPLIES TO THE CONTENTS OF PART A, PART B, AND THE MIXTURE PRODUCED BY COMBINING PARTS A AND B.

THE TECHNOLOGIST MUST MAINTAIN A CONSTANT AWARENESS OF THE CORROSIVE NATURE OF THESE REAGENTS. WHILE PERFORMING THE PREPARATION PROCEDURE, EXERCISE EXTREME CARE TO PREVENT REAGENT SPILLS. WHILE IT IS ALWAYS CONSIDERED GOOD LABORATORY PRACTICE TO WEAR PROTECTIVE GLASSES, THE TECHNOLOGIST SHOULD CONSIDER IT MANDATORY WHILE HANDLING THESE REAGENTS.

-DANGER!-

CAUSES SEVERE BURNS. VAPOR HARMFUL. COMBUSTIBLE. DO NOT GET IN EYES, ON SKIN OR CLOTHING. DO NOT BREATHE VAPOR.

* Available only as a kit, individual components (Part A or Part B) cannot be ordered separately.

-POISON-
(CALL A PHYSICIAN)

FIRST AID

Affected eyes: Irrigate thoroughly with water. In severe cases or where splashing occurred, obtain medical attention.
Skin contact: Drench with water. Remove contaminated clothing and wash before reuse.
Vapor inhaled: Remove from exposure, rest, and keep warm. Give drinks to relieve throat irritation.
If swallowed: Wash out mouth thoroughly with water. Give plenty of water to drink followed by milk of magnesia. Obtain medical attention.

2. Prior to combining Part A and Part B, chill both parts to approximately 4°C. Technicon recommends that the two bottles (Part A and Part B) be stored in a refrigerator overnight prior to use.

3. Open the two bottles (Part A and Part B). Retain the cap from the bottle containing Part B.

4. Carefully pour the contents of the bottle containing Part A into the bottle containing Part B.

   NOTE: When Parts A and B are combined, a slight temperature rise will result. This temperature rise is not detrimental to the product.

5. Tightly replace the cap on the bottle containing the mixture of Parts A and B (working reagent), and invert the bottle three times under a hood.

6. Remove the cap from the bottle, and place the bottle in the system refrigerator. If the system is not equipped with a refrigerator, store the working reagent in an ice bath.

   IMPORTANT: The temperature of the working reagent must be kept at approximately 4°C prior to and during use.

7. Connect the appropriate reagent line.

STABILITY

When handled and stored as directed, the working reagent and its component parts are stable for the periods of time indicated below:

Part A (Technicon Product No. T01-0883) - One year at room temperature*
Part B (Technicon Product No. T01-0890) - One year at room temperature
Working Reagent (Parts A and B combined) - One month when stored at 4°C

IMPORTANT: Tightly cap the bottle of working reagent during storage. This will prevent the reagent from possibly absorbing moisture from the atmosphere.

---

* During storage, Part A may show signs of slight discoloration (yellow). This discoloration, within limits, does not affect the usefulness of the reagent.

WETTING AGENTS

REAGENTS	TECHNICON NO.	WETTING AGENT	INSTRUCTION
Cholesterol Color Reagent (Liebermann-Burchard)	prepared by user	None Required	--

REAGENT CONSUMPTION DATA

Reagent consumption data are provided to assist the technologist in determining his particular reagent requirements. By multiplying the reagent flow rates (ml/min or ml/h) provided in Table 1 by the anticipated average instrument operating time, the technologist can project reagent preparation and reordering schedules.

Table 1. - REAGENT FLOW RATES

REAGENT	FLOW RATE ml/min	ml/h
"Cholesterol Color Reagent" (must be prepared by user)	1.6	96.0

SAMPLE DILUTION

Serum specimens with cholesterol concentrations greater than 500 mg/dl should be diluted with distilled water or saline (0.9% NaCl) and reassayed.

INTERFERING SUBSTANCES

A number of substances have been reported to cause physiological changes in serum cholesterol concentrations.[8,9,10,11] A comprehensive list of these substances, their serum concentrations, and their possible physiological involvements is beyond the scope of this method.

Several substances and conditions have been reported to interfere with the cholesterol (direct) method and, if present in the serum reagents, or system, may interfere in the manner described:

Amphotericin B[8] - Large concentrations (1 mmol/liter) of amphotericin B have been reported to cause elevated serum cholesterol values.

Bilirubin - Empirical data derived from the addition of known amounts of bilirubin to serum indicate that for each mg/dl of bilirubin present in the serum sample, an absorbance equivalent to approximately 4 mg/dl of cholesterol occurs.

Example: A serum containing 5 mg/dl bilirubin will give a 20 mg/dl positive error for cholesterol. If the bilirubin level is known, the correction can be readily made.

Hemolysis[9] - Hemolysis has been reported to cause elevated serum cholesterol values.

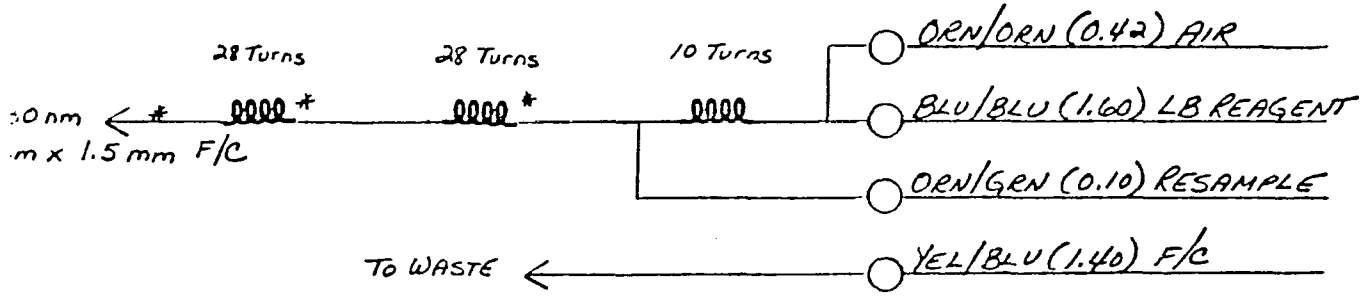
Lipemia[9] - Lipemia has been reported to cause turbidity in nonextraction cholesterol methods, thus elevating the cholesterol values.

Lipochrome[9] - Lipochrome has an appreciable absorbance at 630 nm and can be detected as cholesterol, thus causing an apparent increase in cholesterol concentration values.

Tryptophan[9] - Tryptophan has been reported to elevate cholesterol values.

CHOLESTEROL - SMA MICRO

Manifold #: 157-A058-02



* KEL-F TUBING

SAMPLE INTERACTION STUDY

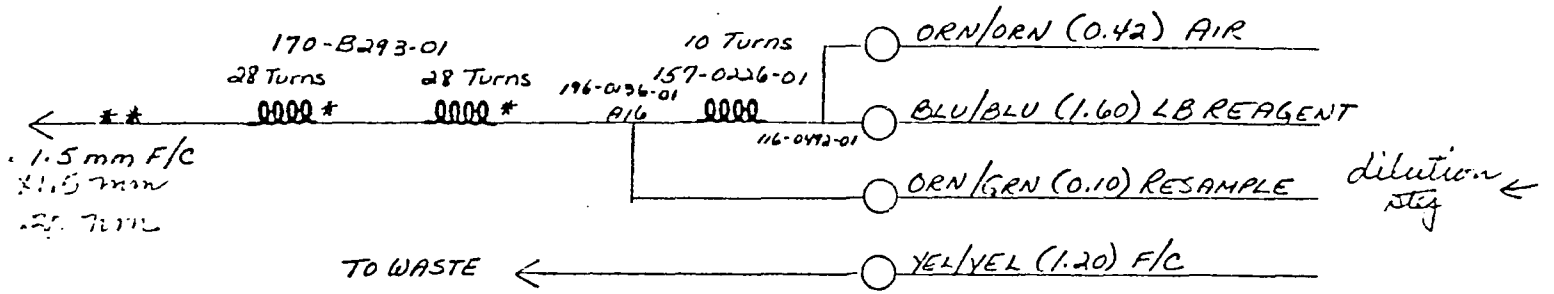
Experimental evidence has indicated that within the expected range of values for the cholesterol method, the amount of sample interaction (carryover) is not clinically significant (less than 5%). In the extreme case where a sample with an extremely high concentration is followed by a sample with a very low concentration, the amount of sample interaction may result in a clincially significant (greater than 5%) elevation of the low-value sample. In routine clinical use, this situation is seldom encountered.

The quantitative effect of carryover at any concentration level is directly related to the operating condition of the system. Conditions such as worn pump tubes, a dirty flowcell, etc., will have a deleterious effect on results. Accordingly, the user is urged to adhere strictly to the maintenance recommendations prescribed in the system operation manual .

METHOD PERFORMANCE CHARACTERISTICS	AAII	SMA
Sensitivity at 500 mg/dl	0.31	
Coefficient of Variation at 195 mg/dl	±0.53%	

CHOLESTEROL - AAII MICRO

Manifold #: 170-A087-01



* KEL-F TUBING, 0.050" ID

** 0.50 KEL-F TUBING – CONNECT DIRECTLY FROM MANIFOLD TO FLOWCELL.

TROUBLESHOOTING

For troubleshooting purposes, note the following information for the cholesterol (direct) method:

	SMA	AAII
1. SMA Reference Serum (200 mg/dl) Absorbance	-	
2. Full Scale (500 mg/dl) Absorbance	-	

NOTE: The absorbances (E) specified are approximate. Minor variations in reagent pump tubes, flowcells, etc., may cause deviations from the specified values. Acceptable ranges should be determined for each system over a period of time.

Table 2 provides a list of possible trouble that could be encountered while running the cholesterol (direct) method. The Table also lists the causes of each difficulty and corrective maintenance procedures:

-WARNING-

THE TECHNOLOGIST MUST MAINTAIN AN AWARENESS OF THE CORROSIVE NATURE OF THE CHOLESTEROL COLOR REAGENT. WHILE PERFORMING THE FOLLOWING TROUBLESHOOTING PROCEDURES, EXERCISE EXTREME CARE TO PREVENT REAGENT SPILLS ON LABORATORY PERSONNEL AND EQUIPMENT. WHILE IT IS ALWAYS CONSIDERED GOOD LABORATORY PRACTICE TO WEAR PROTECTIVE GLASSES, THE TECHNOLOGIST SHOULD CONSIDER IT MANDATORY WHILE TROUBLESHOOTING THE CHOLESTEROL METHOD. REFER TO THE PARAGRAPH TITLED "REAGENT," FOR A COMPLETE DESCRIPTION OF THE FIRST AID TO BE APPLIED IN THE EVENT AN ACCIDENT DOES OCCUR.

Table 2 - TROUBLESHOOTING

TROUBLE	PROBABLE CAUSE	CORRECTIVE MAINTENANCE	NOTE
No reaction	Sample or pull-through line clogged or pinched	Change tubing, and clean out all nipples with a fine wire probe.	Pump platen *SHOULD NOT* be removed before the channel is pumped dry. If removed, there will be a back flow of reagent into the sample line when reagent is left in channel. WARNING REAGENT LEFT IN THE TUBING IS A POTENTIAL PERSONAL HAZARD AND ALSO CAN RESULT IN A RAPID DEGRADATION OF THE PUMP TUBES.
	Defective reagent	Change reagent.	Prepared reagent must be refrigerated.
High results	Bilirubin interference	Subtract bilirubin interfer[illegible]	For each mg/dl of bilirubin there will be [illegible] mg/dl increase [illegible]

Table 2 - TROUBLESHOOTING (Continued)

TROUBLE	PROBABLE CAUSE	CORRECTIVE MAINTENANCE	NOTE
Noise, poor curves (loss of wash)	Defective cholesterol color reagent	Prepare fresh reagent	
	Loss of intersample bubble	Change debubbler line.	Check for presence of intersample bubbles at debubbler. Check bubble integrity at N13 nipple. Check bubble integrity at connections between polyethylene tubing and glassware.
	Precipitate in platinum nipple	The precipitate can be caused by an imbalance between reagent and the sample. The system should be cleaned with 1 *N* NaOH. Flush with air, then with 1 *N* NaOH, again with air, then with 0.25 *N* HCl solution, and finish with air.	

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[4] Burchard H: *Chem Zentr* 61:25-27 (1890) (Ger)

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7. Fredrickson DS, Levy
reaction: A fast kinetic method for serum cholesterol. Clin Chem 19(10):1128-1134 (1973)

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[9] Young DS, Thomas DW, Friedman RB, Pestaner LC: Effect of drugs on clinical laboratory tests. *Clin Chem* 18(10):1041-1303 (1972)

HDL ISOLATION FROM PLASMA

Derived from: Albers, J.J., G. R. Warnick, and M.C. Chenng. Quantitation of High Density Lipoproteins. Lipids 13 (12): 926, 1978.

Principle: The non-HDL apo-B associated lipoproteins are selectively precipitated from plasma by sulfated polysaccharides (heparin) and divalent cations ($Mn^{+}2$), leaving HDL in suspension.

Reagents:

1. Manganese Chloride Solution 1.06M: ($MnCl_2.4H_2$)-MW=197.91) Weigh out 209.78g $MnCl_2.H_2O$ and dissolve in a small amount of distilled water. Dilute to 1 liter volume with distilled water. This solution is thought to be stable indefinitely.

2. Sodium Chloride-0.15M (saline): Weigh out 8.77g NaCl and dissolve in about 500 ml distilled water. Bring up to 1 liter volume with distilled water.

3. Heparin-40,000 units/ml: Commercial heparin preparations contain different strengths of heparin by weight (units per mg). CHECK LABEL ON HEPARIN VIAL BEFORE PREPARATION:

   If heparin preparation contains 140 units/mg, weigh out .286g heparin and dissolve in 1 ml .15 M saline. Since the volume is so slight, use a very small glass vial to prepare solution. The heparin is hard to dissolve so it will be necessary to vortex the solution vigorously. Before removing an aliquot for the combined reagent, let the heparin solution set awhile and check to make sure all the heparin is in solution.

   Prepare fresh for each run.

4. Combined heparin-manganese reagent: Add .6 ml 40,000 units heparin/ml to 10 ml 1.06 M $MnCl_2.4H_2O$. Prepare fresh for each run.

PROCEDURE:

1. Remove plasma samples from refrigerator and allow to warm to room temperature.

2. Mix plasma samples well. Using a calibrated Oxford Macropipet (or volumetric pipet), transfer 2 ml plasma into 12 or 15 ml glass conical centrifuge tubes. Run duplicates.

3. Vortex combined heparin-manganese reagent well. Using a calibrated Oxford or SMI Micropipet, transfer .2 ml combined reagent into each centrifuge tube. A precipitate will form immediately.

4. Vortex each tube lightly and cover with plastic wrap or Parafilm.

5. Allow the tubes to stand at room temperature for 10 minutes.

6. Centrifuge tubes in the refrigerated centrifuge for 30 minutes. A hard pellet will form on the bottom of the tube.

   NOTE: It is necessary to use a swinging bucket rotor for this procedure in order to form a pellet which is lodged in the bottom of the conical centrifuge tube. If a fixed-angle rotor is used, like those on the Sorvall, a firm pellet will not form and the precipitate will smear along the sides of the tube.

7. Supernatants may be directly decanted, but to avoid possible contamination, remove most of the supernatant with a Pasteur pipet and store in a 7 ml or larger vial.

8. HDL are suspended in the supernatant. Cholesterol and triglyceride determinations are run directly on the supernatant following isopropanol extraction; i.e., treat supernatant like unfractionated plasma.

9. Prepare standard solutions of 20, 50 and 100 mg/dl with appropriate dilutions of the test set standards for making the standard curve. Analyze each standard in duplicate with each run.

Final concentrations:

92mM Mn^{2+}, 1.47 mg heparin/ml, 205.8 units heparin/ml

A constant amount of cholesterol in the supernatant has been measured over a heparin concentration range of 92-734 units/ml

(Ishikawa, T. T. et al., Lipids 11(8): 628, 1976) and 1.2 - 2.0 mg/ml (Warnick, G. R. et al., J. Lipid Res. 19: 65, 1978). A final Mn concentration of 92mM has been shown to be more ideal for HDL precipitation from plasma than the commonly used concentration of 46mM (Albers et al., Lipids 13: 926, 1978).

TECHNICON METHOD No. SE4-0036FJ4 DATE RELEASED: SEPTEMBER 1974

GLUCOSE (GLUCOSE-OXIDASE)

HISTORY

The forerunner of the present Technicon glucose method, introduced in 1956 by Keston,[1] determined glucose in biological fluids by a glucose-peroxidase enzymatic procedure. Teller *et al*, in 1856, were the first to apply this procedure to serum and plasma.[2]

o-dianisidine, *o*-anisidine, *o*-toluidine, and indophenol have since been used as O_2 acceptors for the enzymatic reaction, but these chromogens were colloidal and unstable.[3]

Difficulty also had been encountered in the direct application of these reagents to serum or plasma because of the presence of inhibitors, such as ascorbic acid, uric acid, bilirubin, catachols, glutathione, and cysteine or because of hemolysis. These compounds inhibited the determination by competing with the chromogen as proton donors.[3]

In the past decade, semiautomated and completely automated glucose methods have been proposed while the search for a specific enzymatic procedure for measuring the glucose concentration in biological fluids continued. A semiautomated determination requiring a manual protein-free filtrate and the use of ferricyanide as the redox indicator was described by Tucker and Hall.[4] This method likewise was limited by interferences from the reducing substances previously mentioned. Tammes and Nordschow[5] directly measured the hydrogen peroxide which is produced as a ternary complex with titanium and xylenol orange. Serum was recommended for use in this procedure due to negative interferences by common anticoagulants and antiglycolytic agents. A semiautomated procedure for urinary glucose utilizing the fluorometric hexokinase/glucose-6 phosphate dehydrogenase-NADP reaction was described by Scherstein and Tibbling.[6] Excellent adaptation of Calbiochem's hexokinase procedure to the Technicon AutoAnalyzer™ II continuous-flow analytical instrument was reported by Widdowson and Penton[7] and by Neeley[8], even though the method proved expensive for routine use.

The Technicon glucose oxidase method is a modification of the procedure of Gochman and Schmitz.[9] In this colorimetric determination for glucose, the specificity of glucose oxidase is combined with a new peroxidase indicator couple, 3-methyl 2 benzothiaolinone hydrozone (MBTH) and dimethylaniline (DMA). Glucose oxidase initiates the following reactions which generates hydrogen peroxidase that reacts with the peroxidase indicator to form an intensely-colored indamine dye.

$$(1)\ \text{Glucose} + O_2 + H_2O \xrightarrow[\text{Oxidase}]{\text{Glucose}} \text{Gluconic Acid} + H_2O_2$$

$$(2)\ \text{MBTH} + \text{DMA} + H_2O_2 \xrightarrow{\text{Peroxidase}} \text{Indamine Dye} + H_2O + OH$$

MERITS AND LIMITATIONS

The glucose (glucose-oxidase) method provides rapid and precise (SD 0.84 @ 97 mg/dl glucose) results. The method correlates well with the manual hexokinase method (bias −2.05 mg/dl) and the automated *o*-toluidine method (bias −0.1 mg/dl). Comparison studies performed with the Technicon™ SMA 12/60 multichannel biochemical analyzer indicate no significant difference between the two systems for the sample population. The glucose (glucose-oxidase) method was compared also to a neocuproine method. The results indicated a bias, with the glucose-oxidase method giving approximately 5% lower values for the sample population. Refer to "Interfering Substances" for a discussion of those substances that may interfere with this method.

EXPLANATION OF THE METHOD

The glucose method combines the specificity of a glucose oxidase reaction with a peroxidase indicator reaction. In the presence of peroxidase, 3-methyl-2-benzothiazolinone hydrazone oxidatively couples with *N, N*-dimethylaniline to form a stable, intensely colored, water-soluble indamine dye.

Except where noted, the performance data for this method were obtained using instruments, reagents, calibrators, standards, and controls manufactured by the Technicon Instruments Corporation.

TECHNICON INSTRUMENTS CORPORATION/TARRYTOWN, N.Y. 10591

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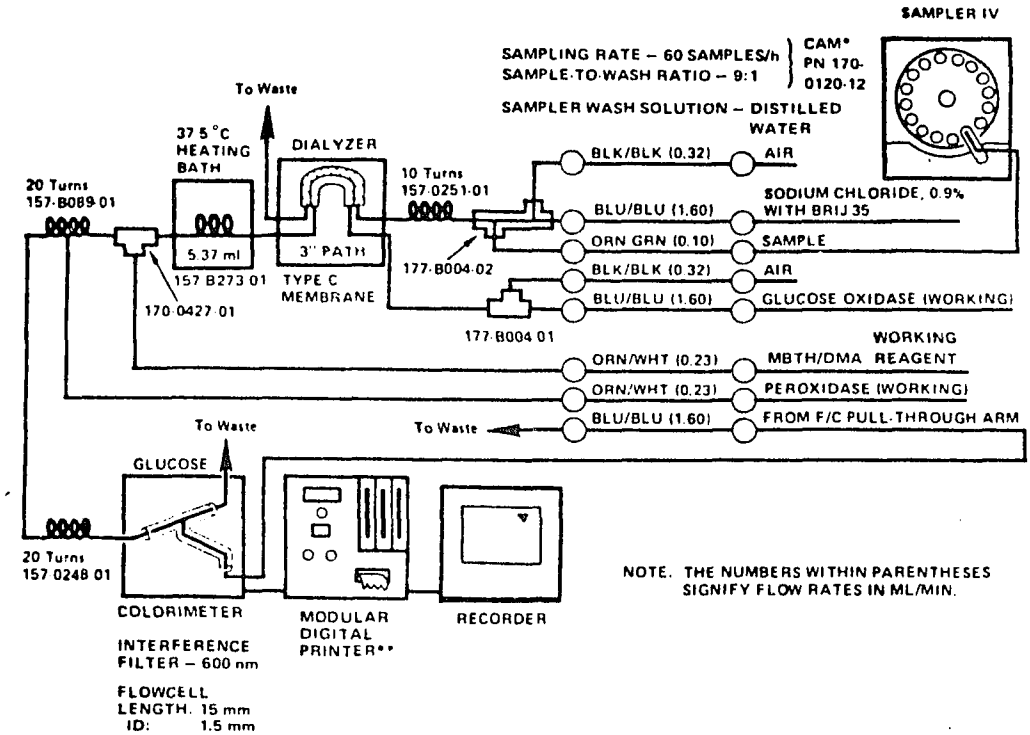

* Only used when Modular Digital Printer is not used

** Refer to "Preparation Procedures" for Modular Digital Printer control settings

Figure 1 GLUCOSE (GLUCOSE OXIDASE) FLOW DIAGRAM

As illustrated in Figure 1, a direct sample is diluted with glucose sample diluent, mixed, and dialyzed into a buffered glucose oxidase reagent. The solution is incubated in a 37.5 °C heating bath to allow the enzymatic reaction to take place.

A solution of 3-methyl-2-benzothiazolinone hydrazone and dimethylaniline (MBTH/DMA) is added to and mixed with a recipient stream containing H_2O_2. Peroxidase reagent is added and mixed with the indicator (H_2O_2) solution. The peroxidase coupling of MBTH and DMA forms the soluble indamine dye. The amount of dye formed is proportional to the amount of glucose present in the original sample. The color reaction takes place at room temperature. The stable blue color of the reaction product is measured at 600 nm in a flowcell that has a 15-mm light path and an inside diameter of 1.5 mm.

EQUIPMENT

The equipment required to perform this method is the Technicon AutoAnalyzer® II continuous-flow analytical instrument for the determination of glucose (glucose oxidase) in serum.

The following modules and accessories comprise the Technicon AutoAnalyzer II system that is used to perform glucose (glucose-oxidase) determinations:

1. Sampler IV with macroprobe
2. Cam 60/Hr, 9:1 (used on systems not equipped with or not operated with a Modular Digital Printer)
3. Proportioning Pump III
4. Glucose (Glucose-Oxidase) Manifold No. 170-A081-01
5. Single-Channel Colorimeter with a 1.5-mm x 15-mm flowcell and two 600-nm interference filters.
6. Modular Digital Printer
7. Technicon AutoAnalyzer II Recorder (chart speed—¶ inch/minute)

REAGENTS

The reagents used in this procedure should be free of particulates and other foreign matter, or obvious color changes. EACH REAGENT SHOULD BE EXAMINED THOROUGHLY BEFORE USE. Reagents displaying any signs of contamination, instability, or color change should be replaced. Refer to the specific reagent container label for the product expiration data and for pertinent storage information.

Sodium Chloride 0.9%, with Brij-35 (Technicon Product No. T01-0381)

Glucose Oxidase (Working) (must be prepared by the user)

Composition

Glucose Oxidase (Reconstituted)	1 ml
Glucose Oxidase Reagent Diluent (Technicon Product No. T21-0588)	QS 100 ml
Brij-35*, 30% Solution (Technicon Product No. T21-0110)	0.1 ml (2 drops)

Preparation Instructions

Place 1 ml of glucose oxidase (reconstituted) in a 100-ml volumetric flask. Dilute to volume (100 ml) with Glucose Oxidase Reagent Diluent (Technicon Product No. T21-0588), and mix. Add 0.1 ml (2 drops) of Brij-35, 30% Solution (Technicon Product No. T21-0110), and mix.

Storage Instructions

Store the glucose oxidase (working) reagent in a refrigerator at a temperature between 4 °C and 6 °C.

Stability

When prepared and stored as directed, the glucose oxidase (working) reagent is stable for 48 hours.

Glucose Oxidase (Reconstituted) (must be prepared by the user)

Composition

Glucose Oxidase (Technicon Product No. T11-0578)	1000 U/ml when reconstituted as directed
Distilled water	25 ml

Preparation Instructions

Reconstitute the lyophilized glucose oxidase by accurately pipetting 25 ml of distilled water into the vial containing the product. Swirl the vial gently until the contents are completely dissolved.

Storage Instructions

Store the glucose oxidase (reconstituted) reagent at a temperature between 2 °C and 8 °C.

Stability

When prepared and stored as directed, this reagent is stable for one month.

Glucose Oxidase (Technicon Product No. T11-0578)

NOTE

This lyophilized product is used to prepare the glucose oxidase (reconstituted) reagent.

*Trademark of ICI America Inc., Wilmington, Del.

Glucose Oxidase Reagent Diluent (Technicon Product No. T21-0588)

NOTE

This diluent is used in the preparation of the glucose oxidase reagent (working).

Brij-35, 30% Solution (Technicon Product No. T21-0110)

NOTE

The Brij-35, 30% Solution is used in the preparation of the glucose oxidase (working) reagent.

CAUTION!
MAY BE HARMFUL IF SWALLOWED
MAY CAUSE EYE IRRITATION

MBTH/DMA Working Reagent (must be prepared by the user)

Composition

Stock MBTH	2.0 ml
Stock DMA	3.0 ml
MBTH/DMA Working Reagent Diluent (Technicon Product No. T21-0577)	5.0 ml
Distilled Water	QS 100 ml

DANGER!
MAY BE FATAL IF
ABSORBED THROUGH SKIN
CAUSES EYE BURNS
HARMFUL IF INHALED

Avoid contact with eyes and skin.
Do not breathe vapor.
Wash thoroughly after handling.

– POISON –
(CALL A PHYSICIAN.)

FIRST AID

Skin contact: Drench with water, and wash thoroughly with soap and water. Remove and wash contaminated clothing before reuse. If contamination has been other than slight, obtain medical attention.
Affected eyes: Irrigate thoroughly with water, and obtain medical attention.
Vapor inhaled: Remove from exposure, rest, and keep warm. In severe cases, obtain medical attention.
If swallowed: Wash out mouth thoroughly with water, and obtain medical attention.

Preparation Instructions

WARNING

DO NOT PIPETTE THE MBTH OR DMA BY MOUTH.

Pipette 2.0 ml of stock MDTH, 3.0 ml of DMA, and 5.0 ml of MBTH/DMA Working Reagent Diluent (Technicon Product No. T21-0577) into a 100-ml volumetric flask containing approximately 50 ml of distilled water. Dilute to volume (100 ml) with distilled water, and mix thoroughly.

Storage Instructions
The reagent must be stored at room temperature in a amber glass bottle.

Stability
When prepared and stored as directed, the reagent is stable for 24 hours.

Stock MBTH (must be prepared by the user)

NOTE

The stock MBTH is used in the preparation of the MBTH/DMA working reagent.

Composition

MBTH (3-Methyl-2-Benzothiazolinone Hydrazone Hydrochloride) (Technicon Product No. T11-0575)	1 vial
MBTH/DMA Stock Reagent Diluent (Technicon Product No. T21-0574)	QS 100 ml

WARNING!
HARMFUL IF SWALLOWED
MAY CAUSE EYE OR SKIN IRRITATION
Wash thoroughly after handling.
Avoid contact with skin and eyes.

Preparation Instructions

WARNING

DO NOT PIPETTE MBTH BY MOUTH.

Empty one vial of MBTH (Technicon Product No. T11-0575) into a 100-ml volumetric flask containing about 50 ml of MBTH/DMA stock reagent diluent, and mix until the MBTH is dissolved. Dilute to volume with MBTH/DMA stock reagent diluent.

Storage Instructions
The reagent must be stored at room temperature in an amber glass bottle.

Stability
When prepared and stored as directed, this reagent is stable for two weeks.

MBTH (3-Methyl-2-Benzothiazolinone Hydrazone Hydrochloride) (Technicon Product No. T11-0575)

NOTE

The MBTH is used in the preparation of the stock MBTH reagent.

WARNING!
HARMFUL IF SWALLOWED
MAY CAUSE EYE OR SKIN IRRITATION
Wash thoroughly after handling.
Avoid contact with skin and eyes.

MBTH/DMA Stock Reagent Diluent (Technicon Product No. T21-0574)

NOTE

The MBTH/DMA stock reagent diluent is used in the preparation of the stock MBTH reagent.

CAUTION!
MAY BE HARMFUL IF SWALLOWED
MAY CAUSE EYE IRRITATION

Stock DMA (must be prepared by the user)

NOTE

The stock DMA is used in the preparation of the MBTH/DMA working reagent.

Composition

DMA (Technicon Product No. T21-0576)	1.0 ml
MBTH/DMA Stock Reagent Diluent (Technicon Product No. T21-0574),	100 ml

DANGER!
MAY BE FATAL IF
ABSORBED THROUGH SKIN
CAUSES EYE BURNS
HARMFUL IF INHALED
Avoid contact with eyes and skin.
Do not breathe vapor.
Wash thoroughly after handling.

– POISON –
(CALL A PHYSICIAN.)

FIRST AID

Skin contact: Drench with water, and wash thoroughly with soap and water. Remove and wash contaminated clothing before reuse. If contamination has been other than slight, obtain medical attention.

Affected eyes: Irrigate thoroughly with water, and obtain medical attention.

Vapor inhaled: Remove from exposure, rest, and keep warm. In severe cases, obtain medical attention.
If swallowed: Wash out mouth thoroughly with water, and obtain medical attention.

Preparation Instructions

WARNING

DO NOT PIPETTE DMA BY MOUTH.

Pipette 1.0 ml of DMA (Technicon Product No. T21-0576) into a 100-ml volumetric flask. Add 75 ml of MBTH/DMA Stock Reagent Diluent (Technicon Product No. T21-0574), and mix by shaking. Dilute to volume (100 ml) using the MBTH/DMA stock reagent diluent.

Storage Instructions
Store the stock DMA reagent at room temperature in an amber glass bottle.

Stability
When prepared and stored as directed, the DMA stock reagent is stable for two weeks.

DMA (Dimethylaniline) (Technicon Product No. T21-0576)

NOTE

The DMA is used in the preparation of the stock DMA reagent.

DANGER!
MAY BE FATAL IF
ABSORBED THROUGH SKIN
CAUSES EYE BURNS
HARMFUL IF INHALED
Avoid contact with eyes and skin.
Do not breathe vapor.
Wash thoroughly after handling.

– POISON –
(CALL A PHYSICIAN.)

FIRST AID

Skin contact: Drench with water, and wash thoroughly with soap and water. Remove and wash contaminated clothing before reuse. If contamination has been other than slight, obtain medical attention.
Affected eyes: Irrigate thoroughly with water, and obtain medical attention.
Vapor inhaled: Remove from exposure, rest, and keep warm. In severe cases, obtain medical attention.
If swallowed: Wash out mouth thoroughly with water, and obtain medical attention.

MBTH/DMA Stock Reagent Diluent (Technicon Product No. T21-0574)

NOTE

The MBTH/DMA stock reagent diluent is used in the preparation of the stock DMA reagent.

CAUTION!
MAY BE HARMFUL IF SWALLOWED
MAY CAUSE EYE IRRITATION

MBTH/DMA Working Reagent Diluent (Technicon Product No. T21-0577)

NOTE

The MBTH/DMA working reagent diluent is used in the preparation of the MBTH/DMA working reagent.

DANGER!
CAUSES SEVERE BURNS
VAPOR HARMFUL
COMBUSTIBLE
Do not get in eyes, or on skin or clothing.
Do not breathe vapor.

– POISON –
(CALL A PHYSICIAN.)

FIRST AID

Affected eyes: Irrigate thoroughly with water. In severe cases or where splashing has occurred, obtain medical attention.
Skin contact: Drench with water. Remove contaminated clothing and wash before reuse.
Vapor inhaled: Remove from exposure, rest, and keep warm. Give soothing drinks to relieve throat irritation.
If swallowed: Wash out mouth thoroughly with water. Give plenty of water to drink followed by milk of magnesia. Obtain medical attention.

Peroxidase (Working) (must be prepared by the user)

Composition

Peroxidase (Technicon Product No. T11-0579)	1 vial
Peroxidase Reagent Diluent (Technicon Product No. T21-0582)	QS 200 ml

Preparation Instructions

1. Fill the vial of Peroxidase (Technicon Product No. T11-0579) to about 3/4 of its volume with Peroxidase Reagent Diluent (Technicon Product No. T21-0582).
2. Mix to dissolve the contents, then transfer into a 200-ml volumetric flask.
3. Rinse the vial three times as described in steps 1 and 2.
4. Dilute to volume with Peroxidase Reagent Diluent (Technicon Product No. T21-0582).
5. Mix gently to prevent foaming. Transfer to an amber glass bottle.

Storage Instructions

Store the peroxidase (working) reagent in a refrigerator at a temperature between 4 °C to 6 °C.

Stability

When prepared and stored as directed, the peroxidase (working) reagent is stable for one week.

Peroxidase (Technicon Product No. T11-0579)

NOTE

The peroxidase is used in the preparation of the peroxidase (working) reagent.

Peroxidase Reagent Diluent (Technicon Product No. T21-0582)

NOTE

The peroxidase reagent diluent is used in the preparation of the peroxidase reagent.

REAGENT CONSUMPTION DATA

The reagent consumption data listed in Table 1 are provided to assist the technologist in determining his particular reagent requirements. By multiplying the applicable reagent flow rate (ml/min or ml/h) by the anticipated average instrument operating time, the technologist can project reagent preparation and reordering schedules.

Table 1 REAGENT FLOW RATES

REAGENT	FLOW RATE ml/min	FLOW RATE ml/h
Sodium Chloride, 0.9% with Brij-35 (Technicon Product No. T01-0381)	1.60	96.0
Glucose Oxidase (Working) Reagent (must be prepared by the user)	1.60	96.0
MBTH/DMA Working Reagent (must be prepared by the user)	0.23	13.8
Peroxidase (Working) Reagent (must be prepared by the user)	0.23	13.8

CALIBRATION REFERENCE

(Refer to Table 2.)

Technicon SMA Reference Serum 2 w/Diluent

Table 2 AVAILABLE TECHNICON SMA REFERENCE SERUM 2 PRODUCTS

PART NUMBER	PRODUCT NAME	UNIT OF SALE
T03-8050-56	SMA Reference Serum 2 and SMA Reference Serum 2 Diluent	6 sets x 50 ml
T03-8025-57	SMA Reference Serum 2 and SMA Reference Serum 2 Diluent	7 sets x 25 ml
T03-8150-56	SMA Reference Serum 2 CPK and SMA Reference Serum 2 Diluent	6 sets x 50 ml
T03-8125-57	SMA Reference Serum 2 CPK and SMA Reference Serum 2 Diluent	7 sets x 25 ml
T03-8250-56	SMA Reference Serum 2 SGPT and SMA Reference Serum 2 Diluent	6 sets x 50 ml
T03-8225-57	SMA Reference Serum 2 SGPT and SMA Reference Serum 2 Diluent	7 sets x 25 ml

Composition of Technicon SMA Reference Serum 2. – Technicon SMA Reference Serum 2 is a lyophilized product that has been formulated for use as a general-purpose reference material for calibrating Technicon AutoAnalyzer II systems. This reference material is prepared from defibrinated human plasma to which various constituents have been added to achieve specific concentration levels. Added enzymes are derived from the following sources: LDH – bovine heart muscle, GOT – porcine heart muscle, alkaline phosphatase – chicken intestine, GPT – porcine heart muscle. Other additives are included to improve and preserve the characteristics of the product.

A complete description of SMA Reference Serum 2 together with suggested procedures for confirming its constituent concentrations are provided in Technical Bulletin No. TT3-0313-20. A copy of this bulletin is provided in VOLUME III of the Product Labeling for the Technicon AutoAnalyzer II system.

CAUTION!

This reference material is processed from human plasma obtained from donors who have been tested for the absence of Australia antigen, using methods approved by, and reagents licensed by, the Bureau of Biologics, Food and Drug Administration. However, the possibility of contaminants cannot be excluded. HANDLE AS YOU WOULD ANY PATIENT SAMPLE. Do not pipette by mouth. Avoid skin contact, and clean up spills immediately.

Refer to the SMA Reference Serum 2 package insert for information concerning the DIRECTIONS FOR USE, STORAGE CONDITIONS, STABILITY, and ASSIGNED VALUES.

CONTROLS
(Refer to Table 3.)

Technicon SCALE I (Low) (Serum Control for Automated Linearity Evaluation) and Technicon SCALE II (High) (Serum Control for Automated Linearity Evaluation)

Composition of Technicon SCALE I and SCALE II Controls. – Technicon SCALE I (Low) and SCALE II (High) serum controls for automated linearity evaluation are lyophilized products that have been formulated as general-purpose control materials for use in Technicon AutoAnalyzer II systems. These controls are prepared from defibrinated human plasma to which various constituents have been added to achieve specific concentration levels. Added enzymes are derived from the following sources: LDH – bovine heart muscle, GOT – porcine heart muscle, alkaline phosphatase – chicken intestine, GPT – porcine heart muscle. Other additives are included to improve and preserve the characteristics of the product.

A complete description of Technicon SCALE I and SCALE II controls together with suggested procedures for confirming their constituent concentrations are provided in Technical Bulletin No. TT4-0302-10. A copy of this bulletin is provided in VOLUME III of the Product Labeling for the Technicon AutoAnalyzer II system.

CAUTION!

Technicon SCALE controls are processed from human plasma obtained from donors who have been tested for the absence of Australia antigen, using methods approved by, and reagents licensed by, the Bureau of Biologics, Food and Drug Administration. However, the possibility of contaminants cannot be excluded. HANDLE AS YOU WOULD ANY PATIENT SAMPLE. Do not pipette by mouth. Avoid skin contact, and clean up spills immediately.

Refer to the appropriate Technicon SCALE control package insert for information concerning the DIRECTIONS FOR USE, STORAGE CONDITIONS, STABILITY, and ASSIGNED VALUES.

SYSTEM PERFORMANCE SPECIFICATIONS

The following specifications apply to the glucose (glucose-oxidase) method as performed on Technicon AutoAnalyzer II systems:

Rate of analysis – 60 samples/h
Sample-to wash ratio – 9:1
Chart speed – 1 inch/minute
Linearity range – 0-500 mg/dl
Sensitivity coefficient – 0.0009 absorbance (*E*) unit per unit of concentration
Minimum sample required – 9 µl (approx)

PATIENT PREPARATION

Patients should be prepared according to the protocol established by the user's laboratory.

SAMPLE COLLECTION AND PREPARATION

Glucose should be assayed using clear, nonhemolyzed sera. The blood sample should be collected in commercially

Table 3 AVAILABLE TECHNICON SCALE CONTROL PRODUCTS

PART NUMBER	PRODUCT NAME	UNIT OF SALE
T03-8610-60	SCALE I (Low) (serum control for automated linearity evaluation and SCALE I Diluent	10 sets x 10 ml
T03-8710-60	SCALE II (High) (serum control for automated linearity evaluation) and SCALE II Diluent	10 sets x 10 ml
T85-8510-70	SCALE I (Low) (serum control for automated linearity evaluation) and SCALE I Diluent; SCALE II (High) (serum control for automated linearity evaluation) and SCALE II Diluent	Package of 20 sets x 10 ml

available collection tubes using routine venipuncture techniques and should be centrifuged at a speed of 2 700 rev/min (RPM) for 10 to 12 min. Following centrifugation, the serum should be carefully decanted to avoid introducing clots into the system. Decant the serum directly into a sample cup.

The use of the Technicon SeraClear filtering device assures the preparation of clot-free serum samples. To ensure optimum performance when using the Technicon SeraClear filtering device, perform the following procedure:

1. After drawing the specimen from the patient, place the collection tube in an upright vertical position, and allow the specimen to stand undisturbed for a period of about 30 minutes prior to centrifugation.
2. Before placing the specimen in the centrifuge, carefully rim the collection tube to free the clot from the glass. Perform this procedure carefully so that the cells are not hemolyzed.
3. Centrifuge the specimen at a speed of 2 700 rev/min (RPM) for 10 to 12 minutes.
4. Slowly insert the SeraClear filtering device into the mouth of the collection tube. During insertion, make certain to keep the filtering device straight and parallel to the long axis of the collection tube. If the SeraClear filtering device is not inserted in this manner, the wiper may be bent; thereby, rendering the filter ineffective.
5. Press the filtering device into the collection tube until the bottom of the filter is positioned approximately 1/2 mm above the packed red cells. If the SeraClear filtering device is pushed past the point specified, the cells may be disturbed; thereby, permitting them to pass through the filter.
6. If the Sampler is equipped with the direct sampling capability, place the collection tubes on the Sampler, and allow the Sampler to aspirate sample directly from the collection tube. If the Sampler is not equipped for direct sampling, pour the filtered serum out of the SeraClear filtering device and into a sample cup. The sample is then aspirated from the sample cup.

SAMPLE STABILITY

When stored at 5 °C, serum samples are stable from 2 to 3 days.

SAMPLE DILUTION

Serum samples with glucose concentrations greater than 500 mg/dl should be diluted with distilled water or saline (0.9% NaCl) and reassayed.

OPERATION

Preoperation Procedure

1. Verify that the interference filters are in the place in the Colorimeter and that both apertures are wide open (controls fully clockwise and down). Verify that the reversing switch is set to position D. Adjust the BASELINE control to midposition (5 turns from either limit).
2. Set the proportioning pump POWER switch to position ON and the recorder SYSTEM switch to position 1 & 2. Press the colorimeter POWER ON push-button switch.
3. Press the modular digital printer POWER ON push-button switch. Verify that all modular digital printer lamps except the POWER ON and WASH lamps are off; then press the applicable CHAN ON push-button switch.
4. Set the following modular digital printer controls to the position indicated:

CONTROL	POSITION FOR 60-SAMPLE/h OPERATION
SAMPLING RATE switch	60
WASH TIME switch	6
MODE switch	NORMAL
RANGE switch	1000
DECIMAL switch	000.
TEST IDENT. control	36
SAMPLE No. switches	000*

5. On systems not equipped with the Modular Digital Printer, install the 60 Hr 9:1 sampler timing cam on the sampler timing motor shaft.
6. Install the proportioning pump cover plate and platen assembly.
7. Connect each reagent line in accordance with Figure 1. Allow the system to warm up for ten minutes with reagents flowing and the sample probe in the wash reservoir. Check for proper bubble pattern.
8. Set the recorder CHART DRIVE switch to position ON.
9. Turn the colorimeter Rotary Display Switch to position ZERO. Adjust the ZERO control (screwdriver adjustment), if necessary, to position the recorder pen at zero on the recorder chart paper.
10. Turn the modular digital printer DISPLAY switch to the appropriate channel, and adjust the applicable ZERO control until the CONCENTRATION indicator displays 000.
11. Turn the colorimeter Rotary Display Switch to position FULL SCALE. Adjust the FULL SCALE control (screwdriver adjustment), if necessary, until the recorder pen is positioned at 100 (full scale) on the recorder chart paper.
12. Adjust the appropriate modular digital printer CAL control until the CONCENTRATION indicator displays 1000.
13. Turn the colorimeter Rotary Display Switch to position NORMAL.
14. Adjust the colorimeter B (reference) Aperture until the modular digital printer CONCENTRATION indicator displays 000. Use the colorimeter BASELINE control for fine adjustment of the CONCENTRATION indicator display.

*These switches can be set to any numerical position that may be required to conform to individual laboratory sample handling and accounting procedures.

On systems not equipped with a Modular Digital Printer, adjust the colorimeter B (reference) Aperture until the recorder pen is positioned at zero on the recorder chart paper. Use the colorimeter BASELINE control for fine adjustment of recorder pen position.

15. Set the recorder CHART DRIVE switch to position OFF.
16. Turn the applicable modular digital printer RANGE switch to position 500.

Daily Calibration and Operating Procedure

1. Refer to and perform the preoperation procedure described in the preceding paragraph.
2. Remove the sample tray cover.
3. Load the sample tray in accordance with the following protocol:

SAMPLE POSITION	SAMPLE
1,2	SMA Reference Serum 2
3-14	Patient samples
15	SMA Reference Serum 2, SCALE I control, or SCALE II control
16-29	Patient samples
30	SMA Reference Serum 2, SCALE I control, or SCALE II control
31-40	Patient samples

NOTE

It is recommended that Technicon SCALE I and SCALE II controls be interspersed randomly among the patient samples. The use of these controls provides additional quality control checks during the processing of each sample tray.

4. Install the end of run pin adjacent to the last sample.
5. Replace the sample tray cover.
6. Press to light the sampler POWER push-button switch, and observe that the sampler probe cycles out of the wash reservoir and into the sample cup; then proceed as applicable:

Initial tray – Press to light the modular digital printer SAMPLE HOLD push-button switch to halt the sampling cycle with the sample probe in the sample cup. After two minutes, press the modular digital printer SAMPLE HOLD push-button switch, and proceed to step 7.

On systems not equipped with a Modular Digital Printer, press the sampler POWER push-button switch to turn the sampler off, thereby halting the sampling cycle with the sample probe in the sample cup. After two minutes, press to light the sampler POWER push-button switch, and proceed to step 7.

Succeeding tray – Proceed to step 7.

7. Press to light the sampler ALARM push-button switch.
8. Set the Recorder Chart Drive switch to position ON.
9. Adjust the colorimeter BASELINE control until the modular digital printer CONCENTRATION indicator displays 000.

On systems not equipped with a Modular Digital Printer, adjust the colorimeter BASELINE control until the recorder pen is positioned at zero on the recorder chart paper.

10. As the first SMA Reference Serum 2 sample reaches steady state, adjust the colorimeter STD CAL control until the modular digital printer CONCENTRATION indicator displays a value equal to the glucose (glucose-oxidase) assay value specified on the SMA Reference Serum 2 package insert. Observe the CONCENTRATION indicator during the steady-state portion of the glucose curve and, when the numbers displayed on the CONCENTRATION indicator start to decrease, press the applicable modular digital printer START PRINT push-button switch. Immediately press and hold the applicable ADV PRINT push-button switch until the light in the push-button flashes three (3) times.

If the system is not equipped with a Modular Digital Printer, observe the steady-state portion of the curve produced by the first SMA Reference Serum 2 sample, and adjust the colorimeter STD CAL control until the recorder pen is positioned to the level on the recorder chart paper which corresponds to the glucose (glucose oxidase) assay value specified on the SMA Reference Serum 2 package insert (each chart recorder unit = 5 mg/dl glucose).

11. As the second SMA Reference Serum 2 sample reaches steady state, the modular digital printer CONCENTRATION indicator should display the glucose (glucose-oxidase) assay value. Adjust the colorimeter STD CAL control to correct for any discrepancy that may exist between the value displayed on the modular digital printer CONCENTRATION indicator and the value specified on the SMA Reference Serum 2 package insert.

A short, sharp deflection of the pen will appear on the recorder trace when the Modular Digital Printer prints. Verify that this pen deflection occurs near the end of the steady-state plateau. Adjustment of the print timing may be made by pressing the appropriate ADV PRINT or RET PRINT push button. (Each flash represents a change of one second. The chart speed of the Recorder is 1/60 of an inch per second.)

If the system is not equipped with a Modular Digital Printer, observe the steady-state portion of the curve produced by the second SMA Reference Serum 2 sample – the recorder pen should be positioned at a level which corresponds to the glucose (glucose-oxidase) assay value specified on the SMA Reference Serum 2 package insert

(each chart recorder unit = 5 mg/dl glucose). If the pen is not tracing along the level which corresponds to the assay value, adjust the Colorimeter STD CAL control to position the pen to the proper level.

12. After all the samples have been recorded, set the recorder CHART DRIVE switch to position OFF.

If the system is not equipped with a Modular Digital Printer, convert the data recorded on the recorder chart paper into concentration units by multiplying the chart recorder units by 5 mg/dl glucose (glucose-oxidase).

Shutdown Procedure

1. Turn off all the modules except the Proportioning Pump.
2. Place the reagent lines into a container of distilled water.
3. Wash out the system with distilled water for 20 minutes.

NOTE

After every other day of operation, wash out the MBTH/DMA and the peroxidase working reagent lines and pump tubes for 15 minutes, using a 1 *N* solution of HCl. Following this wash, rinse out the lines and pump tubes for 10 minutes with distilled water. NEVER use a hypochlorite (Chlorox*) solution to wash out the glucose oxidase analytical cartridge or any of the related lines or pump tubes. Washing out this channel with hypochlorite will produce erroneous results.

4. Remove the reagent lines from the container of distilled water.
5. Remove the sample probe from the sample probe arm, and operate the AutoAnalyzer II system to pump air through all lines until the system is completely dry.
6. Replace the sample probe in the sample probe arm.
7. Release and remove the proportioning pump cover plate and platen assembly. Set the proportioning pump POWER switch to position OFF.
8. Change the dialyzer membrane weekly. An old membrane can cause low results. Before changing the membrane, wash out the sample, 0.9% sodium chloride, and glucose oxidase (working) reagent lines and pump tubes for 20 minutes using a 1 *N* solution of NaOH. Following this wash, rinse the lines and pump tubes for 10 minutes with distilled water. Upon completion of this washout procedure, proceed to change the membrane.
9. The glucose color reagent (MBTH/DMA) line should be changed every six weeks. The line becomes very discolored with time. The shoulders on the quick-disconnect mechanism also should be changed regularly.

METHOD PERFORMANCE CHECKS

Routine Linearity Check

The routine linearity check is a convenient means for monitoring method linearity. This check should be performed on a periodic basis or whenever the linearity of the method is in doubt. To perform the routine linearity check, proceed as follows:

1. Refer to and perform the preoperation procedure previously described in this method.
2. Remove the sample tray cover, and load the sample tray in accordance with the following protocol:

SAMPLE POSITION	SAMPLE
1-3	SMA Reference Serum 2
4,5	SCALE I control
6,7	SMA Reference Serum 2
8,9	SCALE II control

3. Replace the sample tray cover.
4. Press to light the sampler POWER push-button switch.
5. Set the recorder CHART DRIVE switch to position ON.
6. Adjust the colorimeter BASELINE control to position the recorder pen at zero on the recorder chart paper.
7. As the first SMA Reference Serum 2 sample reaches steady state, adjust the colorimeter STD CAL control until the modular digital printer CONCENTRATION indicator displays a value equal to the glucose (glucose-oxidase) assay value specified on the SMA Reference Serum 2 package insert.

On systems not equipped with a Modular Digital Printer, adjust the colorimeter STD CAL control to position the recorder pen to the level on the recorder chart paper which corresponds to the glucose (glucose-oxidase) assay value specified on the SMA Reference Serum 2 package insert (each chart recorder unit = 5 mg/dl glucose).

8. Identify each sample as its curve is traced on the recorder chart paper.
9. After all the samples have been recorded, set the recorder CHART DRIVE switch to position OFF.
10. Refer to the appropriate package insert to obtain the glucose (glucose-oxidase) concentration in each sample.
11. Using linear graph paper, plot the modular digital printer units vs glucose concentration. A typical routine linearity curve is illustrated in Figure 2.

On systems not equipped with a modular digital printer, use linear graph paper to plot the recorder chart units vs glucose concentration. (See Figure 2.)

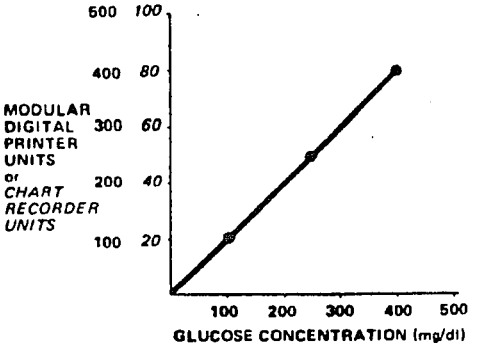

Figure 2 TYPICAL ROUTINE LINEARITY CURVE

*Trademark of the Chlorox Co., Oakland, California.

Conditional Linearity Check

The conditional linearity check provides a rigorous assessment of method linearity. This check should be performed after changing reagent lot numbers, pump tubes, flowcells, colorimeters, etc. To perform the conditional linearity check proceed as follows:

1. Refer to and perform the preoperation procedure previously described in this method.
2. Remove the sample tray cover, and load the sample tray in accordance with the following protocol:

SAMPLE POSITION	SAMPLE
1-3	SMA Reference Serum 2
4	100 mg/dl glucose standard
5	200 mg/dl glucose standard
6	300 mg/dl glucose standard
7	400 mg/dl glucose standard
8	500 mg/dl glucose standard

NOTE

The glucose concentrations specified are approximate.

It is recommended that dilutions of retained patients' sera be used. However, NBS standards or dilutions of commercial standards also are acceptable.

3. Replace the sample tray cover.
4. Press to light the sample POWER push-button switch.
5. Set the recorder CHART DRIVE switch to position ON.
6. Adjust the colorimeter BASELINE control to position the recorder pen at zero on the recorder chart paper.
7. As the first SMA Reference Serum 2 sample reaches steady state, adjust the colorimeter STD CAL control until the modular digital printer CONCENTRATION indicator displays a value equal to the glucose (glucose-oxidase) assay value specified on the SMA Reference Serum 2 package insert.

   On systems not equipped with a Modular Digital Printer, adjust the colorimeter STD CAL control to position on the recorder pen to the level on the recorder chart paper which corresponds to the glucose (glucose-oxidase) assay value specified on the SMA Reference Serum 2 package insert (each chart recorder unit = 5 mg/dl glucose).

8. Identify each sample as its curve is traced on the recorder chart paper.
9. After all the samples have been recorded, set the recorder CHART DRIVE switch to position OFF.
10. Using linear graph paper, plot the modular digital printer units vs glucose concentration. A typical conditional linearity curve is illustrated in Figure 3.

    On systems not equipped with a Modular Digital Printer, use linear graph paper to plot the recorder chart units vs glucose concentration. (See Figure 3.)

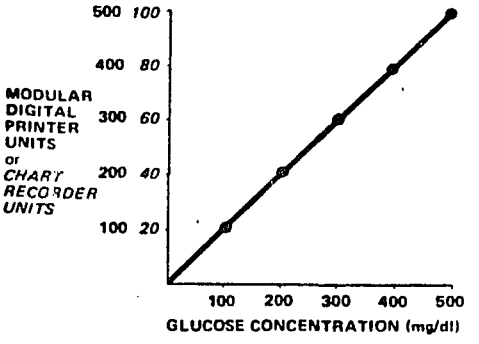

Figure 3 TYPICAL CONDITIONAL LINEARITY CURVE

Reagent vs Water Absorbance Check

The reagent vs water absorbance check is performed to evaluate overall system performance. System optics, hydraulics, and reagents are involved in the performance of this check. A malfunction in any of these items can contribute to a poor reagent absorbance. To perform the reagent vs water absorbance check, proceed as follows:

1. Start the Proportioning Pump, and place all reagent lines into a container of distilled water. After water has reached flowcell (approximately 10 minutes), check for proper bubble pattern.
2. Adjust the colorimeter STD CAL control to 100.
3. Set the recorder CHART DRIVE switch to position ON.
4. Turn the colorimeter Rotary Display Switch to position ZERO and, using a screwdriver, adjust the ZERO control to position the recorder pen at zero on the recorder chart paper.
5. Turn the Rotary Display Switch to position FULL SCALE and, using a screwdriver, adjust FULL SCALE control to position the recorder pen at 100 (full scale) on the recorder chart paper.
6. Turn the Rotary Display Switch to position NORMAL, and adjust the colorimeter Reference Aperture to position the recorder pen at zero on the recorder chart paper. Fine control of this adjustment is obtained by using the colorimeter BASLEINE control.
7. Remove the reagent lines from the container of distilled water, and connect them to their respective reagent container (see Figure 1). After ten minutes, check for proper bubble pattern.
8. Record the absorbance (*E*) units as calculated from the values indicated on the recorder chart paper (10 chart recorder units equal 0.1 absorbance (*E*) unit). The approximate reagent absorbance for the glucose (glucose-oxidase) method is 0.03 *E*. Minor variations in reagents, pump tubes, flowcells, etc. can cause deviations from the specified value; therefore, an acceptable range for each system should be determined over a period of time.

NOTE

Maintain a permanent record of the recorded absorbances – this record will be a valuable troubleshooting aid.

Sensitivity Check

The sensitivity check is a procedure that is performed to monitor the overall performance of the glucose (glucose-oxidase) method. To perform the sensitivity check on the Technicon AutoAnalyzer II system, proceed as follows:

1. Refer to and perform the preoperation procedure previously described in this method.
2. Adjust the colorimeter STD CAL control to 100.
3. Remove the sample tray cover.
4. Place a sample cup of SMA Reference Serum 2 in the first sample position on the sample tray.
5. Replace the sample tray cover.
6. Press to light the sampler POWER ON push-button switch.
7. Set the recorder CHART DRIVE switch to position ON.
8. Record the absorbance (*E*) units as calculated from the value recorded on the recorder chart paper for the SMA Reference Serum 2 sample (10 chart recorder units = 0.1 absorbance (*E*) units). Obtain (from the SMA Reference Serum 2 package insert) the glucose (glucose-oxidase) assay value.
9. Set the recorder CHART DRIVE switch to position OFF.
10. Calculate the sensitivity coefficient by dividing the absorbance (*E*) units recorded in step 8 by the glucose (glucose-oxidase) assay value specified on the SMA Reference Serum 2 package insert. The approximate glucose sensitivity coefficient is 0.0009 absorbance (*E*) unit per unit of concentration. Minor variations in reagents, pump tubes, flowcells, etc. can cause deviations from the specified sensitivity values; therefore, and acceptable range for each system should be determined over a period of time.

NOTE

Maintain a permanent record of sensitivity values – this record will be a valuable troubleshooting aid.

RESULTS

Range of Expected Values

The range of expected values for the glucose (glucose-oxidase) method, as performed on the Technicon AutoAnalyzer II system, is 70 to 100 mg/dl.

Calculation of Results

On the Technicon AutoAnalyzer II system, the test results for each sample is printed in direct concentration units. If the system is operated without the Modular Digital Printer, convert the recorder units into concentration units of glucose by multiplying the chart recorder units by 5 mg/dl.

Interfering Substances

A number of substances have been reported to cause physiological changes in glucose concentration.[10,11,12] A comprehensive discussion of these substances, their serum concentrations, and their possible physiological involvements is beyond the scope of this Product Labeling. However, the following substances, if present in the sample, reagents, or system, may interfere in glucose determinations in the manner described:

Galactose – This substances may cause an elevation (5%) in the determination of glucose levels.

Maltose – Maltose can cause a significant elevation (30%) in the determination of glucose levels.

Diethreitol – This substance may cause an elevation (5%) in the determination of glucose levels.

Hydrazine Sulfate – This substance may cause an elevation (5%) in the determination of glucose levels.

Phenylhydrazine · HCl – This substance may inhibit the glucose-oxidase method, resulting in a significant depression (65%) of glucose levels determined by this method.

Ascorbic Acid – Ascorbic acid may inhibit the glucose-oxidase method, resulting in a depression (10%) of glucose levels determined by this method.

A study performed by Carey, Feldbruegge, and Westgard[13] reported that the glucose-oxidase (MBTH-DMA) method was not significantly affected by uric acid levels of 25 mg/dl or less.

Interpretation of Results

System operators and laboratory supervisors have a responsibility to determine that the Technicon AutoAnalyzer II system is performing according to specification and that the maintenance and troubleshooting procedures outlined in VOLUME I of this Product Labeling are followed. In the event of a grossly aberrant result, as defined by the laboratory protocol, a determination should first be made that the system is performing correctly. The operator then should follow the laboratory's predetermined protocol for advising the clinician of a result which has deviated from the established norm.

Technicon adequately identifies potential aberrant results based on machine function (or reagent function) through the Troubleshooting and Maintenance sections contained in VOLUME I of this Product Labeling. Technicon considers these sections of the Product Labeling to be responsive to the question of how to handle an apparent abnormal result. This troubleshooting and maintenance information is provided in lieu of attempting to assume a responsibility that may involve medical judgement.

The interpretation of results and determinations concerning a need for further testing involve decisions which are best made by the clinician who has the most complete physical and clinical picture of the patient's condition.

METHOD PERFORMANCE CHARACTERISTICS

Within-run Precision Study

The within-run precision of the glucose (glucose–oxidase) method was studied using dilutions of a spiked pool of Technicon SMA Reference Serum as test samples. Each test sample was assayed a minimum of 24 times, consecutively. The results obtained from this study are provided in Table 4.

Table 4 WITHIN-RUN PRECISION STUDY

	TEST SAMPLE 1	TEST SAMPLE 2	TEST SAMPLE 3
Number of Assays	36	24	24
Mean (mg/dl)	97	194	388
Coefficient of Variation (%)	0.86	0.47	0.87
Standard Deviation	0.84	0.92	3.39

Correlation Studies

Technicon SMA 12/60 System vs Manual Hexokinase Method

The performance of the glucose (glucose-oxidase) method was independently compared to a manual hexokinase method by Carey, Feldbruegge, and Westgard [13] The study was performed using a Technicon 12/60 system. However, the results obtained apply equally to the Technicon Auto-Analyzer II system, since both systems perform glucose (glucose-oxidase) determinations by the same method. As shown in Figure 4, the results of the study indicate a small bias of 2.05 mg/dl between the two methods.

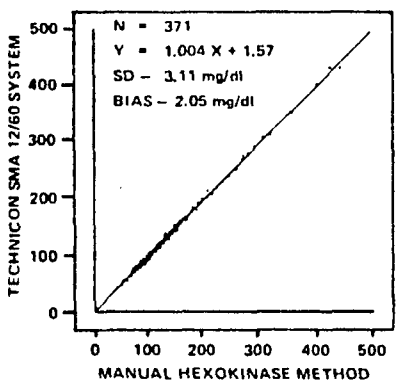

Figure 4 CORRELATION STUDY – TECHNICON SMA 12/60 SYSTEM VS MANUAL HEXOKINASE METHOD

Technicon SMA 12/60 System vs Automated o-Toluidine Method

The performance of the glucose (glucose-oxidase) method was independently compared to an automated *o*-toluidine method by Carey, Feldbruegge, and Westgard.[13] The study was performed using a Technicon 12/60 system. However, the results obtained apply equally to the Technicon Auto-Analyzer II system, since both systems perform glucose (glucose-oxidase) determinations by the same method. As shown in Figure 5, the results of the study indicate that there is no significant bias between the two methods.

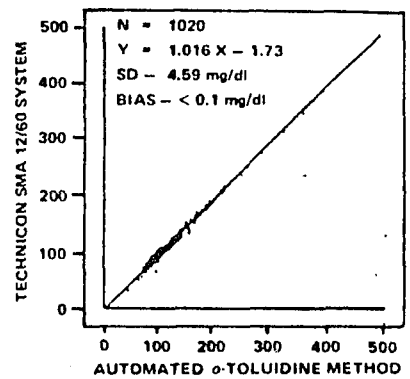

Figure 5 CORRELATION STUDY – TECHNICON SMA 12/60 SYSTEM VS AUTOMATED *o*-TOLUIDINE METHOD

Technicon AutoAnalyzer II System vs Technicon SMA 12/60 System (Glucose-Oxidase)

The performance of the glucose (glucose-oxidase) method was compared to the glucose (glucose-oxidase) method as performed on the Technicon SMA 12/60 system. A sample population of 998 random patients' sera was analyzed consecutively by both systems. The results obtained from this study are provided in Table 5.

Table 5 CORRELATION STUDY – TECHNICON AUTOANALYZER II SYSTEM VS TECHNICON SMA 12/60 SYSTEM (GLUCOSE-OXIDASE)

	TECHNICON AUTOANALYZER II SYSTEM (GLUCOSE-OXIDASE) (*y*-axis)	TECHNICON SMA 12/60 SYSTEM (GLUCOSE-OXIDASE (*x*-axis)
Number of Samples	998	998
Mean (mg/dl)	188	194
Standard Deviation of Sample Population	99.2	101
Correlation Coefficient	0.998	
Slope of Regression Line	0.976	
y-Intercept	1.08	

These statistical data demonstrate a positive correlation between the results obtained from both systems. The correlation study indicates that there is no significant difference in the values obtained from the sample population as assayed on the Technicon AutoAnalyzer II and SMA 12/60 systems.

Technicon AutoAnalyzer II Systems – Glucose-Oxidase vs Neocuproine

The performance of the glucose-oxidase method was compared to the neocuproine method. A sample population of 103 random patients' sera was analyzed consecutively by both methods. As shown in Table 6, a small bias was found to exist between the two methods, with the glucose-oxidase method giving 5% lower values than the neosuproine method for the sample population.

Table 6 GLUCOSE-OXIDASE vs NEOCUPROINE

	TECHNICON AUTOANALYZER II SYSTEM (GLUCOSE-OXIDASE) (*y*-axis)	TECHNICON AUTOANALYZER II SYSTEM (NEOCUPROINE) (*x*-axis)
Number of Samples	103	103
Mean (mg/dl)	110	116
Standard Deviation of Standard Population	60.4	54.3
Correlation Coefficient	0.986	
Slope of Regression Line	1.11	
y-Intercept	-19.3	

Recovery Study

The ability to recover precisely known levels of glucose was studied using dilutions of Technicon SMA Reference Serum. One set of the test samples was spiked with a specific level of a glucose standard. The second set was used as a control. The percentage of glucose concentration recovered, at each test level, was calculated using the following formula:

$$\% \text{ Recovery} = \frac{\text{Value of Test Sample (Spiked)} - \text{Value of Test Sample (Control)}}{\text{Value of Spike}} \times 100$$

The results obtained from this study are listed in Table 7.

Table 7 RECOVERY STUDY

DILUTION OF TECHNICON REFERENCE SERUM (%)	CONCENTRATION OF GLUCOSE (mg/dl)	PERCENT OF GLUCOSE CONCENTRATION RECOVERED
25	60	100
50	125	99.5
100	247	97.5

Sample Interaction Study

Experimental evidence indicates that within the range of expected values for the glucose (glucose-oxidase) method, the amount of sample interaction (carryover) is not clinically significant (less than 5%). In the extreme case, where a sample with an extremely high concentration, well beyond the expected range, is followed by a sample with a very low concentration, the amount of sample interaction may result in a clinically significant (greater than 5%) elevation of the concentration in the sample. In routine clinical use, this situation is seldom encountered.

The quantitative effect of carryover at any concentration level is directly related to the operating condition of the system. Conditions such as worn pump tubes, dirty flowcells, clogged dialyzer membranes, etc. can have a deleterious effect on results. Accordingly, the user is urged to adhere strictly to the maintenance recommendations prescribed in VOLUME I of the Product Labeling for the Technicon AutoAnalyzer II system.

Protein Effect on Dialysis

Experimental evidence has indicated that there is no clinically significant protein effect on dialysis throughout the entire range of the glucose (glucose-oxidase) method. At no time do varying levels of protein concentration decrease test results by more than 5%, not even in the extreme, seldom encountered case of high protein and high sample concentrations.

The quantitative effect of protein on dialysis is directly related to the operating condition of the system. The system should be kept clean and the dialyzer membrane should be inspected and changed in accordance with the maintenance recommendations specified in VOLUME I of the Product Labeling for the Technicon AutoAnalyzer II system.

TROUBLESHOOTING

Troubleshooting should take the path of least resistance and the path of least effort on the part of the operator. Always look for the most obvious and for the most easily repaired source of trouble. When troubleshooting the glucose (glucose-oxidase) method, the first step is to localize the trouble. In the continuous-flow method of analysis, the bubble is an important part of the system operation. Although a number of factors contribute to an

improper bubble pattern, it is always the easiest feature of system operation to check, since the tubing is transparent and most of the pathways are exposed.

For troubleshooting purposes, note the following information for the glucose (glucose-oxidase) method as performed on the Technicon AutoAnalyzer II continuous-flow analytical instrument:

1. SMA Reference Serum (250 mg/dl glucose) Absorbance – 0.225 *E* (approx)
   Full Scale (500 mg/dl glucose) Absorbance – 0.450 *E* (approx)
3. Reagent vs Water Absorbance – 0.03 *E* (approx)

NOTE

The absorbances (*E*) specified are approximate. Minor variations in reagents, pump tubes, flowcells, etc. can cause deviations from the specified values. An acceptable range for each system should be determined over a period of time.

Table 8 provides a list of possible troubles that could be encountered while running the glucose (glucose-oxidase) method. The Table also lists the probabel cause of each difficulty and the suggested corrective maintenance procedures.

Table 8 TROUBLESHOOTING

TROUBLE	PROBABLE CAUSE	CORRECTIVE MAINTENANCE
Air bubbles in system:		
Sudden deflection of recorder pen from baseline	Micro air bubbles passing through flowcell	Check for air leakage into the system, and for constrictions in the flow path.
Slow decrease in recorder pen position from baseline	Excessive air in dialyzer donor stream	Check size of pump tubes associated with donor stream. Check for constriction in the flow path.
Noise		
Random noise	Reagents dirty	Filter the reagents through filter paper.
Chatter noise	Dirt on slidewire of recorder	Clean the slidewire.
Poor sensitivity or linearity	Defective reagents	Inspect each reagent used with this method. Replace or prepare fresh reagent, as applicable.
	Defective dialyzer membrane	Replace dialyzer membrane
	Worn pump tubes	Replace pump tubes, as required.
No reaction	Reagent lines connected to manifold incorrectly	Refer to the flow diagram, Figure 1, for correct reagent line connections.

For a comprehensive list of related publications which provide additional troubleshooting information, refer to VOLUME III of the Product Labeling for the Technicon AutoAnalyzer II system.

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APPENDIX D

Fasting Plasma Total Cholesterol, High Density Lipoprotein Cholesterol, and Glucose Concentrations for Females and Males

Females

Fasting Plasma Glucose (mg%)

Week	0		4		8		12	
Exercise	Pre	Post	Pre	Post	Pre	Post	Pre	Post
ID								
001	107	126	88	128	--	--	88	130
003	99	--	97	--	91	--	94	--
006	90	--	92	101	93	116	89	105
009	87	--	90	--	92	--	99	--
011	95	106	100	116	99	122	93	138
024	104	143	88	145	76	131	75	141
025	88	128	80	108	77	118	71	91
027	96	114	93	133	106	152	94	147
028	89	124	80	120	84	122	68	115
030	84	102	96	127	--	--	87	112
031	86	127	88	135	--	--	86	139

Males

Fasting Plasma Glucose (mg%)

Week	0		4		8		12	
Exercise	Pre	Post	Pre	Post	Pre	Post	Pre	Post
ID								
802	101	100	101	97	103	103	100	98
803	112	132	103	132	105	124	102	145
804	95	132	101	150	98	146	104	162
805	104	198	104	143	115	167	122	157
806	94	84	98	119	101	120	103	136
807	97	89	96	92	91	99	96	100
820	99	--	95	143	92	135	91	135
821	98	113	96	122	97	119	96	123
822	96	115	91	115	87	131	87	131
825	105	117	77	--	103	110	84	89

Females

Fasting Plasma Total Cholesterol (mg%)

Week	0		4		8		12	
Exercise	Pre	Post	Pre	Post	Pre	Post	Pre	Post
ID								
001	161	206	157	175	--	--	192	235
003	88	--	141	--	118	--	153	--
006	214	--	153	158	172	196	201	230
009	196	--	178	--	224	--	209	--
011	200	237	135	200	204	308	292	265
024	175	207	170	158	174	159	177	182
025	173	178	131	135	198	230	166	156
027	182	205	214	223	211	233	178	182
028	190	199	234	223	184	178	189	194
030	308	302	298	271	--	--	266	260
031	228	209	202	199	--	--	186	172

Males

Fasting Plasma Total Cholesterol (mg%)

Week	0		4		8		12	
Exercise	Pre	Post	Pre	Post	Pre	Post	Pre	Post
ID								
802	140	225	163	165	168	247	287	249
803	202	227	228	205	239	303	215	191
804	141	141	116	145	177	204	139	192
805	196	217	236	236	225	155	253	259
806	239	239	218	208	252	225	200	173
807	254	209	203	219	234	287	219	269
820	151	--	165	178	150	194	155	160
821	132	174	178	235	168	186	175	181
822	186	223	213	176	197	218	176	213
825	149	201	138	--	147	158	159	161

Females

Fasting Plasma HDL-Cholesterol (mg%)

Week	0		4		8		12	
Exercise	Pre	Post	Pre	Post	Pre	Post	Pre	Post
ID								
001	34	23	44	44	--	--	47	46
003	48	55	47	27	54	--	44	31
006	47	--	39	41	61	34	42	54
009	--	--	--	--	--	--	--	--
011	21	--	28	--	34	--	43	--
024	45	--	44	51	43	45	56	49
025	45	54	59	42	45	48	43	53
027	48	58	46	50	78	78	55	52
028	85	64	83	86	70	81	74	--
030	65	--	54	--	--	--	55	--
031	37	46	36	31	--	--	60	44

Males

Fasting Plasma HDL-Cholesterol (mg%)

Week	0		4		8		12	
Exercise	Pre	Post	Pre	Post	Pre	Post	Pre	Post
ID								
802	--	--	--	--	--	--	--	--
803	26	27	31	37	32	32	25	30
804	24	30	36	35	--	34	41	41
805	38	42	--	--	57	33	42	50
806	46	59	34	47	--	34	46	38
807	25	28	36	49	49	36	46	46
820	43	--	40	34	40	43	23	33
821	29	32	25	24	21	23	30	30
822	30	40	47	50	47	46	30	39
825	41	36	54	--	48	53	50	55

Females

HDL-Cholesterol:Total Cholesterol

Week	0		4		8		12	
Exercise	Pre	Post	Pre	Post	Pre	Post	Pre	Post
ID								
001	0.21	0.11	0.28	0.28	--	--	0.24	0.20
003	0.55	--	0.33	--	0.46	--	0.29	--
006	0.22	--	0.25	0.26	0.35	0.17	0.21	0.23
009	--	--	--	--	--	--	--	--
011	0.11	--	0.21	--	0.17	--	0.15	--
024	0.26	--	0.26	0.32	0.25	0.28	0.32	0.27
025	0.26	0.30	0.45	0.31	0.23	0.21	0.26	0.34
027	0.26	0.28	0.21	0.22	0.37	0.33	0.31	0.29
028	0.45	0.32	0.35	0.39	0.38	0.46	0.39	--
030	0.21	--	0.18	--	--	--	0.21	--
031	0.16	0.22	0.18	0.16	--	--	0.32	0.26

Males

HDL-Cholesterol:Total Cholesterol

Week	0		4		8		12	
Exercise	Pre	Post	Pre	Post	Pre	Post	Pre	Post
ID								
802	--	--	--	--			--	--
803	0.13	0.12	0.14	0.18	0.13	0.11	0.12	0.16
804	0.17	0.21	0.31	0.24	--	0.17	0.29	0.21
805	0.19	0.19	--	--	0.25	0.21	0.17	0.19
806	0.19	0.25	0.16	0.23	--	0.15	0.23	0.22
807	0.10	0.13	0.18	0.22	0.21	0.13	0.21	0.17
820	0.28	--	0.24	0.19	0.27	0.22	0.15	0.21
821	0.22	0.18	0.14	0.10	0.13	0.12	0.17	0.17
822	0.16	0.18	0.22	0.28	0.24	0.21	0.17	0.18
828	0.28	0.18	0.38	--	0.32	0.34	0.31	0.34