

SERUM INSULIN AND GLUCOSE RESPONSE TO GRADED EXERCISE IN ADULTS

PART I: THE INFLUENCE OF FITNESS STATUS

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ABSTRACT

The effect of acute exercise upon serum immunoreactive insulin levels (IRI) and serum glucose concentrations (GC) was studied in groups of middle-aged men of contrasting physical fitness status. Two groups of subjects, one active and one sedentary (both N = 11, mean age 44 years), performed a graded cycle ergometer exercise test in the post-absorptive state. Venous blood samples were taken at rest, during low and high work intensities, and after recovery. The response of serum IRI to exercise was similar in both groups of subjects with significant increases observed during exercise followed by a return to resting values during recovery. However, the magnitude of serum IRI response was lower in the active group. In contrast, the sedentary group demonstrated little or no change in serum GC during exercise, whereas significant increases in serum GC were observed during exercise in the active group.

INTRODUCTION

Considerable variation in the response of circulating insulin levels has been reported in studies involving human subjects during exercise. Decreased plasma insulin levels have been observed during acute exercise (Cochran *et al*, 1966) and during heavy and prolonged severe exercise (Pruett, 1969; Pruett, 1970; Pruett and Oseid, 1970; Wahren *et al*, 1971) in spite of increase glucose utilisation (Hartley *et al*, 1972; Wahren *et al*, 1971). Moreover decreased serum insulin levels have been demonstrated during moderate work where little or no change occurred in blood glucose concentration (Hartley *et al*, 1972 (a); Métivier *et al*, 1971; Nikkilä *et al*, 1968; Oseid and Hermansen, 1971; Pruett and Oseid, 1970). In contrast some investigations have shown that exercise to fatigue (Rottini *et al*, 1971), intermittent maximal exercise (Hermansen *et al*, 1970), and even moderate exercise (Reinheimer, Davidson and Albrinck, 1968; Schwartz *et al*, 1969) have led to significantly increased serum insulin levels as well as increased serum glucose concentrations.

Such variations in insulin levels during exercise may possibly be explained in terms of the inhibition of insulin by catecholamines (Conard *et al*, 1969), altera-

tions in the catabolic rate of endogenous insulin (Franckson *et al*, 1971), or by variations attributable to differences in the fitness status of the subjects under investigation (Björntorp *et al*, 1972).

In the present investigation we have studied the patterns of serum immuno-reactive insulin level (IRI) and serum glucose concentration (GC) during exercise in order to discern differences related to fitness status.

SUBJECTS

Twenty-two men, aged 27-57 years, were used in this investigation. Two groups were established, one active and one sedentary (both N = 11), based upon the physical fitness criterion of Ismail, Falls and MacLeod (1965). The physiological characteristics of the subjects are given in Table I. The two groups represent non-obese and moderately obese men as opposed to weight-matched sedentary and active individuals, as demonstrated by the differences in weight and percentage of lean body weight. All subjects gave their informed consent and had undergone medical examinations before participating in the investigation.

METHODS

On reporting to the laboratory the subjects were allowed

Index terms: Physical Fitness; insulin; glucose; graded exercise.

TABLE I
Physical characteristics and physiological responses of the two groups of subjects who participated in the study

	Active Group Mean \pm S.E.	Sedentary Group Mean \pm S.E.
Age (yr)	44.6 \pm 2.5 (range 27-54)	43.7 \pm 2.5 (range 28-57)
Height (cm)	181.3 \pm 1.6	182.9 \pm 2.2
Weight (kg)	82.9 \pm 3.4	102.3 \pm 7.8
% Lean	84.2 \pm 1.6	77.1 \pm 1.2
Blood Pressures (mm Hg)		
Systolic	126.4 \pm 4.3	133.1 \pm 3.6
Diastolic	77.8 \pm 1.7	88.7 \pm 3.5
Pulse	47.7 \pm 2.9	44.4 \pm 2.2
Heart Rates (b.min ⁻¹)		
Rest	58.7 \pm 2.4	66.7 \pm 2.8
Low Intensity	110.0 \pm 4.1	124.4 \pm 5.4
High Intensity	157.3 \pm 3.8	154.6 \pm 2.4
$\dot{V}O_2$ ml kg ⁻¹ min ⁻¹		
Low Intensity	22.6 \pm 1.2	24.8 \pm 2.0
High Intensity	45.4 \pm 1.6	36.4 \pm 2.0
Respiratory Quotient		
Low Intensity	0.82 \pm 0.02	0.85 \pm 0.02
High Intensity	0.92 \pm 0.02	0.94 \pm 0.02
% Predicted $\dot{V}O_2$ max		
Low Intensity	45%	59%
High Intensity	92%	88%

Low/high intensity exercise expressed as % predicted $\dot{V}O_2$ max

to rest in the supine position for 10 min before performing graded exercise seated on a cycle ergometer (Monark, Sweden), followed by a 15 min recovery period in the supine position. Venous blood samples were taken from the antecubital vein at rest, during exercise and after recovery, and later analysed for serum IRI and SG as well as serum free fatty acids (FFA) and serum corticosteroids (SC).

The subject's respiratory and cardiac responses were monitored during exercise and $\dot{V}O_2$ and HR were determined. Each subject was studied in the post absorptive state between 08.00 and 12.00 hr and all subjects were familiarised with the cycle ergometer test before the investigation.

Two exercise levels were selected to produce low (\sim 50% predicted $\dot{V}O_2$ max) and high (\sim 90% of predicted $\dot{V}O_2$ max) work intensities in each group of subjects. Maximal oxygen uptake capacity was predicted using the procedure of Åstrand and Ryhming (1954) with a correction factor for age. In view of the limitations of this procedure (Davies, 1968) and in the interests of the safety of the subjects concerned, the upper limit of high intensity exercise was established by an HR in excess of 160 b.min⁻¹ (Balke, 1960), or prior to this if the subject indicated that he was exhausted or could no longer maintain the required work rate, at which point oxygen intake ($\dot{V}O_2$) was measured.

The exercise consisted of a low intensity work of 10 min duration at 100 W (600 kpm/min) and a pedalling frequency of 50 rpm. This involved work at 45% and 59% of predicted $\dot{V}O_2$ max for the active and sedentary groups respectively. Following the low intensity work bout the subjects immediately performed a bout of high intensity exercise which involved an increase in the work load of 25 W/min (150 kpm/min) until the relative work output demanded 92% and 88% of predicted $\dot{V}O_2$ max for the active and sedentary groups, respectively.

Oxygen intake ($\dot{V}O_2$)

The subjects inspired through a 3-way "J" valve and expired air was collected during the last 30 seconds of each exercise bout in a 150 litre Tissot gasometer equipped with a kymograph. All volumes of expired air were corrected to STPD and duplicate samples were analysed for O₂ and CO₂ using a Beckman 777 oxygen analyser and a Beckman BI-I medical gas analyser, respectively. Both instruments were calibrated at frequent intervals using commercial gas mixtures of known concentrations which had been checked using the micro-Scholander apparatus. Respiratory quotients (RQ) were calculated from the O₂ and CO₂ data.

Heart rates (HR) were monitored at rest and throughout the exercise period using a stethoscope placed at the apex of the heart. HR was noted during the last minute of each level of exercise. Blood pressure was recorded under resting conditions using a standard clinical sphygmomanometer and a stethoscope placed over the brachial artery. Systolic and diastolic pressures were noted and pulse pressure determined. The percentage of lean body weight (% LBW) was estimated using the method of Wilmore and Behnke (1969).

Blood samples were centrifuged at 3,000 rpm for 10 min and serum was separated and frozen in aliquots at -20°C until analysed. Serum immunoreactive insulin (IRI) level was determined using the Phadebas R radio-immunoassay test for insulin (Pharmacia Laboratories Inc., Piscataway, N.J.) based on the procedure by Yalow

and Berson (1960). Serum glucose concentration (GC) was determined by a standard clinical laboratory procedure using a 'Technicon' autoanalyser based on a modification of the method of Hoffman (1937). Serum corticosteroid (SC) concentration was determined by a modification (Few and Cashmore, 1971) of the competitive protein binding technique of Murphy (1969). Serum free fatty acid (FFA) concentration was assessed by the method of Dole (1956) as modified by Trout, Estes and Friedberg (1960).

The differences between means were tested using the Student's 't' test with the appropriate degrees of freedom.

RESULTS

The physical characteristics of the two groups of subjects are presented in Table I. The active group had significantly lower body weight and % LBW ($p < 0.05$) and tended to have lower systolic and diastolic pressures but higher pulse pressure than the sedentary group although the differences were not statistically significant. The changes in serum insulin and glucose of the two groups of subjects are presented in Table II, and the time course of changes in mean serum insulin level and glucose concentration for both groups of subjects during the exercise test is presented in Figure 1.

The active group displayed a significant rise in mean insulin level over a resting value of $19.2 \pm 4.5 \mu\text{U/ml}$ during exercise at 92% predicted $\dot{V}O_2 \text{ max}$ ($33.0 \pm 8.0 \mu\text{U/ml}$, $p < 0.05$), followed by a significant fall during recovery to $15.9 \pm 4.2 \mu\text{U/ml}$ ($p < 0.05$). This was accompanied by a progressive, but non-significant rise in mean glucose concentration over the resting value of $82.7 \pm 3.5 \text{ mg\%}$ during exercise, but becoming significantly elevated to $89.9 \pm 2.5 \text{ mg\%}$ during recovery ($p < 0.05$).

The sedentary group showed a similar response pattern in mean insulin levels to exercise, with a significant rise over a resting value of $30.0 \pm 6.7 \mu\text{U/ml}$ during

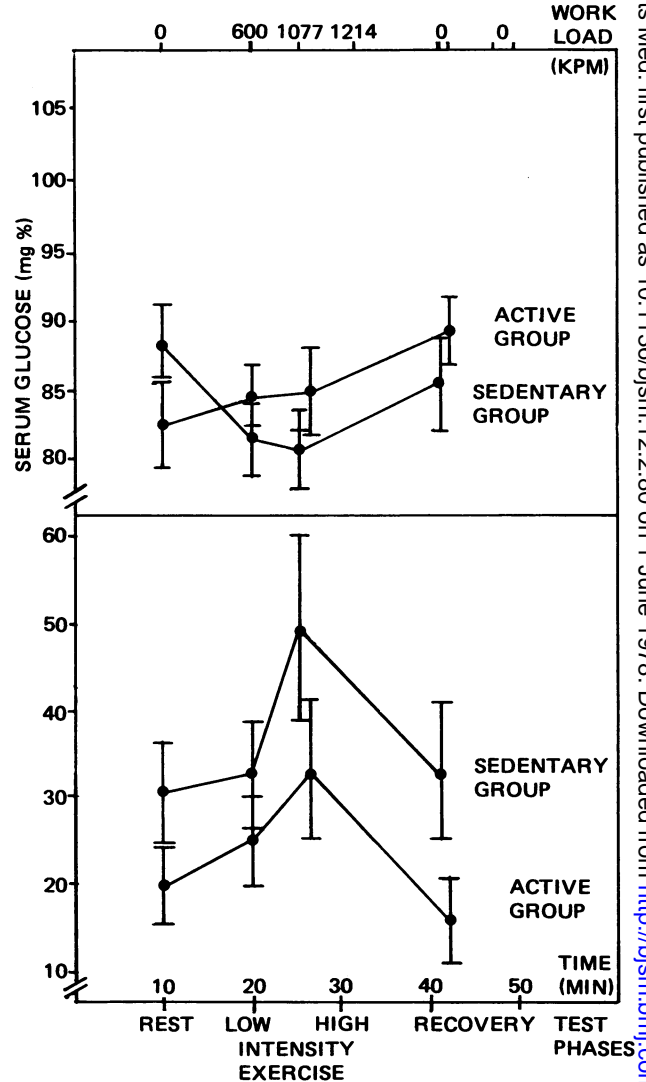


Figure 1. Mean (\pm S.E.) serum insulin level and glucose concentration of the active and sedentary groups.

TABLE II

Serum insulin and glucose values for the active and sedentary groups during exercise

Test Phase	Serum Insulin ($\mu\text{U/ml}$) (Mean \pm S.E.)			% Predicted $\dot{V}O_2 \text{ max}$		Serum Glucose (mg %) (Mean \pm S.E.)		
	Sedentary Group	(P)	Active Group	Sedentary Group	Active Group	Sedentary Group	(P)	Active Group
Rest	30.0 \pm 6.7	N.S.	19.2 \pm 4.5	—	—	88.5 \pm 3.2	N.S.	82.7 \pm 3.5
Low Intensity	31.8 \pm 6.2	< 0.05	24.5 \pm 4.6	59%	45%	81.8 \pm 3.0	N.S.	84.5 \pm 2.4
High Intensity	49.6 \pm 10.8	N.S.	33.0 \pm 8.0	88%	92%	80.8 \pm 2.8	N.S.	85.4 \pm 2.9
Recovery	32.6 \pm 8.6	< 0.05	15.9 \pm 4.2	—	—	86.9 \pm 2.7	N.S.	89.9 \pm 2.5

Low/high intensity exercise expressed as % predicted $\dot{V}O_2 \text{ max}$

exercise at 88% of predicted $\dot{V}O_2$ max (49.6 ± 10.8 , $p < 0.05$), and a corresponding decline to a recovery value of $32.6 \pm 8.6 \mu\text{U/ml}$ ($p < 0.05$). However, in contrast to the active group, mean glucose concentration fell significantly from a resting value of $88.5 \pm 3.2 \text{ mg\%}$ during exercise at 88% of predicted $\dot{V}O_2$ max ($80.8 \pm 2.8 \text{ mg\%}$, $p < 0.05$), but returned towards the resting value during recovery.

The active group had significantly lower mean insulin level than the sedentary group in recovery, although no significant differences in mean glucose concentration were observed between the groups before, during or after exercise (Table II).

DISCUSSION

The results of this investigation demonstrate a pronounced similarity in the response of serum insulin levels in active and sedentary subjects during acute exercise, although differences in the magnitude of insulin levels were observed. However, differences in both the response pattern and the magnitude of serum glucose concentration during acute exercise were noted between active and sedentary subjects.

The variability in the responses of serum insulin and glucose to exercise reported by other studies cited in this investigation may be due to the differences in experimental protocol as well as in the nature of the subjects under investigation. It is possible therefore, that by taking into account the intensity and duration of the exercise procedures employed in other works, as well as the fitness status of the subjects tested, the results of this investigation selectively support, and to some extent help explain, the findings of other studies.

The findings support evidence for an increase in insulin level and a decrease in glucose concentration in untrained subjects exercised to fatigue (Rottini *et al*, 1971) as indicated by the response of our sedentary group. Likewise the insulin and glucose responses of the active group supports work which has suggested similar increases in trained subjects exposed to intermittent "maximal" exercise bouts of short duration (Hermansen *et al*, 1970). Furthermore, increases in serum insulin and glucose have been observed during exercise of moderate intensity in obese subjects or patients with reduced performance capacity due to cardio-vascular disease (Nikkilä *et al*, 1968) as well as "normal healthy" individuals following moderate exercise (Reinheimer, Davidson and Albrinck, 1968).

The importance of blood glucose as an energy substrate in exercise has been demonstrated despite reduced insulin levels (Wahren *et al*, 1971). Furthermore glucose assimilation into exercising muscle and its utilisation rate during exercise has been shown to be somewhat inde-

pendent of systemic or local availability of insulin (Freinkel *et al*, 1959; Rasio *et al*, 1966). Such findings may explain the observations of reduced insulin level occurring with unchanged or minor fluctuations in glucose concentration during exercise (Hartley *et al*, 1972; Métivier *et al*, 1971; Nikkilä *et al*, 1968; Oseid and Hermansen, 1971). Moreover the contribution of glucose to the energy substrate supply of working muscle appears to be related to increased turnover rate rather than elevated blood glucose concentration *per se* (Conard *et al*, 1969; Wahren *et al*, 1971). However the importance of FFA as an energy substrate during moderate and heavy exercise must be considered and the fall in FFA concentration observed in this study (Fig. 2) probably represented increased utilisation during low and high intensity exercise (Horstman *et al*, 1971).

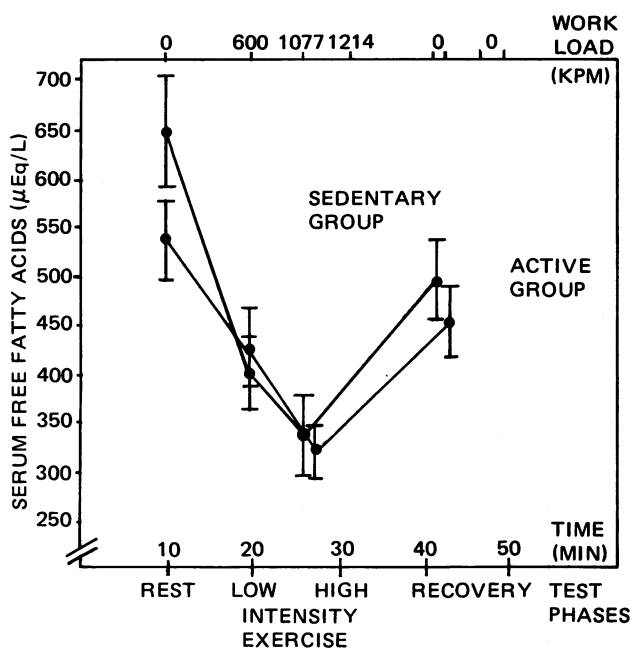


Figure 2. Mean (\pm S.E.) serum free fatty acid concentration of the active and sedentary groups.

In order to explain an increase in insulin level during exercise, it has been postulated that anoxia of the pancreatic islet tissue may be the stimulus for the release of centrally stored insulin (Nikkilä *et al*, 1968). Alternatively theoretical work suggests the storage of labile insulin not in a homogeneous form, but distributed as "packets" with specific sensitivity thresholds relative to varying glucose concentration (Grodsky, 1972). This would appear to contrast with the evidence for widely distributed storage of insulin throughout the body (Rasio *et al*, 1972), and the release of peripherally stored insulin during muscular work (Dieterle *et al*, 1973).

However, under conditions where augmented glucose utilisation and reduced insulin levels have been observed during muscular activity, the existence of local insulin-like activity factors have been suggested but not proven conclusively (Couturier, Rasio and Conard, 1971; Devlin, 1963; Havivi and Wertheimer, 1964; Szabo, Szabo and Mahler, 1972).

In the present study the variability in the magnitude of insulin levels between active and sedentary subjects reflected physiological differences between the two groups of subjects. The sedentary group were heavier, with a lower percentage of lean body weight and a higher resting insulin level than the active group. These observations are consistent with evidence that demonstrates that variability of basal insulin level is a function of relative adiposity (Porte and Bagdade, 1970), although the influence of dietary regime on basal insulin level cannot be overlooked (Muller, Faloona and Unger, 1971). Furthermore the proportionately greater elevation of insulin level observed in the sedentary subjects during exercise, may have been due in part to an increased rate of insulin secretion by these subjects (Nikkilä *et al*, 1968) and possibly to a higher insulin sensitivity on the part of the active subjects (Björntorp *et al*, 1972). Indeed this may explain why, in the case of active subjects, blood glucose concentrations were main-

tained at or increased above resting values during exercise when compared with their sedentary counterparts.

It is possible that the responses observed in the sedentary group may have represented hyperinsulinaemic obese subjects, whereas the active group represented normal insulinaemic non-obese subjects, although no glucose tolerance tests were carried out to determine if any sub-clinical diabetics were included in the sample.

In summary, the variations in serum insulin and glucose observed between active and sedentary subjects in this study, probably represent chronic differences associated with subjects of widely contrasting fitness status (Björntorp *et al*, 1972). Furthermore, it is likely that the differences observed between the two groups reflect not only the long term effects of physical conditioning, but also variations in lifestyle, since it was ascertained by personal interview, that the groups of subjects selected had maintained "active" and "sedentary" lifestyles since early adulthood.

The experiments described in this paper were approved by the Committee on the Use of Human Subjects in Research at Purdue University.

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The second part of this work will be published shortly in another number of B.J.S.M.

Editors

REFERENCES

- Åstrand, P. O. and Ryhming, I. 1954. A nomogram for calculation of aerobic capacity from pulse rate during submaximal work. *J. Appl. Physiol.* **7**: 218-221.
- Balke, B. 1960. The effect of physical exercise on the metabolic potential. In: *Exercise and Fitness*. The Athletic Institute p. 74, Chicago, Illinois.
- Björntorp, P., Fahlen, M., Grimby, G., Gustafson, A., Holm, J., Renström, P. and Scherstén, T. 1972. Carbohydrate and lipid metabolism in middle-aged, physically well-trained men. *Metabolism* **21**: 1037-1044.
- Cochran, B., Marbach, E. P., Poucher, R., Steinberg, T. and Gwinup, G. 1966. Effect of acute muscular exercise on serum immunoreactive insulin concentration. *Diabetes* **15**: 838-841.
- Conard, V., Brunnengraber, H., Vanroux, R., Deschaepdrijver, A., Moermans, E. and Franckson, J. R. M. (Ed. J. R. Poortmans) 1969. Influence of muscular exercise on glucose regulation. In: *Medicine and Sport 3. Biochemistry of Exercise*. Karger. pp. 114-115. Basel.
- Couturier, E., Rasio, E. and Conard, V. 1971. Insulin in plasma lymph and tissue glucose uptake in the exercising hind limb of the dog. *Horm. Metab. Res.* **3**: 382-386.
- Davies, C. T. M. 1968. Limitations to the predictions of maximal oxygen intake from cardiac frequency measurements. *J. Appl. Physiol.* **24**: 700-706.
- Devlin, J. G. 1963. The effect of training and acute physical exercise on plasma insulin-like activity. *Irish J. Med. Sci.* 423-425. (September).

- Dieterle, P., Birkner, B., Gmeiner K-H., Wagner, P., Erhardt, F., Henner, J. and Dieterle, C. 1973. Release of peripherally stored insulin during acute muscular work in man. *Horm.Metab.Res.* **5**: 316-322.
- Dole, V. P. 1956. A Relationship between non-esterified fatty acids in plasma and the metabolism of glucose. *J.Clin.Invest.* **35**: 150-154.
- Few, J. D. and Cashmore, G. C. 1971. The determination of plasma cortisol by competitive protein binding. *Ann.Clin.Biochem.* **8**: 205-209.
- Franckson, J. R. M., Vanroux, R., Leclerq, H., Brunnengraber, H. and Ooms, H. A. 1971. Labelled insulin catabolism and pancreatic responsiveness during long term exercise in man. *Horm.Metab.Res.* **33**: 366-373.
- Freinkel, N., Saunders, C. A., Levinson, G. E. and Abelman, W. H. 1959. The utilisation of glucose and metabolism of insulin in the exercised extremity of man. *J.Clin.Invest.* **38**: 1005-1006.
- Grodsky, G. M. 1972. A threshold distribution hypothesis for packet storage of insulin and its mathematical modeling. *J.Clin.Invest.* **51**: 2047-2059.
- Hartley, H. L., Mason, J. W., Hogan, R. P., Jones, L. G., Kotchen, T. A., Mougey, E. H., Wherry, F. E., Pennington, K. L. and Ricketts, P. T. 1972. Multiple hormonal responses to graded exercise in relation to physical training. *J.Appl.Physiol.* **33**: 602-606.
- Havivi, E. and Wertheimer, H. E. 1964. A muscle activity factor increasing sugar uptake by rat diaphragms in vitro. *J.Physiol.* **172**: 342-352.
- Hermansen, L., Pruett, E. D. R., Osnes, J. B. and Giere, F. A. 1970. Blood glucose and plasma insulin in response to maximal exercise and glucose infusion. *J.Appl.Physiol.* **29**: 13-16.
- Hoffman, W. S. 1937. A rapid photoelectric method for the determination of glucose in blood and urine. *J.Biol.Chem.* **120**: 51-61.
- Horstman, D., Mendez, J., Buskirk, E. R., Boileau, R. and Nicholas, W. C. 1971. Lipid metabolism during heavy and moderate exercise. *Med.Sci.Sports* **3**: 18-23.
- Ismail, A. H., Falls, H. B. and MacLeod, D. F. 1965. Development of a criterion for physical fitness tests. *J.Appl.Physiol.* **20**: 991-999.
- Métivier, G., Poortmans, J., Vanroux, R., Leclerq, P. and Copinschi, G. 1971. Arterial blood plasma cortisol and human growth hormone changes in male trained subjects submitted to various physical work intensities. *Med.Sci.Sports* **3**: (pt. 1)g. (abstract).
- Müller, W. A., Faloona, G. R. and Unger, R. H. 1971. The influence of antecedant diet upon glucagon and insulin secretion. *New Engl.J.Med.* **284**: 1450-1454.
- Murphy, B. E. P. 1969. Protein Binding and the assay of non-antigenic hormones *Rec.Prog.Horm.Res.* **25**: 565-610.
- Nikkilä, E. A., Taskinen, M-R., Meittinen, T. A., Pelkonen, R. and Poppins, H. 1968. Effect of muscular exercise on insulin secretions. *Diabetes* **17**: 209-218.
- Oseid, S. and Hermansen, L. 1971. Hormonal and metabolic changes during and after prolonged muscular work in pre-pubertal boys. *Acta Ped.Scand.Suppl.* **217**: 147-153.
- Porte, D. Jr. and Bagdade, J. D. 1970. Human insulin secretion: an integrated approach. *Ann.Rev.Med.* **21**: 219-240.
- Pruett, E. D. R. 1969. Insulin free fatty acids and glucose during and after long term work in men. *Acta Physiol.Scand., Suppl.* **330**: 110.
- Pruett, E. D. R. 1970. Plasma insulin concentration during prolonged work at near maximal oxygen uptake. *J.Appl.Physiol.* **29**: 155-158.

- Pruett, E. D. R. and Oseid, S. 1970. Effect of exercise on glucose and insulin response to glucose infusion. *Scand.J.Clin.Lab.Invest.* **26**: 227-285.
- Rasio, E., Malaisse, W., Franckson, J. R. and Conard, V. 1966. Serum insulin during muscular exercise in normal man. *Archives Internationales de Pharmacodynamie et de Thérapie* **160**: 485-491.
- Rasio, E., Whichelow, M. J., Butterfield, W. J. H. and Hicks, B. H. 1972. Insulin fixation and glucose uptake by forearm tissues in response to infusions of physiological amounts of insulin in non-diabetic subjects. *Diabetologia* **8**: 244-249.
- Reinheimer, W., Davidson, P. C. and Albrinck, M. J. 1968. The effect of moderate exercise on plasma glucose, insulin and free fatty acids during oral glucose tolerance tests. *J.Lab.Clin.Med.* **71**: 429-437.
- Rottini, E., Cozzolino, G., Dominici, G. and D'Ovido, M. 1971. Physical activity and blood insulin, sugar and non-esterified fatty acid values in normal, untrained subjects. *J.Sports Med.* **11**: 1-5.
- Schwartz, F., Terhaar, D. J., Van Reit, H. G. and Thijssen, J. H. H. 1969. Response of growth hormone, free fatty acid, blood sugar and insulin to exercise in obese patients and normal subjects. *J.Endocrin.Metab.* **18**: 1013-1020.
- Szabo, O., Szabo, A. and Mahler, R. J. 1972. Influence of exercise upon serum factors and its secondary effect on glucose utilisation by resting muscle. *Horm.Metab.Res.* **4**: 139-143.
- Trout, D. L., Estes, E. H., Jr. and Friedberg, S. J. 1960. Titration of free fatty acids of plasma: a study of current methods and a new modification. *J.Lip.Res.* **1**: 199-202.
- Wahren, J., Felig, P., Ahlborg, G. and Jorfeldt, L. 1971. Glucose metabolism during leg exercise in man. *J.Clin.Invest.* **50**: 2715-2725.
- Wilmore, J. H. and Behnke, A. R. 1969. An anthropometric estimation of body density and lean body weight in young men. *J.Appl.Physiol.* **27**: 25-31.
- Yalow, R. S. and Berson, S. 1960. Immunoassay of endogenous plasma insulin in man. *J.Clin.Invest.* **39**: 1157-1159.
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