[3] Wool, I. G.: Insulin and incorporation of radioactivity into nucleic acid fractions of isolated diaphragm. Amer. J. Physiol. *199*:719-21, 1960.

[4] Wool, I. G.: Effect of insulin on nucleic acid synthesis in isolated rat diaphragm. Biochim. Biophys. Acta. *68*:28-33, 1963.

[5] Davidson, M. B., and Goodner, C. J.: Assay of insulin antagonism by serial incubation of paired rat hemidiaphragms. Diabetes *15*:380-87, June 1966.

[6] Schneider, W. C.: Methods in Enzymology, Vol. 3, New York, Academic Press, 1957, p. 680.

[7] Robbins, P. W., Traut, R. R., and Lipmann, F.: Glycogen synthesis from glucose, glucose-6-phosphate, and uridine diphosphate in muscle preparations. Proc. Nat. Acad. Sci. *45*: 6-12, 1959.

[8] Friedman, D. L., and Larner, J.: Interconversion of two forms of muscle UDPG-α-transglucosylase by a phosphorylation-dephosphorylation reaction sequence. Biochim. Biophys. Acta. *64*:185-86, 1962.

[9] Wool, I. G., and Munro, A. J.: An influence of insulin on the synthesis of a rapidly labeled RNA by isolated rat diaphragm. Proc. Nat. Acad. Sci. 50:918-23, 1963.

[10] Ensinck, J. W., Mahler, R. J., and Vallance-Owen, J.: Antagonism of insulin action on muscle by the albumin-bound B chain of insulin. Biochem. J. *94*:150-58, 1965.

[11] Vallance-Owen, J., and Lilley, M. D.: An insulin antagonist associated with plasma-albumin. Lancet *1*:804-06, 1961.

[12] Vallance-Owen, J., Dennes, E., and Campbell, P. N.: The nature of the insulin-antagonist associated with plasma albumin. Lancet *2*:696, 1958.

[13] Recant, L., and Alp, H.: Insulin inhibition and adipose tissue metabolism. Ann. N.Y. Acad. Sci. *131*:334-43, 1965.

[14] Buse, M. G., and Buse, J.: Insulin antagonism associated with albumin. Fifth Congress of the International Diabetes Federation. Abstract No. 336, p. 169, 1964.

[15] Unpublished observations.

[16] Davidson, M. B., and Goodner, C. J.: Effects of insulin antagonistic albumin on transport, glycogen synthetase, RNA synthesis and QO_2 in rat diaphragm. Clin. Res. *14*:176, 1966.

[17] Lowy, C., Blanshard, G., and Phear, D.: Antagonism of insulin by albumin. Lancet *1*:802-04, 1961.

[18] Alp, H., and Recant, L.: Effect of the insulin-inhibitory albumin fraction from normal and diabetic subjects on adipose tissue. Metabolism *13*:609-19, 1964.

[19] Davidson, M. B., and Goodner, C. J.: The failure of synalbumin to demonstrate insulin antagonism in vivo. Diabetes (Submitted).

Effect of Acute Muscular Exercise on Serum Immunoreactive Insulin Concentration

Burt Cochran, Jr., M.D., Edward P. Marbach, Ph.D., Russell Poucher, M.D., Terry Steinberg, M.D., and Grant Gwinup, M.D., Los Angeles

SUMMARY

The change in circulating levels of immunoreactive insulin (IRI) was studied in a group of six normal, three obese, and eight obese diabetic subjects undergoing the acute exercise of stair climbing. Bloods were sampled immediately before and after the exercise period. Serum IRI concentration decreased in ten subjects and did not increase in the remainder of the subjects. The data suggest an exercise metabolite rather than increased insulin secretion accounts for the enhanced glucose assimilation produced by muscular activity. DIABETES *15*:838-41, November, 1966.

It has been demonstrated that the energy expenditure of muscular exercise is accomplished by utilization of glucose as a part of the source of fuel in the laboratory animal[1-4] and in man,[5,6] and that prolonged and severe exercise may produce hypoglycemia.[7] The precise role of body insulin in the metabolism of carbohydrate during exercise has, however, not been defined.

It has been reported that serum insulin-like activity (ILA) as assayed on the rat diaphragm is diminished by muscular activity.[8] In the present study we have utilized a more specific assay for immunoreactive insulin (IRI) to study the effect of acute muscular exercise on serum insulin concentrations in human subjects.

MATERIAL AND METHODS

Ambulatory normal, as well as hospitalized obese subjects, both diabetic and nondiabetic, were studied. All participants fasted overnight for ten to fourteen hours and rested at least fifteen minutes prior to the beginning of exercise. No hypoglycemic or other medication was being used by the group. Serum concentrations of glucose, insulin, lactate and pyruvate were determined on venous blood obtained by the method of Friedemann[9] immediately before and after the acute exercise study. Exercise consisted of climbing up and down six ¾-inch steps, at a rate of seventy to 100 steps per minute.

From the Department of Medicine, University of California, California College of Medicine and the Los Angeles County General Hospital, Los Angeles, California.

TABLE 1

Subject number	Age (yrs.)	Sex	Weight (lb.)	Glucose (mg./100 ml.) Pre	Glucose (mg./100 ml.) Post	Insulin (μU./ml.) Pre	Insulin (μU./ml.) Post	Lactate (mg. per cent) Pre	Lactate (mg. per cent) Post	Pyruvate (mg. per cent) Pre	Pyruvate (mg. per cent) Post	Lactate/Pyruvate ratio Pre	Lactate/Pyruvate ratio Post
1	25	M	165	71	70	5	<5	5.4	34.0	.32	1.2	17	28
2	25	M	178	73	69	<5	<5	5.8	13.0	.45	.63	13	21
3	25	M	164	73	70	<5	<5	2.7	11.0	.36	.41	8	27
4	24	M	150	60	66	15	10	8.1	22.0	.49	1.2	17	18
5	26	M	188	78	74	10	<5	5.4	11.0	.31	.95	17	12
6	24	M	165	72	73	5	5	8.1	9.7	.44	.41	18	24
7*	31	F	209	88	78	<5	<5	10.0	19.0	.91	2.5	11	8
8*	22	F	220	86	86	<5	<5	9.1	51.0	.58	2.4	16	21
9*	26	F	300	83	78	10	5	9.0	44.0	.91	2.2	10	20
10†	45	F	240	87	87	10	10	10.4	41.0	.82	2.2	13	19
11†	59	F	204	226	198	10	5	14.0	48.0	1.5	2.2	9	22
12†	50	M	248	102	99	50	20	11.1	41.0	1.0	2.3	11	18
13†	48	M	329	212‡	197‡	20	15	11.6	34.0	—	—	—	—
14†	45	F	200	202‡	176‡	40	35	8.9	42.0	.78	1.9	11	22
15†	46	F	264	109	109	<5	<5	8.8	19.6	—	—	—	—
16†	25	M	207	122	122	5	<5	5.2	31.0	.78	2.2	7	14
17†	48	M	255	106*	100*	15	10	9.6	—	.76	—	—	—

*Obese nondiabetic subjects.
†Diabetic patients.
‡Sundermann and Fuller glucose method.[11]

The normal subjects climbed continuously but the obese and obese diabetic subjects alternated one minute of climbing with one minute of seated rest. The period of exercise was terminated in twenty-five minutes or, in the case of the obese subjects, when distressing fatigue developed.

Serum glucose was determined by the glucose oxidase method of Ware and Marbach[10] except for three instances in which the method of Sundermann and Fuller[11] was used. Serum insulin was measured by a modification of the immunoassay of Meade and Klitgaard[12] in which free insulin is separated from antibody-bound insulin by adsorption to the ion exchange resin Amberlite CG-400. Blood lactate was determined by the method of Barker and Sumerson[13] and pyruvate by the method of Segal et al.,[14] except in the normal subjects where a common metaphosphoric acid filtrate was assayed enzymatically[15] for both. Standard oral glucose tolerance tests were done on each of the obese and obese diabetic subjects and the designation of "diabetic" was made on the basis of the criteria suggested by Conn and Fajans.[16]

RESULTS

As shown in table 1 serum glucose decreased with exercise in eleven of the studies, remained unchanged in four and increased in two. The magnitude of change was of questionable significance and similar for all three clinical groups.

The concentration of serum insulin decreased in ten of the seventeen studies and appeared to remain unchanged in the remainder. With the lower limit of sensitivity of the insulin assay 5 μU. per milliliter, it is not certain that there was no change. It can, however, be stated that in the five studies in which there was no apparent change the insulin level did not rise above 5 μU. per milliliter with exercise, and in none of the studies was an increase in serum insulin demonstrated. Among the obese diabetics, the somewhat higher basal levels of serum insulin resulted in an apparent greater magnitude of change.

In each study in which blood lactate and pyruvate were measured both increased with acute exercise. The increase of lactate was relatively greater than that of pyruvate in twelve of fourteen studies.

DISCUSSION

At one time it was proposed[17,18] that increased insulin secretion might account for the carbohydrate utilization observed in muscular exercise. This hypothesis became untenable when it was demonstrated that exercise produced hypoglycemia in eviscerated or pancreatectomized animals.[4,19,20] More recently, Goldstein,[21] Wertheimer[22] and others[4] have contributed to our understanding of the mechanism by which skeletal muscle contraction enhances glucose assimilation. Their studies of an evanescent metabolite produced by contracting muscle might also suggest that the effect on glucose metabolism is not mediated through increased insulin secretion. Devlin[8] studied the effects of acute exercise on the concentration of serum ILA as assayed on the rat diaphragm. In ten normal volunteers acute exercise caused a reduction of serum ILA in five, an increase in two, and no significant change in three subjects. The mean change for the group was a decrease of 15 per cent. In a recent abstract Schalch[23] reported serum IRI was not significantly altered by vigorous, competitive exercise.

Since our data reflect conditions present immediately before and after exercise, no conclusions can be reached regarding any dynamic changes which might have occurred during the exercise period. In view of marked differences in serum growth hormone concentration found immediately upon awakening, and after arising and coming to the laboratory,[24-26] it might be interesting to repeat these studies in a more truly basal state. It has been demonstrated, however, that even a brisk walk will not usually elevate blood lactate levels.[9] The assumption that the exercise stress reported here was adequate to produce measurable metabolic changes is corro-

borated by the increases in postexercise lactate, and particularly, by the increasing lactate/pyruvate ratio, which has been suggested as an index of anaerobiosis.[27,28]

Our data suggest, therefore, that acute exercise stress of the type used in this study results in no measurable change or a decrease in serum insulin concentration which accompanies insignificant changes in serum glucose and a rise in levels of lactate and pyruvate. These data must be interpreted in light of the demonstrated enhanced glucose utilization accompanying muscular exercise. We suggest that the stability of glucose concentration is accomplished by both the known counter-regulatory mechanisms which accompany exercise,[29-31] as well as the reduction in the concentration of serum insulin which we have demonstrated.

The decrease in serum IRI and the insignificant changes in blood glucose concentration at a time when there is increased glucose utilization might be principally due to increased sympathetic activity. This is supported by the recent work of Porte et al. who have shown that epinephrine inhibited an expected increase in serum IRI during the administration of substances (glucose, glucagon and tolbutamide) known to elevate serum insulin.[30] Following the acute exercise in the present study, all subjects exhibited profuse perspiration and tachycardia. Sanders et al.[5] have demonstrated augmented glucose utilization by exercised muscles and suggested that this may be due to hypoxia per se which would also produce an elevated serum lactate level. The role that the increase in serum lactate itself might play must remain speculative. However, it is conceivable that the increase in lactate concentration might affect the cell membrane transport system to promote an influx of glucose into the muscle and that the resulting reduction of circulating glucose levels would constitute a decreased stimulus for insulin secretion. Gourley and Fisher[33] have demonstrated that the oxygen consumption of a skeletal muscle preparation incubated with insulin is enhanced by the addition of lactate. Clinical investigations under way at this center include an attempt to define serum insulin and glucose responses to lactic acid infusions. From this may evolve a more precise definition of the relationship of serum insulin, lactate and glucose in response to acute muscular exercise.

ACKNOWLEDGMENT

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REFERENCES

[1] Ingle, D. J., Nezamis, J. R., and Morley, E. H.: Work output and blood glucose in values in severely diabetic rats with and without insulin. Amer. J. Physiol. *165*:469, 1951.

[2] Goldstein, M. S., Mullick, V., Huddlestun, G., and Levine, R.: Action of muscular work on transfer of sugars across cell barriers: Comparison with action of insulin. Amer. J. Physiol. *173*:212, 1953.

[3] Ingle, D. J., Morley, E. H., and Stetten, D., Jr.: Metabolism of glucose in resting and worked rats. Amer. J. Physiol. *182*: 263, 1955.

[4] Dulin, W. E., and Clark, J. J.: Studies concerning a possible humoral factor produced by working muscles. Diabetes *10*:289, 1961.

[5] Sanders, C. A., Levinson, G. E., Abelmann, W. H., and Freinkel, N.: Effect of exercise on the peripheral utilization of glucose in man. New Eng. J. Med. *271*:220, 1964.

[6] Reichard, G. A., Issekutz, B., Jr., Kimbel, P., Putnam, R. C., Hochella, N. J., and Weinhouse, S.: Blood glucose metabolism in man during muscular work. J. Appl. Physiol. *16*:1001, 1961.

[7] Levine, S. A., Gordon, G., and Derick, C. L.: Some changes in chemical constituents of blood following marathon races: With special reference to the development of hypoglycemia. JAMA *82*:1778, 1924.

[8] Devlin, J. G.: The effect of training and acute physical exercise on plasma insulin-like activity. Irish J. Med. Sci. *6*:423, 1963.

[9] Friedemann, T. E., Haugen, G. E., and Kmieciak, T. C.: Pyruvic acid III. The level of pyruvic and lactic acids, and the lactic-pyruvic ratio, in the blood of human subjects. The effect of food, light muscular activity, and anoxia at high altitude. J. Biol. Chem. *157*:673, 1945.

[10] Ware, A. G., and Marbach, E. P.: Application of a glucose oxidase method to serum. Clin. Chem. *11*:792, 1965 (Abstract).

[11] Sundermann, F. W., and Fuller, J. B.: A modification of the Benedict method for measuring blood glucose. Amer. J. Clin. Path. *21*:1077, 1951.

[12] Meade, R. C., and Klitgaard, H. M.: A simplified method for immuno-assay of human serum insulin. J. Nucl. Med. *3*: 407, 1962.

[13] Barker, S. B., and Sumerson, W. H.: Colorimetric determination of lactic acid in biological material. J. Biol. Chem. *138*:535, 1941.

[14] Segal, S., Blair, A. E., and Wyngaarden, J. B.: An enzymatic spectrophotometric method for the determination of pyruvic acid in blood. J. Lab. Clin. Med. *48*:137, 1956.

[15] Marbach, E. P., Goodell, D. E., and Weil, M. H.: Rapid determination of lactate-pyruvate during shock. Fed. Proc. *24*: 341, 1965 (Abstract).

[16] Fajans, S. S., and Conn, J. W.: The early recognition of diabetes mellitus. Ann. N.Y. Acad. Sci. *82*:208, 1959.

[17] Dill, D. B., Edwards, H. T., and Mead, S.: Blood sugar regulation in exercise. Amer. J. Physiol. *111*:21, 1935.

[18] Issekutz, B., Jr., Miller, H. I., and Rodahl, K.: Effect of exercise on FFA metabolism of pancreatectomized dogs. Amer. J. Physiol. *205*:645, 1963.

[19] Ingle, D. J., Nezamis, J. E., and Rice, K. L.: Work output and blood glucose values in normal and in diabetic rats subjected to the stimulation of muscle work. Endocrinology *46*:505, 1950.

[20] Moxness, R. E., Molnar, G. D., and McGuckin, W. F.: Exercise and blood glucose concentration in intact and pancreatectomized dogs. Diabetes *13*:37, 1964.

[21] Goldstein, M. S.: Humoral nature of the hypoglycemic factor of muscular work. Diabetes *10*:232, 1961.

[22] Wertheimer, H. E., and Havivi, E.: The in vitro release of a product of muscular activity which increases the transport of sugars into diaphragm. Harefuah *62*:251, 1962.

[23] Schalch, D. S.: The effect of physical stress and exercise in the human on growth hormone and insulin secretion. Clin. Res. *13*:344, 1965 (Abstract).

[24] Roth, J., Glick, S. M., Yalow, R. S., and Berson, S. A.:

Secretion of human growth hormone: physiologic and experimental modification. Metabolism *12*:577, 1963.

[25] Roth, J., Glick, S. M., Yalow, R. S., and Berson, S. A.: The influence of blood glucose on the plasma concentration of growth hormone. Diabetes *13*:355, 1964.

[26] Hunter, W. M., Fonseka, C. C., and Passmore, R.: Effect of exercise and feeding on plasma growth hormone levels. Biochem. J. *96*:75 p. 1965.

[27] Hill, A. V., Long, C. N. H., and Lupton, H.: Muscular exercise, lactic acid, and supply and utilization of oxygen. V. The recovery process after exercise. Proc. Roy. Soc., London, Ser. B *97*:96, 1924.

[28] Huckabee, W. E.: Relationships of pyruvate and lactate during anaerobic metabolism. II. Exercise and formation of O_2-debt. J. Clin. Invest. *37*:255, 1958.

[29] Krebs, H. A., and Yoshida, T.: Muscular exercise and gluconeogenesis. Biochem. Z. *338*:241, 1963.

[30] Nikkila, E. O., Torsti, P., and Penttilla, O.: Effects of fasting, exercise and reserpine on cathecholamine content and lipoprotein lipase activity of rat heart and adipose tissues. Life Sci. *4*:27, 1965.

[31] Cornil, A., De Coster, A., Copinschi, G., and Franckson, J. R. M.: Effect of muscular exercise on the plasma level of cortisol in man. Acta Endocr. *48*:163, 1965.

[32] Porte, D., Jr., Graber, A. L., Kuzuya, T., and Williams, R. H.: The effect of epinephrine on immuno-reactive insulin levels in man. J. Clin. Invest. *45*:228, 1966.

[33] Gourley, D. R. H., and Fisher, K. C.: The role of lactate in the stimulation of the oxygen consumption of frog muscle by insulin. Canad. J. Physiol. Pharmacol. *42*:139, 1964.

REPORT TO THE EDITOR

1966 Award Essays of the New York Diabetes Association

Prize-winning entries in the Third Annual Prize Essay Contest on diabetes mellitus, sponsored by the Clinical Society of the New York Diabetes Association for House Staff Officers and Fellows of hospitals in the metropolitan New York area, were presented on May 19 at a meeting of the Society at the New York Academy of Medicine.

Judges of the contest were James Berkman, M.D., Martin Goldner, M.D., Irving Graef, M.D., and Harold Rifkin, M.D.

The abstracts of the winning papers follow.

MAX ELLENBERG, M.D.
Chairman, Prize Essay Contest Committee

FIRST PRIZE

Effects of Diazoxide Administration on Plasma Glucose, Insulin, and Lipids in Von Gierke's Disease

Gabriel Spergel, M.D., Fellow in Diabetes and Endocrinology, Metabolic Research Unit, The Jewish Hospital of Brooklyn, Brooklyn, New York.

Diazoxide, a nondiuretic thiazide, has been noted to cause hyperglycemia and has been used therapeutically in the treatment of the hypoglycemia associated with leucine sensitivity and insulinomata.

The mechanisms by which this drug induces hyperglycemia were studied during the treatment of a twelve-year-old boy with type I glycogen storage disease and marked hyperlipemia.

(1) Inhibition of the plasma immunoassayable insulin response to administered glucose was shown, as well as an increase of 30 to 70 mg. per 100 ml. in postprandial plasma glucose levels. Oral glucose tolerance decreased during treatment with diazoxide. These changes in plasma glucose were not due to glycogenolysis, by virtue of the enzymatic defect present in this patient.

(2) During treatment, skin xanthomata disappeared although plasma triglycerides rose. Serum cholesterol was unchanged while plasma free fatty acids initially rose but subsequently spontaneously declined.

(3) Analysis of serial oral glucose tolerance tests suggests that diazoxide inhibits hepatic glucose uptake, as part of its hyperglycemic action.

SECOND PRIZE

Reevaluation of the Role of Calcium Ion in Corticotropin-induced Lipolysis in Vitro

Melvyn Klein, Fourth year student, Downstate Medical Center, Brooklyn, New York.

The calcium-dependent nature of corticotropin (ACTH) induced lipolysis in vitro, shown by Engel