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SERUM LIPIDS AND CHOLESTEROL DISTRIBUTION IN LIPOPROTEINS OF EXERCISE-TRAINED FEMALE RATS FED SUCROSE.

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Summary

This study was undertaken to evaluate the combined effects of sucrose feeding and exercise training on serum insulin, triglycerides, as well as cholesterol and its distribution into lipoproteins of female Wistar rats. The animals were fed *ad libitum* either laboratory chow alone, or chow and a 32% aqueous sucrose solution. Half of each dietary group was submitted to an exercise-training program. Both sucrose feeding and exercise training elicited greater energy intake. Sucrose feeding produced a marked elevation in triglyceridemia that was prevented by exercise training. Insulin levels paralleled those of triglycerides. The sucrose-fed animals had higher total cholesterol levels than the animals given chow. Although exercise training did not affect total cholesterol in the chow-fed animals, it partly prevented the sucrose-induced elevation in total cholesterol. Cholesterol in the lipoproteins of lower densities was increased significantly with sucrose feeding, and exercise training totally prevented this augmentation. The amount of cholesterol carried by high-density lipoprotein (HDL) was not affected by exercise training in the chow-fed animals. In contrast, sucrose feeding increased HDL-cholesterol in sedentary animals, whereas exercise training partly prevented this increase. The HDL/total cholesterol ratio was similar in all groups. Changes in insulin concentration underline the importance of this hormone in the regulation of blood lipid levels.

The amount and type of carbohydrate in the diet can influence serum lipid levels and the composition and distribution of lipoproteins (1-3). Indeed, serum triglycerides have been shown to be higher when sucrose is given instead of starch (4,5). In man and in the rat, some studies have reported that sucrose feeding does not alter total cholesterol concentration (4,6,7), while others with different experimental conditions have shown an increase in this parameter (2,8-10). A redistribution of cholesterol in the various high-density lipoprotein (HDL) subfractions have also been observed after sucrose feeding (11). On the other hand, exercise training is known to decrease serum triglycerides in man (12) and in the rat (13). While physical activity does not seem to affect serum total cholesterol (14,15), it has been shown to increase cholesterol carried by HDL in man under a variety of experimental conditions (12,16,17), and in some, but not all, studies in the rat (15,18,19).

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Because of the apparent antagonism between the effects of sucrose consumption and exercise training on lipid metabolism, it was decided to evaluate the interactive effects of sucrose feeding and exercise training on serum levels of triglycerides and cholesterol as well as its distribution into serum lipoproteins of female rats.

Methods

Female Wistar rats initially weighing 175-190 g were used. They were housed individually in a room kept at 25°C and with a 12-12 hour light-obscurity cycle. Half of the animals were fed Purina rat chow *ad libitum* (C group). In addition to the chow, the other half was given free access to a 32% aqueous sucrose solution (S group). Half of the animals from both C and S groups were submitted to an exercise-training program which consisted of a daily three-hour long swimming bout (0900-1200), five days a week, for five to seven weeks. The animals swam in three feet of water maintained at 36°C and agitated continuously by a motor-driven panel immersed at one end of the water bath. Body weights and food consumption were recorded twice a week.

A few days before the end of the treatment, the animals were cannulated (20) and blood was drawn for the measurement of various parameters. A sample of fasting blood drawn at that time was used to determine insulin levels by radioimmunoassay. Rats were fasted the night following the last day of treatment. The next morning, they were decapitated and blood was collected. After clotting was achieved, serum was separated by centrifugation at 1,500xg, 4°C, for 20 minutes. Samples for determinations on total serum were stored under nitrogen at -80°C for later use. HDL was separated from lipoproteins of lower densities by ultracentrifugation within 48 hours of blood collection, and serum samples were stored at 4°C when necessary. The density of one mL of serum was raised to 1.063 by addition of solid KBr, and the sample was overlayered with one mL of an NaCl-KBr solution of density 1.063 containing 0.01% EDTA. The tubes were centrifuged at 18°C in a Beckman type L5-65B centrifuge using a 50 rotor, at 122,000xg for 18 hours. The tubes were then cut at about half their length with a tube slicer, and the upper portion containing lipoproteins of lower densities was discarded. The lower portion containing HDL was pipetted into a graduated tube; its volume was adjusted to one mL, and a sample was frozen under nitrogen at -80°C. Separation of HDL was verified by agarose gel electrophoresis of fresh samples. Triglycerides and cholesterol in whole serum and HDL-cholesterol were determined enzymatically using reagents purchased from Boehringer (Montreal, Canada). Cholesterol carried by very low and low density lipoproteins (VLDL+LDL) was obtained by subtraction. Results were statistically analyzed using a 2x2 analysis of variance, covariance analysis and the Duncan's multiple-range test.

Results

Table 1 shows that sucrose feeding produced a slight, statistically insignificant increase in the average daily body weight gain in sedentary animals. Exercise training prevented this increase, resulting in a significant difference between sedentary and exercise-trained groups fed sucrose. The exercise-trained rats ingested more food than their sedentary counterparts in both the chow-fed and the sucrose-fed groups. Food intake, both in terms of grams and kiloJoules, was also significantly higher in sucrose-fed groups. Diet and exercise had no overall effect on body weight gain, but a significant overall effect ($p<0.0001$) on energy intake. Figure 1 shows that all groups had similar fasting serum glucose levels. Triglycerides were slightly, but not significantly lower in the trained animals fed chow alone, as compared to their sedentary counterparts, whereas sucrose feeding produced a marked hypertriglyceridemia that was prevented by exercise training. Serum

TABLE 1

Average Daily Body Weight Gain (BWG), and Food and Energy Intake of Sedentary and Exercise-Trained Female Rats Fed Chow Alone, or Chow and a 32% Sucrose Solution.

		Chow-Fed		Sucrose-Fed	
		Sedentary (12)*	Exercised (12)	Sedentary (11)	Exercised (11)
BWG (g)		1.10 ± 0.07	1.07 ± 0.09	1.27 ± 0.10	0.97 ± 0.08[c]
FOOD INTAKE (g)	Chow	15.1 ± 0.3[a]	18.1 ± 0.3[b]	5.7 ± 0.3[b]	6.6 ± 0.3[bc]
	Sucrose	—	—	12.2 ± 0.4	13.0 ± 0.4
	Total	15.1 ± 0.3	18.1 ± 0.3[b]	17.9 ± 0.4[b]	19.6 ± 0.3[bd]
ENERGY INTAKE (kJ)	Chow	210 ± 4	258 ± 7[b]	79 ± 4[b]	93 ± 4[bc]
	Sucrose	—	—	205 ± 7	217 ± 7
	Total	210 ± 4	258 ± 7[b]	284 ± 6[b]	310 ± 6[bd]

* Number of animals.
[a] Mean ± S.E.M.
[b] Different from chow sedentary at $p<0.01$ (Duncan's multiple range test).
[c] Different from sucrose sedentary at $p<0.05$.
[d] Different from sucrose sedentary at $p<0.01$.
Note: Analysis of variance was performed first for the total group of animals before the Duncan test was applied.

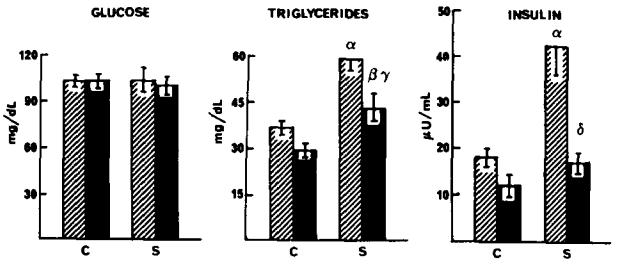

FIG. 1

Fasting glucose, triglyceride and insulin levels in sedentary (hatched bars) and exercise-trained (full bars) female rats fed chow alone (C), or chow and a 32% sucrose solution (S). Bars represent the mean ± S.E.M.

α Different from chow sedentary at $p<0.01$ (Duncan's test).
β Different from chow exercised at $p<0.05$.
γ Different from sucrose sedentary at $p<0.05$.
δ Different from sucrose sedentary at $p<0.01$.

triglycerides were affected overall by diet ($p<0.0002$) and exercise ($p<0.01$). Since the amount of energy ingested could possibly influence serum lipids, a covariance analysis was performed in addition to the analysis of variance, using the average daily energy intake as covariate. In this analysis, the diet effect on serum triglycerides remained significant ($p<0.04$), but the exercise effect did not.

Fasting insulin levels were slightly, but not significantly, lower in the trained animals fed chow alone. Sucrose feeding produced a large increase in insulin levels, which was prevented by exercise training. Of the overall effects of diet ($p<0.0001$) and exercise training ($p<0.0004$) on insulin levels, only the latter remained significant in the covariance analysis ($p<0.0001$).

The sucrose-fed animals also had higher total cholesterol levels than the animals given chow alone, as shown in Figure 2. Although exercise did not affect total cholesterol levels in the chow-fed groups, it partly prevented the sucrose-induced hypercholesterolemia. Both diet and exercise had a significant effect ($p<0.0001$ and $p<0.006$, respectively) on serum total cholesterol. A covariance analysis using energy intake as covariate revealed that diet had a significant effect ($p<0.001$), while the exercise effect lost its significance. An interaction was observed between diet and exercise, and remained significant in the covariance analysis ($p<0.03$). The overall effects on (VLDL+LDL)-cholesterol paralleled those on total cholesterol. Cholesterol in the lipoproteins of lower densities (VLDL+LDL) was increased by 78% with sucrose feeding, and exercise prevented this augmentation (Fig. 2). On the other hand, HDL-cholesterol levels were not affected by exercise in the chow-fed groups, and were higher in the sedentary sucrose-fed animals (36% over sedentary chow-fed group). There was a significant difference between HDL-cholesterol levels in the sedentary and exercise-trained animals given sucrose (12% lower in the latter). Only diet had a significant overall effect upon HDL-cholesterol

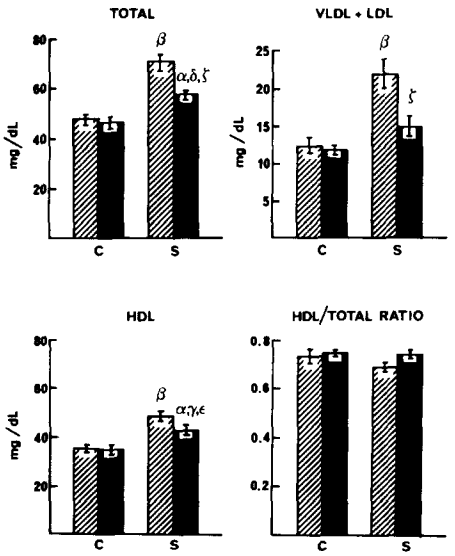

FIG. 2

Fasting total cholesterol and its distribution in lipoproteins of sedentary (hatched bars) and exercise-trained (full bars) female rats fed chow alone (C), or chow and a 32% sucrose solution (S). Bars represent the mean ± S.E.M.

Greek letters mean that group is different from:
α C-sedentary at $p<0.05$.
β C-sedentary at $p<0.01$.
γ C-exercised at $p<0.05$.
δ C-exercised at $p<0.01$.
ε S-sedentary at $p<0.05$.
ζ S-sedentary at $p<0.01$.
(Duncan's test)

TABLE 2

Pearson Correlation Coefficients between Serum Cholesterol Fractions, Daily Energy Intake, Serum Triglycerides and Insulin.

	Energy Intake	Triglycerides	Insulin
Total Cholesterol	0.40 p<0.008	0.52 p<0.002	0.61 p<0.002
(VLDL+LDL)-Cholesterol	0.25 N.S.*	0.66 p<0.0001	0.47 p<0.006
HDL-Cholesterol	0.42 p<0.005	0.32 N.S.	0.52 p<0.006
HDL/Total Cholesterol Ratio	0.007 N.S.	-0.50 p<0.002	-0.16 N.S.
Triglycerides	0.28 N.S.	——	0.57 p<0.003

* Not significant at the p<0.05 level.

levels (p<0.0001), and it remained significant (p<0.04) when energy intake was used as covariate. The HDL/total cholesterol ratio was similar in all four groups. Covariance analysis of the lipid parameters using body weight as covariate showed that this variable had no effect.

Table 2 shows that while total and HDL-cholesterol were correlated to energy intake, the (VLDL+LDL)-cholesterol fraction was correlated with triglyceride levels. Insulin was significantly correlated with all cholesterol fractions and triglycerides. The HDL/total cholesterol ratio was inversely related to triglyceride levels.

Discussion

Sucrose feeding produced a slight increase in body weight gain in sedentary animals, as was reported by others (10), while exercise training prevented this weight increase, probably by reducing adipose tissue mass (21). Exercise training did not affect body weight in chow-fed animals, thus confirming the findings of Oscai and co-workers (22). Sucrose feeding and the exercise-training program used in the present study both induced higher food consumption. The influence of exercise training on food consumption by female rats seems to depend on the intensity and duration of the training period, since food intake was not increased in animals that swam two hours daily for four weeks (19). The animals fed chow and sucrose ingested approximately two thirds of their calories in the form of sucrose. Nevertheless, previous experiments in our laboratory have shown that animals fed sucrose in a similar manner get amounts of the various nutrients that exceed their minimum requirements (23). Adequate nutrient intake was also reflected by body weight gains comparable to that of the chow-fed animals. Total and HDL-cholesterol were both positively associated with the amount of energy intake. This fact underlines the importance of considering total energy intake when studying the influence of various nutrients on serum lipids.

The production of hypertriglyceridemia resulting from sucrose feeding has been reported in several other studies (4,5,24). Elevated triglycerides after chronic sucrose feeding have been shown to result at least partly from an increase in the secretion of VLDL-associated triglycerides by the liver (24,25). Altered activity of lipoprotein lipase, which is found in several tissues including skeletal muscle, heart and adipose tissue, may also be involved, since it is responsible for the clearance of postprandial chylomicrons and endogenous VLDL (26-28). The lowering of triglycerides by exercise in normal rats has been reported in several studies (15,18,24). In the present study, triglycerides were only slightly lowered by exercise in the chow-fed animals, perhaps because of their already low triglyceridemia. However, exercise prevented the sucrose-induced hypertriglyceridemia. Even if the overall exercise-training effect on serum triglycerides lost its significance when energy intake was used as covariate, there is evidence that exercise *per se* prevents the increase in triglycerides caused by sucrose feeding. Indeed, Zavaroni *et al.* (24) reported that the rate of VLDL-triglyceride secretion is reduced in exercise-trained rats fed sucrose, when compared to that of sedentary animals. An increased rate of catabolism of endogenous triglyceride-rich lipoproteins by lipoprotein lipase in exercise-trained rats is another possible mechanism. Although the above cited study by Zavaroni (24), as well as others (29,30), did not report any alterations in lipoprotein lipase activity of rat tissues following exercise training, higher activity of the enzyme has been observed in adipose tissue and skeletal muscle of physically active men and women (31), after acute exercise in the rat (32), as well as in skeletal muscle of exercised-trained rats (33). Although there may exist species differences in the response of the enzyme to exercise, the time after exercise at which the activity of the enzyme is measured could be of importance.

Sucrose feeding produced a significant increase in total and HDL-cholesterol, although the HDL/total cholesterol ratio remained unchanged. While some studies (7,8) report a decrease in HDL-cholesterol following sucrose feeding, others have reported otherwise. For instance, in carbohydrate-sensitive subjects, Reiser *et al.* (2) found an increase in the cholesterol content of all the lipoprotein fractions, including HDL, despite a decrease in the HDL/total cholesterol ratio, when sucrose replaced starch as the source of carbohydrates. Høstmark *et al.* (9,11) found that there was an increase in the HDL_2 subfraction in rats fed sucrose.

The inverse relationship often reported between HDL-cholesterol and triglycerides in man (2,34) was not observed in this study. However, the HDL/total cholesterol ratio was negatively correlated, and the (VLDL+LDL) fraction of cholesterol was positively correlated to serum triglycerides. Since the diets used in the present study contained virtually no cholesterol, the increase in total cholesterol after sucrose feeding must have resulted from increased synthesis and secretion by the liver, and/or from a diminished capacity for cholesterol disposal in the sucrose-fed animals, although the possible role of the level of dietary fiber intake on serum cholesterol concentration (35) cannot be disregarded. The mechanism by which sucrose feeding may influence cholesterol concentration is still unclear. However, it may involve interactions with triglyceride-rich lipoprotein metabolism. VLDL secretion is enhanced by sucrose feeding (24,25), thereby raising the amount of cholesterol carried by the lipoproteins of lower densities. The HDL-cholesterol concentration could be related to VLDL catabolism by lipoprotein lipase. Indeed, HDL_2 has been shown to be formed by the fusion of HDL_3 with surface components of triglyceride-rich lipoproteins during catabolism by lipoprotein lipase (36). HDL *de novo* synthesis by the liver and/or the gut could also have been influenced by diet and exercise training.

The sucrose-induced hyperinsulinemia and its prevention by exercise training observed in the present study is in accordance with other reports (24,37). Furthermore, correlations between insulin levels, triglycerides and cholesterol fractions underline the important role of this hormone in the control of blood lipid levels. Indeed, hepatic VLDL-triglyceride synthesis and secretion are stimulated by insulin (3,24,38). On the other hand, insulin could influence VLDL removal by stimulating lipoprotein lipase activity (39).

Exercise had no effect on total or HDL-cholesterol concentration in the chow-fed animals, while it reduced the latter by 13% in the sucrose-fed animals. The lack of effect of exercise on total cholesterol has been observed in man and in the rat (14,15). On the other hand, higher HDL-cholesterol concentrations have been measured in a variety of physical activity conditions in humans (12,16,17), while in the rat the results are less consistent. Some investigators have reported a lack of change in HDL cholesterol following exercise training (15,40), and this tends to be confirmed by the present experiment. However, in a recent study in this laboratory (19), exercise training increased the HDL/total cholesterol ratio in normal rats, and prevented the drop in the ratio caused by a fat-rich diet. Differences between the latter and the present study may come from the fact that, while animals in the previous experiment swam for two hours daily and did not eat more than the sedentary animals, they were exercised for three hours in the present study, and this resulted in a higher food intake. Thus, higher caloric intake could possibly have masked the exercise effect on HDL-cholesterol. These results stress the importance of the intensity of exercise training and its consequences on food intake in the female Wistar rat, in relation to cholesterol response.

In conclusion, this study demonstrated that exercise training prevented the sucrose-induced hypertriglyceridemia and attenuated the concomitant increase in serum cholesterol in female Wistar rats. Sucrose feeding increased the cholesterol content of HDL and lipoproteins of lower densities. Exercise training prevented the increase in the cholesterol content of the latter, and slightly but significantly lowered HDL cholesterol in the sucrose-fed animals. The concomitant changes observed in insulin concentrations underline the importance of this hormone in the regulation of blood lipid levels.

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References

1. J.A. LITTLE, V. MC GUIRE and A. DERKSEN, Nutrition in Health and Disease. Vol. 1: Nutrition, Lipids, and Coronary Heart Disease, R. Levy, B. Rifkind, D. Dennis and N. Ernst, p. 119-148, Raven Press, New York (1979).
2. S. REISER, M.C. BICKARD, J. HALLFRISCH, O.E. MICHAELIS IV and E.S. PRATHER, J. Nutr. 111 1045-1057 (1981).
3. J.W. FARQUHAR, A. FRANK, R.C. GROSS and G.M. REAVEN, J. Clin. Invest. 45 1648-1656 (1966).
4. S. REISER, J. HALLFRISCH, O.E. MICHAELIS IV, F.L. LAZAR, R.E. MARTIN and E.S. PRATHER, Am. J. Clin. Nutr. 32 1659-1669 (1979).
5. S.S. KANG, K.R. BRUCKDORFER and J. YUDKIN, Nutr. Metab. 23 301-315 (1979).
6. A.T. HØSTMARK and O. GRØNNERØD, Nutr. Rep. Internat. 19 445-450 (1979).
7. R. FEARS, H.P. GLENNY, J.A. TREDGER and R. LINDSAY, Nutr. Rep. Internat. 24 909-917 (1981).

8. I. MACDONALD, Nutr. Rep. Internat. 17 663-668 (1978).
9. A.T. HØSTMARK, E. EILERTSEN and O. GRØNNERØD, J. Nutr. 109 1073-1078 (1979).
10. K.C. ELLWOOD and OE MICHAELIS IV, Nutr. Rep. Internat. 21 113-122 (1980).
11. A.T. HØSTMARK, Ø. SPYDEVOLD, E. LYSTAD, E. KRISTENSEN and I.G. BAY, Lipids 17 489-499 (1982).
12. P.D. WOOD, W. HASKELL, H. KLEIN, S. LEWIS, M.P. STERN and J.W. FARQUHAR, Metabolism 25 1249-1257 (1976).
13. G.M. REAVEN and E.P. REAVEN, Metabolism 30 982-986 (1981).
14. R.J. MOFFATT and J.B. GILLIAM, Artery 6 1-19 (1979).
15. A.K. NARAYAN, J.J. MC MULLEN, D.P. BUTLER, T. WAKEFIELD and W.K. CALHOUN, J. Nutr. 105 581-587 (1975).
16. S.C. ENGER, K. HERBJORNSEN, J. ERIKSSEN and A. FRETLAND, Scand. J. Clin. Lab. Invest. 37 251-255 (1977).
17. M.P. SMITH, J. MENDEZ, M. DRUCKENMILLER and P.M. KRIS-ETHERTON, Am. J. Clin. Nutr. 36 251-255 (1982).
18. C. SIMONELLI and R.P. EATON, Am. J. Physiol. (Endocrinol., Metab., Gastrointest. Physiol.) 234 E221-E227 (1978).
19. Y. DESHAIES, J. LEBLANC and D. RICHARD, Metabolism 32 62-65 (1983).
20. V. POPOVIC and P. POPOVIC, J. Appl. Physiol. 15 727-728 (1960).
21. D. RICHARD and J. LEBLANC, Am. J. Clin. Nutr. 33 2588-2594 (1980).
22. L.B. OSCAI, P.A. MOLE, L.M. KRUSACK and J.O. HOLLOSZY, J. Nutr. 103 412-418 (1973).
23. L.J. BUKOWIECKI, J. LUPIEN, N. FOLLEA and L. JAHJAH, Am. J. Physiol. (Regulat., Integrat., Comp. Physiol.) *in press* (1983).
24. I. ZAVARONI, Y.I. CHEN and G.M. REAVEN, Metabolism 30 476-480 (1981).
25. M.G. RICE and R.P. ROBERTSON, Clin. Res. 27 49A (1979).
26. D.S. ROBINSON and D.R. WING, Adipose Tissue. Regulation and Metabolic Functions. B. Jeanrenaud and D. Hepp, p.41, Academic Press, New York (1970).
27. G. SCHONFELD and B. PFLEGER, Am. J. Physiol. 220 1178-1181 (1971).
28. P. NILSSON-EHLE, A.S. GARFINKEL and M.C. SCHOTZ, Annu. Rev. Biochem. 49 667-693 (1980).
29. N. FUKUDA, T. IDE, Y. KIDA, K. TAKAMINE and M. SUGANO, Nutr. Metab. 23 256-265 (1979).
30. E.M. JONES, H.J. MONTOYE, P.B. JOHNSON, M.J. MARTIN, W.D. VAN HUSS and D. CEDERQUIST, Am. J. Physiol. 207 460-466 (1964).
31. E.A. NIKKILA, M.R. TASKINEN, S. REHUNEN and M. HARKONEN, Metabolism 27 1661-1671 (1978).
32. E.W. ASKEW, G.L. DOHM, R.L. HUSTON, T.W. SNEED and P. DOWDY, Proc. Soc. Exp. Biol. Med. 141 123-129 (1972).
33. J. BORENSZTAJN, M.S. RONE, S.P. BABIRAK, J.A. MC GARR and L.B. OSCAI, Am. J. Physiol. 229 394-397 (1975).
34. G.J. MILLER and N.E. MILLER, Lancet 1 16-19 (1975).
35. J.M. MUNOZ, H.H. SANDSTEAD, R.A. JACOB, G.M. LOGAN, S.J. RECK, L.M. KLEVAY, F.R. DINTZIS and G.E. INGLETT, Am. J. Clin. Nutr. 32 580-592 (1979).
36. J.R. PATSCH, A.M. GOTTO, T. OLIVECRONA and S. EISENBERG, Proc. Nat. Acad. Sci. U.S.A. 75 4519-4523 (1978).
37. S. REISER, H.B. HANDLER, L.B. GARDNER, J.G. HALLFRISCH, O.E. MICHAELIS IV and E.S. PRATHER, Am. J. Clin. Nutr. 32 2206-2216 (1979).
38. H. GINSBERG, J.M. OLEFSKY and G. KIMMERLING, J. Clin. Endocrinol. Metab. 42 729-735 (1976).
39. O.J. PYKALISTO, P.H. SMITH and J.D. BRUNZELL, J. Clin. Invest. 56 1108-1117 (1975).
40. M.H. TAN, A. BONEN, J.B. GARNER and A.N. BELCASTRO, J. Appl. Physiol. 52 1514-1518 (1982).