Insulin Effect on Adipose Tissue Sodium and Potassium.* (29047)

D. R. H. Gourley and Margaret D. Bethea
Department of Pharmacology, University of Virginia School of Medicine, Charlottesville

The electrolyte content of adipose tissue has attracted relatively little attention, although it has been shown that both Na and K are present in adipose tissue in man and that, in contradistinction to most other mammalian tissues, adipose tissue contains more Na than K(1,2). Similar observations have been made in the epididymal adipose body of the rat in which it was shown further that Na, K and water content per g (wet weight) of adipose tissue are inversely related to the total body weight of the rat(3). We have confirmed these observations in freshly dissected epididymal adipose tissue of young rats and describe here the effect of insulin on Na and K content of the tissue when it is incubated *in vitro* in Krebs-Ringer's solution with or without glucose.

Methods. Young male Holtzman rats weighing 80-171 g were decapitated after a 2-hour fast and each epididymal adipose body was removed by a single cut just above the epididymis. Care was taken to minimize crushing damage to the cells. When incubated, the tissue was placed in modified Krebs-Ringer's solution containing 0.73% NaCl, 0.035% KCl, 0.016% KH_2PO_4, 0.029% $MgSO_4 \cdot 7H_2O$, 0.028% $CaCl_2$, 0.16% $NaHCO_3$ ([K] = 5.9, [Na] = 136 meq/liter). The solution was equilibrated with 5% CO_2 + 95% O_2 and had a pH of 7.4 at 37°C. The incubation period was 3 hours at 37.0 ± 0.02°C. The water content of the tissue was determined by drying to constant weight at 105° C. [Na] and [K] were determined in the ashed adipose body and [K] in the incubation fluid by flame photometry. As mentioned earlier, the Na, K and water content per g wet weight of freshly dissected adipose tissue vary inversely with the total body weight of the rat and the data are less variable when the cation concentrations are expressed in terms of the water content of the tissue at the end of the incubation period. However, even when calculated on this basis, [Na] and [K] are significantly correlated with the total body weight of rats weighing 97-171 g ($N = 18$; for Na, $r = -0.81$, $P < 0.001$; for K, $r = -0.47$, $P = 0.05$-0.02). Evidently, the cells of adipose tissue from rapidly growing young rats not only increase their fat content(4) but actually lose Na and K as the body weight increases, at least up to a body weight of 171 g. Further reduction in the variability of the cation concentrations was achieved by selecting rats within as narrow a weight range as was practicable (Table I, exp 2-4).

Crystalline zinc insulin (beef) was supplied by the Lilly Laboratory for Clinical Research through the courtesy of Dr. W. R. Kirtley (lot no. 535664, activity = 25.6 units/mg, zinc = 0.49%, glucagon = 0.4-0.5%). A concentration of 50 milliunits/ml was chosen on the assumption that it would produce a maximum effect on adipose [K] and no study of other concentrations was made.

In experiments in which glucose was added, the amount of glucose remaining in the fluid surrounding the adipose tissue at the end of the incubation period was determined by the Glucostat® method.

Results. A comparison of the [Na], [K] and [H_2O] of freshly dissected adipose bodies is made in Table I (Exp. 1). There is no significant difference in the initial [Na], [K] or [H_2O] of the right and left adipose bodies from each rat. The values for [K] and [H_2O] in freshly dissected adipose tissue agree fairly well with those reported by Martin, Tranquada, Beigelman and Jones(3) for epididymal adipose tissue from rats weighing 180 g or less, but the [Na] is less than half the concentration found by Martin *et al.* It is not clear whether the values reported by Martin *et al.* represent the concentrations of electro-

* This investigation was supported in part by PHS grant from Nat. Inst. of Arthritis and Metab. Dis., U.S.P.H.S.

TABLE I. Sodium, Potassium and Water Content of Epididymal Adipose Tissue of the Young Rat.

Exp*	No. of rats	Rat wt, avg & range (g)	Treatment of adipose body	[Na] (μEq/g H_2O)	[K] (μEq/g H_2O)	Water (mg/g wet wt)
1	18	132 (97-171)	Control (right)	90.2 ± 4.8	67.0 ± 3.0	214 ± 13
			" (left)	88.8 ± 4.9	68.3 ± 3.4	208 ± 13
				(.4)†	(.4)†	(.2)†
2	39	112 (80-145)	Control	109 ± 3	36.1 ± .7	330 ± 6
			Insulin‡	104 ± 2	38.3 ± .8	324 ± 6
				(.01)†	(.01)†	(.1)†
3	20	90 (85-102)	Control	127 ± 5	37.6 ± .8	363 ± 10
			Glucose‡	127 ± 3	41.0 ± 1.3	360 ± 9
				(.9)†	(.05)†	(.6)†
4	20	104 (94-118)	Glucose	121 ± 2	41.2 ± .8	315 ± 6
			" + Insulin‡	118 ± 3	49.9 ± 1.1	287 ± 7
				(.3)†	(<.001)†	(<.001)†

* In Exp 1, means (± standard errors) represent concentrations in freshly dissected tissue; all other means represent concentrations in tissue after incubation at 37.0 ± 0.02°C for 3 hours.

† P value for comparison of paired concentration values.

‡ Glucose concentration, 8.5 mM; insulin concentration, 50 milliunits/ml.

lytes immediately after dissection or after a period of incubation in Krebs-Ringer's solution.

In Exp. 2, one adipose body was incubated in Krebs-Ringer's solution as a control while the opposite adipose body from the same animal was incubated in the same solution in which 50 milliunits/ml of insulin had been dissolved. No carbohydrate substrate was present in either solution. By comparing these means with the data for the initial cation and water contents of adipose tissue in rats of approximately the same weight (Exp. 1), it is apparent that during incubation for 3 hours in this medium the tissue gained Na and water and lost K. In the tissue treated with insulin, there was a small but statistically significant increase in [K] and a decrease in [Na] compared with the K and Na in the control tissues at the end of the incubation period. However, these differences are not consistently observed in smaller groups of animals. The water content of the tissue was not affected by insulin alone.

It is well known that the uptake of glucose by epididymal adipose tissue is greatly accelerated by insulin. It was of interest, therefore, to determine the effect of insulin on adipose tissue [K] and [Na] in the presence of glucose. Glucose alone had no effect on Na or water content, but the [K] of the tissue exposed to glucose was significantly greater than that of the corresponding controls (Exp. 3). The addition of both glucose and insulin (Exp. 4) further increased the [K] by 21% over the [K] in tissue exposed to glucose alone. Concomitantly, the water concentration of the tissue exposed to glucose and insulin was 10% lower than the corresponding tissue exposed to glucose alone but the [Na] was not significantly affected. Significant amounts of glucose were taken up by the tissue only when insulin was present (in glucose alone, uptake = 0.6 ± 0.8; in glucose + insulin, uptake = 40.9 ± 1.3 μmoles/g wet wt.).

Discussion. The adipose tissue used to determine initial [Na], [K] and [H_2O] (Exp. 1) was taken from rats whose average weight was somewhat greater than that of the rats from which the incubated adipose bodies (Exp. 2-4) were obtained. Since there is a significant negative correlation between the Na and water content of adipose tissue and the rat body weight, it is not possible to be certain from the present experiments that the apparent increases in [Na] and [H_2O] are entirely due to incubation in Krebs-Ringer's solution. However, there is no doubt that there is a significant loss of K from adipose tissue during incubation under the experimental conditions used here and extra K appeared in the Krebs-Ringer's solution in all of the incubation experiments summarized in Table I.

The loss of K from adipose tissue during

incubation was slightly retarded by insulin. The magnitude of this insulin effect in adipose tissue is of the same order as the corresponding effect in frog muscle(5). In the latter tissue, insulin appears to increase [K] by increasing K influx without affecting K efflux (D. R. H. Gourley, unpublished experiments), but K fluxes in adipose tissue have not yet been studied. Although the insulin effect on adipose tissue [K] can occur in the absence of added glucose, glucose itself retards the loss of K during incubation. However, in the presence of glucose, the effect of insulin on adipose tissue [K] is more than 3 times greater than it is in the absence of glucose.

The effect of insulin on adipose tissue [Na] is less clear. Unfortunately, the extracellular space of the adipose tissue was not measured in these experiments but it is possible that a considerable fraction of the tissue Na is extracellular. Small changes in intracellular Na would therefore be more difficult to detect. The significance of the reduction in adipose tissue water by insulin in the presence of glucose is unknown but this response of adipose tissue to insulin seems to be unique.

Beigelman and Hollander(6,7) have reported that insulin, with or without added glucose, increases the resting potential of epididymal adipose tissue from rats weighing not more than 270 g. Without knowing the intracellular concentrations of Na and K, rigid comparisons with their data are not possible, but the net increase in adipose tissue [K] observed in the present experiments in the presence of insulin is consistent with an insulin-induced increase in the membrane potential of individual cells. Nevertheless, these two effects may not be related causally because there is no evidence that glucose, which also increases the [K], has any effect on the resting membrane potential of adipose tissue.

Summary. In rats weighing 97-171 g, there is a significant negative correlation between the [Na] and [K] per g of water in freshly dissected epididymal adipose tissue and total body weight. During *in vitro* incubation in Krebs-Ringer's solution at 37°C, epididymal adipose tissue loses K and probably gains water and Na. After incubation, insulin-treated adipose tissue contains slightly more K and less Na than the corresponding control tissue. Glucose alone also increases the [K] of incubated adipose tissue. In the presence of glucose, the effect of insulin on adipose tissue K is considerably enhanced and the water content of the insulin-treated adipose tissue is significantly lower.

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Received December 2, 1963. P.S.E.B.M., 1964, v115.

Muscle Protein Extractability as Influenced by Conditions of Post-Mortem Glycolysis.* (29048)

ERNEST J. BRISKEY AND ROBERT N. SAYRE (Introduced by P. H. Phillips)
Department of Meat and Animal Science, University of Wisconsin and American Meat Institute Foundation, Chicago

Muscle protein extractability at the time of death has been used as an indicator of *in vivo* protein composition(1). Mirsky(2) suggested that a large proportion of muscle

* Published with the approval of Director, Wisconsin Agri. Exp. Station. This investigation was supported, in part, by a research grant from Dept. of H.E.W., P.H.S., Nat. Inst. Health.