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Utilization of Pyruvate, Alanine and Glutamate by Isolated Fat Cells and their Effects on Glycerol Metabolism *

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To study the metabolic interactions of different substrates in adipose tissue *in vitro*, isolated fat cells from fed rats were incubated in medium containing either (U-^{14}C)-pyruvate, L-(U-^{14}C)-alanine, L-(U-^{14}C)-glutamate or (1-^{14}C)-glycerol, and supplemented or not with 5 mM glucose or with non-radioactive pyruvate, alanine or glutamate (2 or 10 mM). The utilization of pyruvate for CO_2 or fatty acid formation was greater than that of alanine and glutamate, both in the absence and presence of glucose. Glucose enhanced the formation of fatty acids from all the labelled substrates, decreased the synthesis of glyceride glycerol from pyruvate and glycerol, and enhanced it from alanine and glutamate. Pyruvate and glutamate enhanced the utilization of glycerol by the adipocytes and these effects were significantly reduced in the presence of glucose. Thus, the metabolic fate of the glycerol taken up by the cells varied according to the nature of the available substrates.

Although it is well known that glucose is the main substrate for the *in vivo* synthesis of triglycerides in adipose tissue, it has been shown that both fatty acids and glyceride glycerol may be synthesized *in vitro* from other substrates, such as pyruvate (14, 15, 26, 29, 32), fructose (9, 10, 23), amino acids (13, 24), and glycerol (3, 4, 17-19). Glucose enhances the conversion of pyruvate (15, 16), amino acids (13), and glycerol (3) into fatty acids

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by providing NADPH for lipogenesis. On the contrary, glucose inhibits the conversion of these substrates into glyceride glycerol, as it has been shown for fructose (9, 11) and glycerol (3, 5, 19), by competing in the synthesis of α-glycerophosphate. In order to gain a better understanding of these interactions among different metabolites for the synthesis of glycerides in adipose tissue *in vitro*, in the present work a comparative study has been made of the metabolization of pyruvate, alanine and glutamate in isolated rat fat cells, incubated both in the absence and presence of glucose, together with the effects of these metabolites on glycerol utilization by the same adipose tissue preparations.

Materials and Methods

White fat cells were obtained from the parametrial adipose tissue of 152-183 g female Wistar rats fed *ad libitum* on laboratory rat chow. They were killed by cervical fracture and the fat cells were isolated by a modification of the method of RODBELL (28). Approximately 1 g of adipose tissue pieces were incubated for 60 min in 3 ml of Krebs Ringer bicarbonate buffer, pH 7.4, containing defatted (1) bovine serum albumin (20 mg/ml), glucose (3 mM), ovomucoid trypsin inhibitor (Sigma) (3.33 mg/ml) and crude collagenase (Worthington Biochem. Co.) (3.33 mg/ml). The isolated cells were washed four times at room temperature with warm (37° C) plain Krebs Ringer bicarbonate buffer for protein evaluation (21, 25). Aliquots of 1 ml of the cell suspension (125-194/μg of protein) were incubated for 120 min in Krebs Ringer bicarbonate buffer containing defatted bovine albumin (20 mg/ml) and 0.5 μCi of (U-^{14}C) pyruvate (8.86 mCi/mmol), L-(U-^{14}C) glutamate (275 mCi/mmol), or L-(U-^{14}C) alanine (164 mCi/mmol), supplemented with non-radioactive substrates to give the desired final concentration, either in the presence or absence of 5 mM glucose. In another series of experiments, the medium contained 0.5 μCi/ml (1-^{14}C) glycerol (31 mCi/mmol) as the labelled substrate, supplemented with some of the above mentioned metabolites. All the labelled substrates were purchased at The Radiochemical Center. The incubations were carried out at 37° C in sealed siliconized glass vials, gassed for 5 min with O_2/CO_2 (95:5), in a Dubnoff shaking incubator at 100 cycles/min. The incubations were terminated by the addition of 0.5 ml of 10 % (w/v) $HClO_4$. The $^{14}CO_2$ evolved was trapped in Hyamine-10 × hydroxide by gentle shaking at room temperature for 60 min (17, 18). The lipidic fraction was separated from the medium by centrifugation at 1.000 × *g* for 20 min, in the same incubating vials. Glycerol was determined in aliquots of the infranatants by an enzymatic method (12), and lipids were extracted from the washed supernatants (7) and purified and fractionated as previously described (3, 17). The uptake of (^{14}C) glycerol was quantified as the sum of the label found as $^{14}CO_2$ and ^{14}C-total lipids at the end of the incubating. These methods were validated by recovery experiments (3).

Results

In table I are summarized the results of the amounts of (U-^{14}C) pyruvate, (U-^{14}C) alanine and (U-^{14}C) glutamate oxidized to CO_2 or converted into glyceride-glycerol and fatty acids by isolated fat cells incubated *in vitro* in the absence or in the presence of the 5 mM glucose. Of the three substrates studied, pyruvate is the one metabolized in greater proportion by the adipocytes, being mainly converted to CO_2 and fatty acids and, in a lower proportion, to glyceride-glycerol. By increasing the concentration of pyruvate in the medium from 2 to 10 mM, the net amount of substrate converted to

Table I. *Effect of glucose on the utilization of (U-^{14}C)-pyruvate, L(U-^{14}C) alanine and (U-^{14}C) glutamate by isolated fat cells from fed rats incubated* in vitro.

Isolated adipocytes were incubated for 120 min in Krebs Ringer bicarbonate buffer containing defatted bovine albumin (20 mg/ml) and 0.5 μCi/ml of (U-^{14}C) pyruvate (8.86m Ci/mmol), L-(U-^{14}C) glutamate (275m Ci/mmol) or L-(U-^{14}C) alanine (164m Ci/mmol) supplemented with non radioactive substrate, either in the presence or in the absence of 5 mM glucose. The data are expressed as means ± S.E.M. of 8 rats/group. The statistical differences between each group with glucose and that without glucose are shown by the P values and those between each group with 10 mM substrate and that with 2 mM of the same substrate are shown in the P′ values (N.S. = not significant, $p < 0.05$).

	(nmoles of substrate utilized/100 μg of cell proteins)					
Additions to the medium	Formation of CO_2	P′	Formation of glyceride glicerol	P′	Formation of fatty Acids	P′
(U-^{14}C)-Pyruvate (2 mM)	438±20.4		34.6±2.71		270±21.2	
(U-^{14}C)-Pyruvate (2 mM)+Glucose (5 mM)	461±24.5		24.1±1.18		359±18.7	
P	N.S.		$p < 0.01$		$p < 0.01$	
(U-^{14}C)-Pyruvate (10 mM)	444±24.6	N.S.	71.7±3.15	< 0.001	515±24.6	< 0.001
(U-^{14}C)-Pyruvate (10 mM)+Glucose (5 mM)	812±29.1	< 0.001	55.1±1.23	< 0.001	1,230±27.5	< 0.001
P	$p < 0.001$		$p < 0.001$		$p < 0.001$	
(U-^{14}C)-Alanine (2 mM)	34.8±0.98		6.13±0.59		6.29±0.42	
(U-^{14}C)-Alanine (2 mM)+Glucose (5 mM)	73.7±2.08		19.7±3.03		71.4±2.63	
P	$p < 0.001$		$p < 0.001$		$p < 0.001$	
(U-^{14}C)-Alanine (10 Mm)	39.5±2.33	N.S.	17.1±2.95	< 0.01	8.45±0.55	< 0.01
(U-^{14}C)-Alanine (10 mM)+Glucose (5 mM)	91.8±1.11	< 0.001	33.1±3.47	< 0.05	70.4±2.22	N.S.
P	$p < 0.001$		$p < 0.001$		$p < 0.001$	
(U-^{14}C)-Glutamate (2 mM)	41.3±1.23		7.14±1.58		1.98±0.26	
(U-^{14}C)-Glutamate (2 mM)+Glucose (5 mM)	38.9±1.95		11.2±1.77		8.40±1.22	
P	N.S.		N.S.		$p < 0.001$	
(U-^{14}C)-Glutamate (10 mM)	73.4±1.97	< 0.001	19.6±1.01	< 0.001	4.63±0.22	< 0.001
(U-^{14}C)-Glutamate (10 mM)+Glucose (10 mM)	66.1±3.31	< 0.001	25.3±2.47	< 0.001	15.7±1.93	< 0.001
P	N.S.		$p < 0.05$		$p < 0.001$	

Table II. *Effect of glucose, pyruvate, alanine and glutamate on the utilization of (1-^{14}C)- glycerol by isolated fat cells from rats incubated* in vitro.

Isolated adipocytes were incubated for 120 min in Krebs Ringer bicarbonate buffer containing defatted albumin (20 mg/ml), 0.5 μCi/ml of (1-^{14}C), glycerol (31 mCi/mmol) and non radioactive substrates. Values are expressed as means ± S.E.M. of 5-12 rats/group. The uptake correspond to the amount of (1-^{14}C) glycerol converted to $^{14}CO_2$ and ^{14}C-lipids. The statistical differences between each group with glucose and that without glucose are shown by the P values and those between each group with 10 mM non radioactive substrate concentration and that with 2 mM of the same substrate are shown by P′ values. Asterisks correspond to the difference between each group and the basals (no addition) (N.S. or no asterisks, not significant, $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

	(% of initial radioactivity in the medium/100 μg of cell protein)							
Additions to the medium	Uptake	P′	Formation of ^{14}CO	P′	Formation of ^{14}C-glyceride glycerol	P′	Formation of ^{14}C-fatty acids	P′
None	1.29±0.29		0.35±0.03		0.87±0.04		0.07±0.01	
Glucose (5 mM)	1.51±0.19		0.52±0.11		0.70±0.13		0.26±0.01	
Pyruvate (2 mM)	5.44±0.73 ***		1.02±0.12 ***		4.24±0.14 ***		0.18±0.02 ***	
Pyruvate (2 mM) + Glucose (5 mM)	1.84±0.54		0.61±0.07 **		1.02±0.08		0.20±0.03 ***	
P	P < 0.01		P < 0.05		P < 0.001		N.S.	
Pyruvate (10 mM)	2.52±0.65	N.S.	0.38±0.06	P < 0.001	2.01±0.07 ***	P < 0.001	0.90±0.02	P < 0.01
Pyruvate (10 mM) + Glucose (5 mM)	0.81±0.14	N.S.	0.20±0.03	P < 0.01	0.54±0.05 **	P < 0.01	0.06±0.01	P < 0.01
P	N.S.		N.S.		P < 0.001		N.S.	
Alanine (2 mM)	0.73±0.32		0.10±0.03 **		0.60±0.03 **		0.02±0.01 *	
Alanine (2 mM) + Glucose (5 mM)	0.71**0.17		0.25±0.01		0.34±0.02 ***		0.12±0.02 *	
P	N.S.		P < 0.001		P < 0.001		P < 0.01	
Alanine (10 mM)	0.70±0.24	N.S.	0.09±0.02 ***	N.S.	0.57±0.02 **	N.S.	0.02±0.01 *	N.S.
Alanine (10 mM) + Glucose (5 mM)	1.64±0.70	N.S.	0.34±0.02	P < 0.05	1.08±0.13	P < 0.001	0.22±0.08 **	N.S.
P	N.S.		P < 0.001		P < 0.01		P < 0.05	
Glutamate (2 mM)	4.78±1.35 ***		0.42±0.06		4.30±0.07 ***		0.05±0.02	
Glutamate (2 mM) + Glucose (5 mM)	1.27±0.41		0.43±0.09		0.57±0.09 **		0.28±0.05 ***	
P	P < 0.05		N.S.		P < 0.001		P < 0.01	
Glutamate (10 mM)	4.31±1.36 **	N.S.	0.44±0.09	N.S.	3.83±0.09 ***	P < 0.01	0.40±0.01	N.S.
Glutamate (10 mM) + Glucose (5 mM)	1.14±0.37	N.S.	0.35±0.03	N.S.	0.54±0.04 ***	N.S.	0.25±0.04 ***	N.S.
P	P < 0.05		N.S.		P < 0.001		P < 0.01	

CO_2 remains unchanged, but the fraction converted to fatty acids and to glyceride-glycerol increases significantly. Glucose enhances the amount of (U-^{14}C) pyruvate metabolized by the tissue, being its effect greater at high substrate concentrations (10 mM) than at low (2 mM) (table I). In the presence of glucose a greater proportion of pyruvate is converted to CO_2 and fatty acids, but a smaller proportion is used for the synthesis of glyceride glycerol. The total proportion of (U-^{14}C) alanine metabolized by the adipocytes is much lower than that of pyruvate, being most of it converted to CO_2 (table I). With great difference respect to pyruvate, the incorporation of alanine into fatty acids is not greater than into glyceride-glycerol. The amount of alanine metabolized by the fat cells is increased with higher concentrations of alanine in the medium, being further increased by the presence of glucose. The greater effect of glucose on the metabolization of alanine is observed in its conversion to fatty acids that increases almost ten times over the values observed in the absence of glucose. Contrarily to what happens when the substrate is pyruvate, glucose also enhances the conversion of alanine to glyceride-glycerol. (U-^{14}C) glutamate is metabolized by the adipocytes in a similar proportion to that of alanine (table I). The conversion, of glutamate to all fractions studied increases when its concentration in the medium is also increased, but its relative utilization for the different parameters differs substantially from that of the other substrates. Thus, most of the glutamate is oxidized to CO_2, and the small proportion appeared in the lipidic fraction is mainly in glyceride-glycerol form, being very little found in the fatty acid one. Glucose does not affect the conversion of glutamate to CO_2, and increases very slightly its conversion to glyceride glycerol. But glucose enhances significantly the synthesis of fatty acids from glutamate (table I).

In table II are shown the effects of glucose, pyruvate, alanine and glutamate on the utilization of (1-^{14}C) glycerol by the isolated fat cells. In all different conditions studied, the production of non-radioactive glycerol by the cells during the incubation did not differ from that found in the basal controls (data not shown). Thus, the amount of labelled glycerol metabolized under the different conditions does not have to be corrected for differences in the glycerol specific activity between the groups. From all the conditions studied, the presence of 2 mM pyruvate in the medium produces the greatest increase in the uptake of (1-^{14}C) glycerol by the adipocytes, being its effect mainly observed in the formation of CO_2 and the synthesis of glyceride glycerol. Increasing the concentration of pyruvate in the medium from 2 to 10 mM it appears a decrease in its effect on the (^{14}C) glycerol metabolization by adipocytes. The conversion of glycerol to glyceride glycerol remains however significantly increased in the cells incubated with 10 mM pyruvate, as compared to that of the basals. Contrarily to what it was observed in the metabolization of labelled pyruvate, glucose inhibits very intensely the effect of pyruvate on the metabolism of labelled glycerol (table II). Alanine at concentrations of 2 and 10 mM decreases the amount of (^{14}C) glycerol metabolized by the adipocytes, the effect being observed in the conversion of the labelled substrate to CO_2, fatty acids and glyceride-glycerol. Glucose partially compensates the effects of alanine on the metabolism of (^{14}C) glycerol. This effect is better observed in the presence of high alanine concentrations than low ones. Glutamate enhances very significantly the total amount of (^{14}C) glycerol used by the adipocytes for glyceride-glycerol synthesis, but does not affect glycerol oxidation to CO_2, nor its conversion to fatty acids. In the presence of glucose this effect of glutamate on the synthesis of glyceride-

glycerol from glycerol disappears completely (table II). Actually, the fate of the glycerol metabolized in the presence of both glutamate and glucose seems to be the same as that observed with glucose alone in the medium.

Discussion

Metabolism of pyruvate in adipocytes. In the present study it is seen that pyruvate is used in a considerable proportion by adipose tissue cells incubated *in vitro* without affecting the production of glycerol by the tissue. These results confirm previous observations from others (14-16, 20, 26, 29, 32). Even in the absence of glucose pyruvate is mainly used for both oxidation to CO_2 and conversion to fatty acids, more than for the syntheses of glyceride glycerol. These results allow to suggest that although the synthesis of fatty acids may be limited by the availability of NADPH (21, 33), the malate cycle (6) may be able to partially supply this reduced coenzyme, since in the absence of glucose the pentose phosphate pathway is negligible in adipose tissue, where glycogen stores are small (8).

Glucose markedly increases both pyruvate oxidation and conversion to fatty acids. Very probably, in this condition the availability of NADPH for lipogenesis is enhanced (16), and the fatty acids are esterified with glycerophosphate derived from glucose rather than from pyruvate. Actually, it has been shown that the conversion of pyruvate to glyceride glycerol is reduced in the presence of glucose. On the other hand, pyruvate alone enhances very intensely the conversion of glycerol to glyceride glycerol. This would mean that pyruvate facilitates the phosphorylation of glycerol, which suggests that it enhances the esterification of fatty acids in adipose tissue incubated in the presence of glucose, not only by its role as glycerogenetic substrate (14, 26, 29), but also by its effect on the metabolization of the glycerol released into the medium through lipolysis. In the presence of glucose, the effect of pyruvate enhancing the incorporation of glycerol into glyceride-glycerol disappears. In this condition glucose is assumed to become the main source of glycerophosphate for the fatty acids esterification.

Metabolism of alanine in adipocytes. Although it is know that adipose tissue contains alanine aminotransferase (2), the net amount of alanine metabolized by the adipocytes is much lower than that of pyruvate. Its proportional intrinsic conversion into the different fractions studied also differs between both metabolites. In the absence of glucose, the amount of alanine converted to fatty acids is low and very similar to that converted to glyceride glycerol. Probably, the limited metabolism of alanine by the adipocytes does not allow the formation of enough pyruvate to feed the malate cycle and thus most of the substrate is then completely oxidized to CO_2.

Glucose enhances the alanine metabolization as has been shown for other amino acids in adipose tissue (27). These results can be related to those of SHAFRIR *et al.* (30), showing that alanine itself stimulates the glucose metabolism by adipose tissue. Contrarily to the glucose effects on pyruvate utilization by the adipose tissue cells, glucose enhances the conversion of alanine to all studied fractions, including the synthesis of glyceride glycerol. Thus, it seems that glucose acts more upon the alanine uptake by the tissue than on its intrinsic metabolization. In the presence of glucose probably more α-keto acids are available to allow the transamination of alanine which would also contribute to the effects of glucose enhancing the metabolization of that amino acid by adipocytes.

The effects of alanine on glycerol utilization by adipocytes are more difficult to explain. Contrarily to the effects of

pyruvate, alanine inhibits the metabolization of glycerol. Although further studies are required, the possibility exists that the initial metabolization of alanine requires its ATP-dependent transport, which would reduce the availability of ATP for glycerol phosphorylation. It has been previously shown that the metabolism of glycerol in adipocytes is very much dependent on the availability of energy (4). The effect of glucose compensating the inhibitory action of high alanine concentrations on glycerol utilization agrees with this hypothesis.

Metabolism of glutamate in adipocytes. Glutamate during its metabolism is mainly transformed into α-ketoglutarate by glutamate dehydrogenase which is present in adipose tissue and located exclusively in the mitochondria (2). LEVEILLE and HANSON (24) have studied the incorporation of L-(^{14}C) glutamate into fatty acids by adipose tissue, concluding that although some fatty acid synthesis can be carried out from α-ketoglutarate via a *reversal* of the Krebs cycle to citrate, most of the glutamate is oxidized to CO_2 in adipose tissue. Our results on the utilization of L-(^{14}C) glutamate by isolated adipocytes agree with this view, due to the fact that in the absence of glucose more than 75 % of the substrate is oxidized to CO_2. The glutamate converted to lipids appears to be preferentially in the form of glyceride glycerol instead of in the fatty acids fraction. In the *reversal* of the Krebs cycle α-ketoglutarate is transformed to citrate, which in the extramitochondrial space is cleavaged into oxaloacetate and acetyl-CoA. Due to the limited availability of NADPH when glucose is absent, it is not surprising that glycerogenesis from that oxaloacetate will be favored over lipogenesis. Contrarily to alanine, a considerable proportion of the glutamate metabolism is intramitochondrial and does not require keto acids for transamination. These points could explain the smaller effect of glucose on the metabolism of glutamate than on the metabolism of alanine. Actually, the metabolism of glutamate is closely related with the metabolism of α-ketoglutarate, which is known to be unaffected by glucose in fat cells (31).

The effects of glutamate on the glycerol metabolism by the adipocytes allow to partially complete this picture. Glutamate produces an intense increase in the amount of glycerol metabolized by fat cells, but this effect is only observed in its conversion to glyceride glycerol and not in its oxidation to CO_2 nor in its conversion to fatty acids. As in the presence of glutamate the central pathways of the fat cells are fed with enough substrate by the preferent oxidation of glutamate, it is not surprising that once glycerol is phosphorylated, it is not taken by the lower glycolytic pathways, but is directly used for fatty acids esterification. In the presence of glucose, the effect to glutamate on the synthesis of glyceride glycerol disappears. In this condition glycerophosphate is mainly formed from glucose, the oxidative pathways are fulfilled by glutamate, and the fat cell does not require the use of an extrasubstrate, such as glycerol, to feed its main metabolic rutes.

All these metabolic interactions of the adipose tissue cell has been studied *in vitro* and demonstrate the selective use of substrates for the main pathways, depending upon the type and amount of substrate available.

Very probably the *in vivo* events are quite different, as many other factors can be affecting these interactions and thus, the physiological implications of these findings remain to be established.

Resumen

Con el fin de estudiar las interacciones metabólicas de diferentes substratos en tejido adiposo incubado *in vitro*, se incubaron adipocitos aislados de ratas alimentadas con medio

conteniendo uno de los siguientes substratos radioactivos: piruvato-U-C^{14}, L-alanina-U-C^{14}, L-glutamato-U-C^{14} o glicerol-1-C^{14}, siendo suplementado a su vez con piruvato, alanina o glutamato no radioactivos (2 ó 10 mM), en presencia o en ausencia de glucosa 5 mM. La utilización de piruvato para la formación de CO_2 o ácido graso fue superior que las de alanina y glutamato, tanto en ausencia como en presencia de glucosa. La glucosa produjo un aumento de la formación de ácidos grasos a partir de todos los substratos, disminuyendo a su vez la síntesis de glicerol de glicéridos a partir de piruvato y de glicerol, y aumentándola a partir de alanina y glutamato. El piruvato y el glutamato aumentaron la utilizaciión de glicerol por los adipocitos, y estos efectos fueron inhibidos por la presencia de glucosa en el medio. En consecuencia, el destino metabólico del glicerol captado por las células varía en función de la naturaleza de los substratos disponibles.

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