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EFFECTS OF INSULIN ON THE UTILIZATION OF ^{14}C-GLYCEROL AND ^{14}C-GLUCOSE IN HEPATECTOMIZED NEPHRECTOMIZED RATS

Effet de l'insuline sur l'utilisation du glycérol-C^{14} et du glucose-C^{14} chez les rats hépatectomies néphrectomisés

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SUMMARY

Insulin (i.v.) administration to functionally hepatectomized-nephrectomized rats did not alter circulating levels of glycerol and only slightly affected plasma radioactivity when animals received (U-^{14}C)-glycerol, whereas after (U-^{14}C)-glucose administration insulin enhanced hypoglycemia and greatly accelerated the rate of radioactivity loss from plasma. At 15 min after i.v. injection of (U-^{14}C)-glycerol, radioactivity in total lipids was reduced in heart and lungs by insulin administration and enhanced in carcass and brown adipose tissue. These effects involved the ^{14}C-glyceride glycerol fraction in the case of heart and ^{14}C-fatty acids in carcass and adipose tissue. When (U-^{14}C)-glucose was administered, insulin enhanced the appearance of ^{14}C-water-soluble material in heart and carcass and ^{14}C-total lipids in heart, carcass, and both brown and white adipose tissue. The effect in heart corresponded mainly to the ^{14}C-glyceride glycerol fraction whereas it corresponded to the ^{14}C-fatty acids in the other tissues. Therefore, insulin effects on glycerol metabolism substantially differ from those on glucose. Opposite effects on heart and lung glycerol utilization as compared to those in carcass and brown adipose tissue may account for the difficulties in observing changes in plasma glycerol levels after insulin treatment.

Key words : Extrahepatic metabolism. Insulin effects. Blood glucose. Blood glycerol. Lipogenesis. Glycerolgenesis.

Abbreviations used :

BAT : brown adipose tissue ; WAT : white adipose tissue ; VLDL : very low density lipoproteins.

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RÉSUMÉ

L'administration d'insuline (i.v.) à des rats fonctionnellement hépatectomisés néphrectomisés ne modifie pas les taux de glycérol circulant et n'affectant que légèrement, chez les animaux recevant de l'U-C^{14}-glycérol la radioactivité plasmatique. Par contre, après administration d'U-C^{14}-glucose, l'insuline augmente l'hypoglycémie et accélère fortement la vitesse de déperdition de la radioactivité du plasma. Quinze minutes après injection i.v. d'U-C^{14}-glycérol, la radioactivité dans les lipides totaux était réduite dans le cœur et les poumons par l'administration d'insuline et augmenté dans la carcasse et le tissu adipeux brun. Ces effets concernant les fraction glycérol des C^{14}-glycéride dans le cas du cœur et les acides gras-C^{14} dans la carcasse et le tissu adipeux. Quand le glucose-U-C^{14} a été administré, l'insuline augmente l'apparition d'un matériel C^{14} soluble dans l'eau dans le cœur et la carcasse ainsi que les lipides-C^{14} totaux dans le cœur et la carcasse et aussi dans les tissus adipeux brun et blanc. Les effets sur le cœur correspondent principalement à la fraction glycérol des C^{14}-glycérides alors qu'ils correspondent à celle des acides gras-C^{14} dans les autres tissus. Cependant les effets de l'insuline sur le métabolisme du glycérol difère de façon substantielle de ceux observés sur le glucose. Les effets opposés de l'utilisation du glycérol dans le cœur et les poumons, comparés à ceux rencontrés pour la carcasse et le tissu adipeux brun rend compte des difficultés rencontrées dans l'observation des modifications obtenues dans les taux plasmatiques de glycérol après traitement insulinique.

Mots clés : Métabolisme extra-hépatique. Effets de l'insuline. Glucose sanguin. Glycérol sanguin. Lipogénèse. Glycérolgénèse.

Basal concentration of plasma glycerol is normally very low, of the order of 10-200 uM in both adult humans (1-3) and experimental animals (4-8), due to its rapid turnover (9-12). The main endogenous source of circulating glycerol is lipolysis in adipose tissue while its main utilization sites are liver and kidney (8,13) where it is converted mainly into

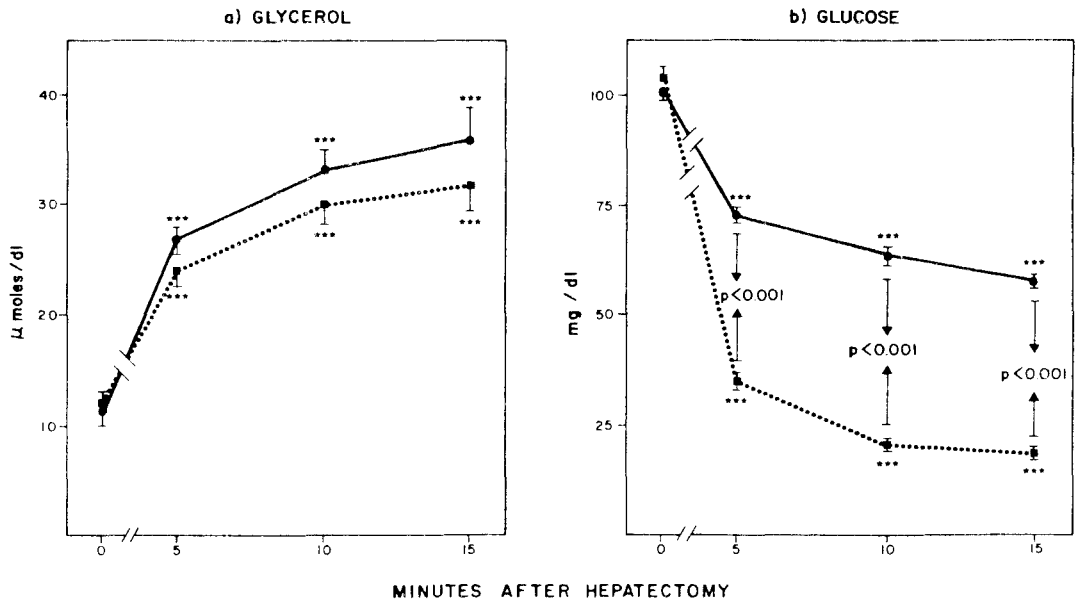

FIG. 1. — Blood concentration of glycerol (Fig. 1a) and glucose (Fig. 1b) in the hepatectomized-nephrectomized rat after the treatment with saline (●——●) or insulin (1 IU/kg) (■·····■).

The significance of the difference at each time point from before evisceration (time 0) is shown as *** = $p < 0.001$ and that of insulin treated versus untreated rats by the p values. For the concentrations of glycerol, differences between values of the insulin treated and untreated animals are not significant at any of the time points studied. Means ± SEM, n = 5-9/group.

enhanced by insulin. Tissue radioactivity and values of experiments with (U-^{14}C)-glycerol 15 min after administration of the tracer are shown in *Fig. 2.* Radioactivity in watersoluble material expressed per g of fresh tissue weight, was similar in heart, lungs and carcass, and lower in brown adipose tissue (BAT) and white adipose tissue (WAT). Insulin treatment did not affect this parameter in any of the

TABLE I. — *Plasma ^{14}C-components after the intravenous administration of either (U-^{14}C)-glycerol or (U ^{14}C)-glucose in the hepatectomized-nephrectomized rat treated or not with insulin (1 IU/Kg)*

	Animals receiving (U-^{14}C)-glycerol			Animals receiving (U-^{14}C) glucose		
	Controls	Insulin treated	P	Controls	Insulin treated	P
Total radioactivity (x10^{-3})						
5 min	16.5 ± 1.7	11.3 ± 0.8	<0.05	30.3 ± 1.6[3a]	11.0 ± 1.1	<0.001
10 min	13.2 ± 1.4	9.2 ± 0.7	<0.05	24.8 ± 2.3[2a]	9.7 ± 1.5	<0.001
15 min	10.9 ± 1.2	9.0 ± 0.7	N.S.	21.4 ± 2.3[3a]	8.3 ± 1.0	<0.001
Percentual distribution of ^{14}C-components						
^{14}C-glucose						
5 min	2.3 ± 0.4	4.2 ± 1.1	N.S.	59.3 ± 2[3a]	44.3 ± 4.6[3a]	<0.01
10 min	3.0 ± 0.6	4.7 ± 1.3	N.S.	54.5 ± 1.8[3a]	32.9 ± 4.4[3a]	<0.001
15 min	3.9 ± 0.5	4.7 ± 0.7	N.S.	49.5 ± 2.9[3a]	26.1 ± 3.1[3a]	<0.001
^{14}C-lactate						
5 min	15.5 ± 0.6	14.8 ± 2.2	N.S.	8.5 ± 0.9[3a]	20.7 ± 1.7	<0.001
10 min	23.1 ± 1.4	25.4 ± 2.8	N.S.	14.8 ± 1.5[2a]	34.2 ± 3.9	<0.001
15 min	28.6 ± 1.3	27.4 ± 2.0	N.S.	20.5 ± 2.4[a]	38.5 ± 6.3	<0.05
^{14}C-glycerol						
5 min	72.4 ± 1.2	68.3 ± 2.2	N.S.	—	—	—
10 min	62.5 ± 1.0	60.9 ± 2.1	N.S.	—	—	—
15 min	59.2 ± 1.0	53.7 ± 2.5	N.S.	—	—	—

Results are expressed as disintegrations. min^{-1}. ml^{-1} or as percentage of total plasma radioactivity (mean ± SEM, n = 5-9). P values correspond to the significance of the difference of treated versus untreated rats, whereas the significance of values from rats receiving (U-^{14}C)-glucose versus (U-^{14}C)-glycerol are shown by *a* : *a* = $p < 0.05$; 2*a* = $P < 0.01$; 3*a* = $P < 0.001$.

tion of α-glycerol-phosphate used for fatty acids and glycerid glycerol synsthesis. In both heart and lungs, most radioactivity in lipids after injection of (U-^{14}C)-glycerol appeared in the form of glyceride glycerol, and administration of insulin decreased this parameter. This effect was similar to the recently reported (35) hormonal suppression of ^{14}C-esterified fatty acids in these two tissues after the administration of ^{14}C-VLDL-triglycerides in hepatectomized rats, and the two effects may be interrelated. In heart, fatty acids are normally utilized as a primary source of energy production in basal conditions (36) and insulin is known to inhibit lipoprotein lipase activity (37, 38). This effect together with the observed insulin reduction of heart glycerol utilization for glyceride glycerol formation (or fatty acids esterification), may represent a compensatory action of the hormone to its effect of enhancing glucose utilization in this tissue, decreasing the use of circulating fatty acids in favor of other tissues (for example, for their deposit in adipose tissue).

Lung tissue incorporated (U-^{14}C)-glycerol into glyceride glycerol and this result is in agreement with the reported presence of glycerolkinase activity in cells from this tissue (39). The pathway was decreased by insulin whereas the hormone affected the utilization of glucose only slightly. These findings are in agreement with the reported presence of insulin receptors in crude membrane preparations from normal rat lung (40) and with the small hormone effect on glucose utilization and lactate production in perfused lung preparation (41, 42). The reduction of glyceride glycerol formation from glycerol in lung produced by insulin may also be related with a similar effect of the hormone on the uptake of ^{14}C-fatty acids from ^{14}C-VLDL-triglycerides already reported by us (35). The combination of these two effects suggests that, in these conditions, insulin decreases the use of these extracellular precursors for phospholipid synthesis by the lung. The responses to insulin by carcass and BAT concerning glycerol incorporation into lipids was contrary to that of heart and lung. In the former tissues, the insulin effect corresponded specifically to an increase in the synthesis of fatty acids from glycerol. A similar effect was observed when glucose was the administered tracer, indicating that the hormone enhanced overall lipogenesis in those two tissues, carcass (mainly, skeletal muscle), and BAT. The effects were striking in BAT, where it is known that glycerolkinase activity is substantial (43) and the tissue lipogenetic reaction to insulin is intense (44). Therefore, our results indicate that insulin acutely enhances glycerolkinase activity in BAT. This action occurs approximately 15 min after the hormone administration and is not accompanied by changes in total tissues enzyme activity (umpublished observations), suggesting that insulin action is exerted by modifying the catalytic efficiency of the enzyme rather than its concentration. The low uptake of either (U-^{14}C)-glycerol or (U-^{14}C)-glucose

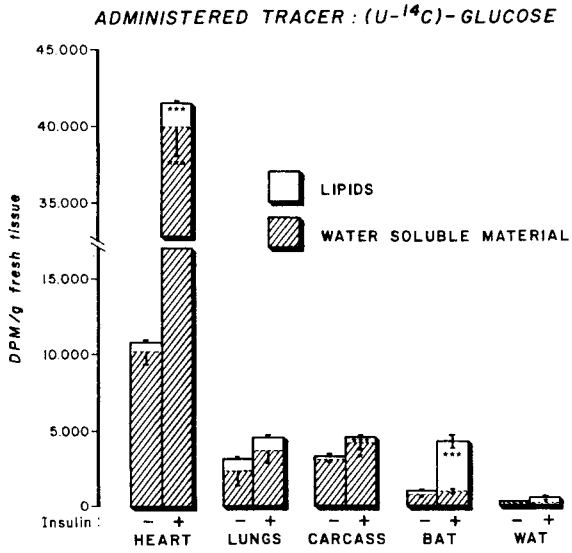

FIG. 3. — Tissue radioactivity 15 min after the intravenous administration of (U-^{14}C)-glucose in hepatectomized-nephrectomized rats treated or not treated with insulin (1 IU/kg). Open part of the bars correspond to values of ^{14}C-lipids whereas shadowed part corresponds to values of ^{14}C-water soluble material. Significant differences between insulin treated and untreated rats are shown by asterisks in the corresponding part of the bar : * = $p<0.05$, *** = $p<0.001$. Means ± SEM, n = 6-9/group.

TABLE III. — *Percentual distribution of tissular ^{14}C-lipidic fractions 15 min after intravenous administration of (U-^{14}C)-glucose in the hepatectomized-nephrectomized rat treated or nor with insulin (1 IU/Kg).*

Tissue	*Fatty acids* (%)	*Glyceride glycerol* (%)
Heart		
Controls	3.0 ± 0.3	95.9 ± 0.5
Insulin	2.3 ± 0.4	96.4 ± 1.3
p	N.S.	N.S.
Lungs		
Controls	33.9 ± 4.1	66.1 ± 4.0
Insulin	44.0 ± 1.6	55.9 ± 1.9
p	<0.05	0.05
Carcass		
Controls	39.9 ± 1.7	53.9 ± 2.4
Insulin	51.9 ± 2.4	46.2 ± 2.5
p	<0.01	N.S.
Brown Adipose Tissue		
Controls	24.8 ± 2.7	74.6 ± 4.3
Insulin	44.2 ± 1.4	47.6 ± 5.9
p	<0.001	<0.01
White Adipose Tissue		
Controls	47.1 ± 2.2	52.5 ± 1.8
Insulin	67.0 ± 2.6	35.1 ± 2.7
p	<0.001	<0.001

Results are expressed as percentage of ^{14}C-total lipids in the fractions (mean ± SEM, n = 6-9). P values correspond to the significance of the difference between treated and untreated rats.

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