L-Leucine-^{14}C (UL) Metabolism in Isolated Fat Cells* (33382)

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Insulin increases protein synthesis by both muscle (1–3) and fat pad preparations (4–6) from amino acids, carbohydrates, and carbohydrate precursors. The muscle preparations do not require glucose for this insulin effect, but glucose (6) or glucose metabolites, pyruvate or acetate (7), have been required for the fat pad. With increasing concentrations of glucose in the incubation media, the insulin effect appears to be enhanced (8).

This paper presents studies of metabolic activity of isolated fat cells. Protein synthesis, lipid synthesis, and carbon dioxide production from L-leucine-UL-^{14}C were studied at varying concentrations of glucose to determine whether the rate of glucose metabolism by the fat cells altered the pathways of amino acid metabolism. The results indicate that varying levels of carbohydrate metabolism, resulting from alteration of the levels of glucose concentration in the incubation medium, modified greatly the effect of insulin on amino acid metabolism.

Methods and Materials. L-leucine-^{14}C (UL) (1 μCi) equivalent to 0.005 μmole of L-leucine was used in each test tube. Beef insulin (Lilly)[1], 24 units/mg, was prepared in Krebs–Ringer buffer to a working concentration of 500 μU/ml. The isolated fat cells were prepared by the method of Rodbell (9) as modified by Miller and Beigelman (10) using male Sprague-Dawley rats (110–180 g) in the fed state. The constituents of each vial consisted of (i) 0.5 ml of fat cell preparation; (ii) 0.3 ml of Krebs–Ringer buffer with or without glucose. The glucose was so adjusted that the glucose concentrations of the incubation medium were: 0.1, 1, and 3 mg/ml; (iii) 0.05 ml of insulin solution or control Krebs–Ringer; (iv) 0.1 ml of isotopic amino acid; and (v) 0.05 ml of amino acid mixture. The control tubes and insulin tubes at each concentration of glucose were run in triplicate or quadruplicate, and each experiment was repeated three to four times. The fat cells were incubated for 2 hr at 37° in the Dubnoff metabolic shaker.

The $^{14}CO_2$ and incorporation of ^{14}C into lipid were determined by techniques previously outlined by Stock and Beigelman (11). The technique of Mans and Novelli (12), modified by Miller and Beigelman (10) for use with isolated fat cells, was employed for determining the amount of isotopic amino acid incorporated into fat cell protein.

Considerable daily variation occurred in the control values despite adjustment for the different volumes of cells used, but the relationship of the controls to the insulin effect was relatively constant. The results are, therefore, expressed as percentage difference between controls and the insulin effect at each glucose concentration. In performing this computation, the percentage variation in the control tubes from the mean was calculated to give a standard error for the controls. The difference demonstrated by the insulin effect was calculated by subtracting, in each experiment, the mean of the control tubes from each of the insulin values and pooling from the total experience all of these values to give a mean insulin response with the standard error of that mean.

Results (Table I). In the presence of 0.1 mg/ml of glucose and glucose omitted from the media there is a significant increase of $^{14}CO_2$ production in the presence of insulin.

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This insulin augmentation is greater in the presence of 0.1 mg/ml of glucose as compared with omitted glucose. There is a reversal of this insulin effect at higher concentrations of glucose.

In the presence of glucose and also with glucose omitted from the medium, insulin significantly stimulates incorporation of L-leucine into lipid. At higher concentrations of glucose, the insulin effect is reversed or is not evident.

Insulin stimulated protein synthesis at all levels of glucose concentration tested. There is augmentation of protein synthesis in response to insulin as glucose concentration is increased. This is marked as glucose concentration is increased from 0 to 0.1 mg/ml, but the increment is considerably diminished as glucose concentration is increased from 0.1 to 3.0 mg/ml.

Discussion. Insulin stimulated selected amino acid intracellular pathways in the isolated fat cell at certain concentrations of glucose. This effect may be secondary to a number of changes occurring simultaneously as the carbohydrate pathways are influenced by insulin. With increasing concentrations of glucose, the fatty acid and Krebs cycle pathways become more active. The major effect of insulin on glucose metabolism is to increase the amount of two carbon molecules entering the acetate pool. If it is assumed that insulin does not influence leucine break down to acetate, then the production of two carbon fragments from the amino acid pool will remain constant. Hence, at high levels of glucose, there will be an apparent decreased incorporation of labeled two-carbon fragments from leucine as they are swamped by the increase of nonlabeled two-carbon fragments resulting from insulin stimulating the rate of nonlabeled glucose turnover. This probably explains the reversal of insulin effect on lipid synthesis and CO_2 production from leucine in these experiments. Similar findings were reported by Goodman (13), using leucine at various levels of glucose.

Insulin stimulated protein synthesis at all levels of glucose concentration. It has been shown that protein synthesis increases as the medium glucose concentration is increased

TABLE I. Effect of Glucose Concentration and Insulin upon Formation of CO_2, Protein, and Lipid from Leucine-^{14}C by Isolated Fat Cells.

Glucose concentration (mg/ml)	Insulin concentration (microunits/ml)	CO_2			Lipid			Protein		
		No.[a]	Change (%)[b]	p[c]	No.[a]	Change (%)[b]	p[c]	No.[a]	Change (%)[b]	p[c]
0	0	20	0 ± 2.2	<.01	8	0 ± 6.5	>.02, <.05	12	0 ± 4.0	<.01
	500		16.6 ± 2.9			21 ± 7.3			18 ± 4.0	
0.1	0	20	0 ± 1.1	<.01	8	0 ± 4.0	<.01	11	0 ± 4.5	<.01
	500		47.9 ±13.7			194 ±42.0			41.5 ± 7.2	
1.0	0	5	0 ± 2.7	<.01	4	0 ±13.1	>.02, <.05	8	0 ± 9.3	<.01
	500		—43.0 ± 7.1			—36.0 ± 3.5			45.0 ± 9.4	
3.0	0	15	0 ± 1.2	<.01	8	0 ± 3.0	>.40	11	0 ± 3.0	<.01
	500		—27.8 ± 3.2			—6.2 ± 4.2			58.3 ± 6.8	

[a] Number of determinations.
[b] Mean ± SE of mean.
[c] Probability of difference between means being significant (from Fisher's table).

(10). This suggests that the effect of carbohydrate metabolism on protein synthesis may not require insulin specifically, but that insulin increases carbohydrate metabolism and also probably stimulates protein synthesis directly. Evidence that insulin directly stimulates protein synthesis from ^{14}C-amino acids has been presented previously, puromycin being shown to inhibit protein synthesis (10).

Insulin stimulation of fat cell protein synthesis in the absence of glucose has not been reported with intact rat fat pad preparations (6–9), but only in the isolated fat cell preparation (10). The method of protein determination used with the isolated fat cells is probably more sensitive to small changes in protein synthesis than the extraction techniques utilized with intact fat pads and may, in part, explain this discrepancy. In all other respects studied, the metabolic activity of isolated fat cells and of the intact fat pad would appear to be similar.

Summary. Effects were studied of insulin and of varying the concentration of glucose upon L-leucine-^{14}C (UL) metabolism by isolated rat epididymal adipose tissue cells. At lower glucose concentrations, insulin increased CO_2 formation and lipid synthesis from labelled L-leucine. At higher glucose concentrations insulin decreased apparent CO_2 formation and lipid synthesis. Insulin stimulated protein synthesis at all levels of glucose concentration studied, but the increment was less at higher glucose concentrations.

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Effects of Methylprednisolone on Plasma Lipids and Aortic Mucopolysaccharides of Normal and Cholesterol-Fed Rabbits* (33383)

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The administration of glucocorticoids to cholesterol-fed rabbits markedly reduces the incidence and severity of aortic atherosclerotic lesions despite a marked elevation in the plasma levels of cholesterol and triglycerides (1–3). Adlersberg *et al.* (4) attributed the protective effect of cortisone to a decreased permeability of the arterial wall resulting from alterations in the ground substance of

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