DEUTERIUM OXIDE STIMULATES GLUCOSE METABOLISM AND INHIBITS LIPOLYSIS IN RAT EPIDIDYMAL ADIPOSE TISSUE

Richard Schimmel

Department of Physiology
University of Pittsburgh
School of Medicine
Pittsburgh, Pennsylvania 15261

Received March 21,1975

Summary:

Incubation of segments of rat epididymal adipose tissue in media prepared with deuterium oxide results in increased glucose oxidation, increased lipogenesis, accelerated sugar transport and decreased lipolysis in response to epinephrine or theophylline. In view of the well documented action of heavy water to "stabilize" cytoplasmic microtubules, the foregoing observations are in support of a link between cytoplasmic microtubules and metabolic process in adipose tissue.

In recent years the physiological role of cytoplasmic microtubules in various tissues has been the subject of much research and speculation (1-3). A function for microtubules in adipose tissue has been postulated because the outward movement of free fatty acids from adipose tissue (4) and insulin stimulated lipogenesis (5) are inhibited by colchicine, a drug known to disrupt microtubules. In an effort to gain additional insight into the role of microtubules in adipose tissue function, a study of the effects of deuterium oxide (D_2O) on adipose tissue was initiated. D_2O was selected for this study because it has been found to "stabilize" cytoplasmic microtubules and interfere with cell functions dependent upon these cellular organelles (6,7).

Materials and Methods

Normal male rats (160-200g) of the Sprague-Dawley strain were purchased from Zivic-Miller Lab, Inc. The animals were fasted overnight, sacrificed by cervical dislocation and the two epididymal fat bodies rapidly excised. Segments weighing between 30 and 70 mg were cut from the thin distal regions of the fat pads and distributed among incubation vessels containing 1.0 ml of Krebs-Ringer bicarbonate buffer (pH, 7.4) and incubated for 60 minutes under an atmosphere of O_2: CO_2, 95/5 (V/V). Bovine serum albumin (Sigma, Fraction V), insulin and glucose were present in some media at concentrations indicated in the text. ^{14}C-glucose labelled in the 1 (ICN, 45 mci/mMole) or in the 6 (Schwarz-Mann, 51.2 mci/mMole) position was present where indicated at a concentration of 0.20μci/ml. D_2O was obtained from Sigma and used at various concentrations up to 99% (V/V). Because the dissociation constant for D_2O (0.2×10^{-14}) is less than that of H_2O, it is not possible to prepare D_2O containing solutions having a pD and pOD equal simultaneously to the pH and pOH of a comparable water solution. For this reason, the effects of D_2O were examined under two different conditions: once with pD = pH and again with pOD = pOH. The pD of D_2O solutions was measured using a standard pH meter and the relation that pD = pH + 0.4 (8). Incorporation of radioactivity from glucose into lipid and CO_2 was determined as described by Bray (9), glycerol was measured enzymatically (10) and free fatty acids by the method of Dole (11). In studies on the effects of D_2O on glucose transport, the intracellular accumulation of D-xylose, which appears to share a common transport mechanism with glucose in many tissues, was used as an indication of sugar transport (12,13).

Results and Discussion

Table I shows the effects of D_2O on lipolysis. In the absence of albumin

TABLE I

Effects of Deuterium Oxide on Lipolysis
in Epididymal Adipose Tissue

Additions to Incubation Buffer	Measurement: Glycerol Release H_2O	Glycerol Release D_2O	FFA Release H_2O	FFA Release D_2O	Tissue FFA H_2O	Tissue FFA D_2O
1. None	1.3 ± 0.3	1.1 ± 0.2			4.5 ± 0.5	4.9 ± 0.6
epinephrine, 0.05μg/ml	3.1 ± 0.4	1.3 ± 0.3*			10.2 ± 1.2	4.7 ± 0.7*
epinephrine, 0.10μg/ml	4.6 ± 0.5	1.8 ± 0.3*			13.8 ± 2.1	6.1 ± 0.8*
theophylline, 0.10mg/ml	5.8 ± 0.7	3.1 ± 0.4*			13.2 ± 2.2	7.2 ± 1.1*
2. Glucose	1.0 ± 0.3	1.2 ± 0.3			3.4 ± 0.9	2.1 ± 0.4
epinephrine, 0.05μg/ml	3.3 ± 0.3	2.6 ± 0.2			8.1 ± 0.7	2.9 ± 0.5*
epinephrine, 0.10μg/ml	4.5 ± 0.5	3.7 ± 0.4			9.5 ± 1.3	4.1 ± 0.4*
3. Glucose & albumin	1.8 ± 0.3	1.6 ± 0.3*	3.8 ± 0.5	0.2 ± 0.4*	4.2 ± 0.5	1.8 ± 0.3*
epinephrine, 0.05	4.0 ± 0.5	1.8 ± 0.3*	5.8 ± 0.7	1.2 ± 0.5*	7.1 ± 0.8	2.3 ± 0.4*
epinephrine, 0.10	7.2 ± 0.8	3.2 ± 0.4*	7.1 ± 1.1	0.8 ± 0.4*	9.1 ± 1.2	2.8 ± 0.4*

Segments of rat epididymal adipose tissue were incubated in Krebs-Ringer bicarbonate buffer prepared with H_2O or with 50% (V/V) D_2O (pH = pD = 7.4) for 60 minutes. Glucose was presented where indicated at a concentration of 1 mg/ml; bovine serum albumin at 40 mg/ml. The units of glycerol and free fatty acid release are μmoles/g tissue wet wt-hr; tissue free fatty acid content is expressed as μmoles/g tissue wet wt. Each value represents the mean ± SE of 12 tissues.

* Significantly different from corresponding values obtained from tissues incubated in H_2O - buffer; $p < .05$.

and glucose, a 50% solution of D_2O markedly inhibited the lipolytic effects of epinephrine or theophylline. When glucose was present, the antilipolytic effect of D_2O was much less pronounced although accumulation of free fatty acids in the tissues remained depressed suggesting that esterification of free fatty acids was accelerated in the presence of D_2O. The antilipolytic effect of D_2O was also evident in medium containing albumin. These, and all subsequent data were obtained from tissues incubated in media with pD adjusted to 7.4 (pOD, 7.3); however, virtually identical results were obtained in media with pOD adjusted to 6.6 (pD, 8.1).

TABLE II

Effect of Deuterium Oxide on Metabolism
of Specifically Labelled [^{14}C] - glucose by Adipose Tissue

Incorporation of ^{14}C into (CPM/mg-hr)

D_2O o/o	Insulin mμ/ml	CO_2	Fatty acids	Glyceride-Glycerol	Total
		[Glucose-1 ^{14}C]			
0	-	10.8 ± 1.5	1.1 ± 0.1	10.6 ± 1.2	22.5 ± 2.8
33	-	10.5 ± 2.1	1.1 ± 0.1	18.0 ± 1.9*	29.6 ± 2.8*
66	-	19.6 ± 1.2*	3.0 ± 0.5*	28.5 ± 3.1*	51.1 ± 7.2*
99	-	30.1 ± 2.5*	7.6 ± 1.3*	35.6 ± 3.8*	73.3 ± 9.1*
0	0.50	18.8 ± 2.1†	4.2 ± 1.1†	21.4 ± 3.1†	44.4 ± 5.2†
33	0.50	23.6 ± 2.7†	5.9 ± 1.1†	25.3 ± 3.8†	54.8 ± 5.1†
66	0.50	34.6 ± 4.1*†	9.0 ± 1.2*	33.3 ± 3.8*	76.9 ± 6.8*†
99	0.50	33.3 ± 2.1*	9.3 ± 1.1*	41.9 ± 5.1*	81.5 ± 10.2*
		[Glucose-6 ^{14}C]			
0	-	6.0 ± 1.9	3.3 ± 0.1	21.7 ± 2.3	31.0 ± 4.0
33	-	6.9 ± 0.8	4.6 ± 1.1	26.3 ± 2.8	37.8 ± 4.2
66	-	9.8 ± 1.0*	11.2 ± 1.3*	29.2 ± 2.8	50.2 ± 4.1*
99	-	11.8 ± 0.8*	25.2 ± 1.9*	38.1 ± 3.1*	75.1 ± 8.8*
0	0.50	8.6 ± 0.9†	22.1 ± 1.4†	34.3 ± 4.4†	65.0 ± 6.7†
33	0.50	9.2 ± 0.5†	30.3 ± 7.3†	35.5 ± 4.2	75.0 ± 8.1†
66	0.50	11.3 ± 1.1*	29.1 ± 2.1*†	35.6 ± 3.9	76.0 ± 8.2†
99	0.50	12.1 ± 1.3*	31.9 ± 2.4*	45.3 ± 5.2	89.3 ± 10.0*

Segments of rat epididymal adipose tissue were incubated in Krebs-Ringer bicarbonate buffer containing various amounts of D_2O for 60 minutes. Glucose was present at a concentration of 1 mg/ml; ^{14}C-glucose at a concentration of 0.20 μci/ml. Each value represents the mean ± SE of 12 tissues.

* Significantly different from value obtained in H_2O - buffer

† Significantly different from corresponding value obtained with no insulin present; $p < .05$.

The metabolism of glucose appears to be increased when tissue segments are incubated in media prepared with increasing amounts of D_2O (Table II) . This accelerated glucose metabolism caused by D_2O probably accounts for the greater esterification of free fatty acids in tissues incubated in D_2O

(Table I). Increased metabolism of glucose was reflected in enhanced oxidation to CO_2, enhanced lipogenesis and increased formation of glyceride-glycerol. Conversion of glucose-1 ^{14}C to $^{14}CO_2$ was increased to a greater extent than glucose-6 ^{14}C to $^{14}CO_2$, implying a greater flux of glucose through the pentose-shunt pathway in tissues incubated in media prepared from D_2O. When insulin was present, a pattern of glucose metabolism qualitatively similar to that produced by D_2O was evident. In addition, the tissue responses to insulin decreased as the concentration of D_2O in the medium increased; in the presence of 99% D_2O, insulin failed to accelerate glucose metabolism significantly.

Insulin increases glucose metabolism in adipose tissue, in part, by accelerating the transport of the sugar into adipocytes (14). The data in Table III show that D_2O may have a similar action. D_2O produced a dose-dependent increase in the xylose space of adipose tissues, suggesting an accelerated entry of xylose into the intracellular compartment. In contrast, incubation of tissues in D_2O did not result in a measurable change in either the sucrose space or the total tissue water content. Exposure of tissues to insulin also resulted in increased xylose transport; however, as was seen when glucose metabolism was measured, the insulin response was lost when tissues were incubated in D_2O.

The salient observation reported herein is that incubation of segments of rat epididymal adipose tissue in buffers prepared with heavy water exerts effects upon the metabolism of the tissue which are remarkably similar to those changes caused by exposure to insulin. These metabolic effects of D_2O include increased glucose oxidation, increased lipogenesis, accelerated sugar transport and decreased lipolysis in response to lipolytic agents.

Interpretation of these data requires consideration of the mechanism of action of D_2O. To date, the best-known cellular action of heavy water is

TABLE III

Effects of Deuterium Oxide on the Distribution of [^{14}C] - Xylose and [^{14}C] - Sucrose in Water of Epididymal Adipose Tissue

D_2O (o/o)	Insulin (mμ/ml)	Total water (μl/100mg)	Xylose space (% of Total water)	Sucrose Space
0	-	25 ± 1	66.6 ± 4.2	58.2 ± 6.2
33	-	24 ± 2	71.1 ± 6.1**	59.1 ± 5.1
66	-	26 ± 1	82.9 ± 6.6*	61.2 ± 5.8
100	-	23 ± 2	84.3 ± 6.8*	60.4 ± 5.1
0	0.50	28 ± 2	86.1 ± 5.2†	60.1 ± 4.9
33	0.50	27 ± 2	84.5 ± 4.1†	63.2 ± 6.1
66	0.50	26 ± 3	89.4 ± 5.8	61.4 ± 6.2
100	0.50	26 ± 2	90.7 ± 8.2	59.5 ± 5.5

Segments of rat epididymal adipose tissue were incubated in Krebs-Ringer bicarbonate buffer containing various amounts of D_2O for 60 minutes. ^{14}C-Xylose or ^{14}C-Sucrose were present at concentrations of 1.0 mg/ml and 0.20 μci/ml. Each value represents the mean ± SE of 16 tissues.

* Significantly different from value obtained in H_2O - buffer; $p < .05$.

† Significantly different from corresponding value obtained with no insulin present, $p<.05$.

**Mean difference, 4.5 ± 1.9; $p< .05$.

---

to foster the polymerization and increase the strength of microtubules (6,7). It follows that the metabolic effects of D_2O could result from a functional alteration in the microtubular system in adipose tissue cells and that microtubule

assembly may, in some as yet undefined manner be an essential component in the regulation of metabolic activity in adipose tissue cells. This explanation is in accord with the view advanced independently from this laboratory (4) and by Soifer et al (5) linking metabolic processes in adipose tissue cells with microtubules. It should be noted that Soifer et al (5) presented evidence that insulin stimulates the formation of microtubules in adipocytes, an action similar to that attributed to D_2O.

However, D_2O exerts other general actions on tissues incubated in vitro (15-18) and in view of the apparent diversity of action of D_2O it may be premature to ascribe its actions on adipose tissues to assembly of microtubules.

Acknowledgements:

The excellent technical assistance rendered by Mr. Dennis McKeag is greatly acknowledged.

This research was supported by a grant from the National Institutes of Health, AM 15466.

References

1. Olmsted, J.B. and Borisy, G.G., (1973) Ann. Rev. Biochem., 42, 507-539.
2. Lacy, P.E. and Malaisse, W.J., (1973) Recent Prog. Horm. Res. 29, 199-228.
3. Wolff, J. and Williams, J.A., (1973) Recent Prog. Horm. Res. 29, 229-285.
4. Schimmel, R.J., (1974) J. Lipid Res. 15, 206-210.
5. Soifer, D., Braun, T. and Hechter, O. (1971) Science 172, 269-271.
6. Marsland, D. and Zimmerman, A.M., (1965) Exptl. Cell. Res. 38, 306-313.
7. Inoue, S. and Sato, H., (1967) J. Gen. Physiol. 50, 259-288.
8. Glasoe, P.D. and Long, F.A., (1960) J. Phys. Chem. 24, 188-190.
9. Bray, G.A., (1967) J. Lipid Res. 8, 300-307.
10. Wieland, O., (1957) Biochem. Z. 329, 313-319.
11. Dole, V.P., (1956) J. Clin. Invest. 35, 150-154.
12. Park, C.R., Reiwein, D., Henderson, M.J. Codenas, E. and Morgan, H.E., (1959) Am. J. Med. 26, 675-684.

13. Schimmel, R.J. and Goodman, H.M., (1971) Biochim. Biophys. Acta, 329, 9-15.
14. Crofford, O.B. and Renold, A.E., (1965) J. Biol. Chem. 240, 3237-3244.
15. Bigeleisen, J., (1965) Science 147, 463-471.
16. Malaisse, W.J., (1973) Nature, New Biology 242, 109-190.
17. Muraoka, S. and Slater, E.C., (1968) Biochim. Biophys. Acta 162, 170-174
18. Kaminer, B. and Kimura, J. (1972) Science 176, 406-407.