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Study of the Metabolism in vitro of Glucose in the Rat Epididymal Fat Tissue.

Stimulating Effect of Palmitic Acid*,**

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Summary. The rat epididymal fat tissue was incubated with glucose differently labelled with ^{14}C, and the effect of palmitate bound to albumin was studied in the absence or presence of insulin. No inhibition by palmitate of the glucose metabolism was observed. On the contrary, it was demonstrated that palmitate stimulates the different pathways of the glucose metabolism, particularly the fatty acid neosynthesis. The results are discussed in relation to the glucose and palmitate metabolism in the adipose cell, and in relation to the role *in vivo* of the adipose tissue in the decrease of the glucose assimilation observed in conditions which abnormally increase the serum level of free fatty acids.

Etude du métabolisme du glucose in vitro dans le tissu épididymaire du rat. Effet stimulant de l'acide palmitique

Résumé. Le tissu adipeux épididymaire du rat a été incubé avec du glucose différemment marqué au ^{14}C et l'effet du palmitate lié à l'albumine a été étudié en l'absence et en présence d'insuline. Aucune inhibition, par le palmitate, du métabolisme du glucose n'a été observée; au contraire, il a été démontré que le palmitate stimule les différentes voies du métabolisme du glucose, en particulier la néosynthèse des acides gras. Les résultats sont discutés en fonction du métabolisme du glucose et du palmitate dans la cellule adipeuse, et en fonction du rôle tenu *in vivo* par le tissu adipeux dans la diminution de l'assimilation du glucose observée dans des conditions qui élèvent anormalement le taux des acides gras libres sériques.

Untersuchungen zum Glucose-Stoffwechsel des epididymalen Fettgewebsanhangs der Ratte in vitro. Zum Stimulationseffekt von Palmitinsäure.

Zusammenfassung. Das epididymale Fettgewebe der Ratte wurde mit verschieden markierter ^{14}C-Glucose inkubiert, und der Effekt von mit Eiweiß gebundenem Palmitat in Ab- und Anwesenheit von Insulin untersucht. Es wurde keine Hemmung des Glucosemetabolismus durch Palmitat beobachtet; hingegen wurde erwiesen, daß Palmitat die verschiedenen Wege des Glucosemetabolismus anregt, besonders die Neosynthese der Fettsäuren. Die Resultate werden wie folgt diskutiert: 1. im Hinblick auf den Metabolismus von Glucose und Palmitat in der Fettzelle, 2. im Hinblick auf die *in vivo*-Rolle des Fettgewebes bei der Verminderung der Glucoseassimilierung. Diese Verringerung wurde unter Bedingungen beobachtet, die den Serumspiegel der freien Fettsäuren ungewöhnlich stark erhöhen.

Key-words: Glucose, insulin, palmitate, fatty acids, CO_2, glycogen, lipogenesis, esterification.

Introduction

An alteration in glucose tolerance has often been shown under experimental and pathological conditions where there was an abnormal elevation of serum FFA[1]: fasting or a lack of carbohydrates [45, 14, 30]; infusion of a lipolytic hormone such as growth hormone [53, 31, 63] or norepinephrine [50]; infusion of an emulsion of triglycerides [18]; ingestion of a meal rich in fat, followed by an injection of heparin [59]; repeated injections of glucagon [19] etc.; and lastly, diabetes and obesity [30, 6, 1, 20].

This observation led Randle et al. [55, 56] to put forward the hypothesis of the "glucose-fatty acid cycle" in which a diminished peripheral utilization of glucose is caused by high serum FFA levels.

The blood glucose level depends, on the one hand, upon the uptake of glucose by the peripheral tissues, especially muscle and adipose tissue, and on the other hand, upon the variations in the balance of glycolysis-gluconeogenesis in the liver.

In perfused heart and in incubated diaphragm of the rat [51, 54, 25, 26], the inhibition *in vitro* of glycolysis by long chain fatty acids has been well established [51, 54, 25, 26, 61].

In the perfused liver, the inhibition by free fatty acids of certain key enzymes of glycolysis [68], the inhibition of acetyl-CoA carboxylase [8, 47] and of lipogenesis [37], and a stimulation of gluconeogenesis were observed [23, 65, 17, 11].

In vivo, however, Seyffert et al. [64] showed that though a sharp rise in FFA levels decreases the peripheral utilization of glucose, it also decreases the release of glucose by the liver; but they considered this last effect secondary to an increased release of insulin and a decreased secretion of glucagon.

The role of adipose tissue in the peripheral utilization of glucose is likely to be important, but is generally little taken into account. It has been mentioned that about 90% of glucose carbon retained in the body is kept in reserve in the form of fat [62].

The changes provoked by fatty acids in the glucose metabolism of adipose tissue have been less studied

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[1] Free Fatty acids.

than those in other tissues. Using very high levels of fatty acid, outside the physiological range, LEBOEUF and CAHILL [41] showed an increased uptake of glucose and an inhibition of the synthesis, stimulated by insulin, of fatty acids and of glycogen. Other workers found no effect *in vitro* of free fatty acids on glucose metabolism in adipose tissue [24, 27].

The purpose of our work was to complete these observations in the hope of proposing a mechanism by which the metabolism of free fatty acids could influence that of glucose in adipose tissue.

Materials and Methods

Wistar rats WAG, of 170 to 200 g, received a glucose-rich diet for 10—15 days before the experiment. The epididymal adipose tissue from each of 12 rats was rapidly removed, cut into 6 fragments and placed in previously weighed flasks containing the complete incubation medium. Normally 24 incubation flasks were prepared for each experiment, and the pieces randomized so that in the end each bottle contained 6 different pieces from 6 different rats. The bottles were again weighed and the tissue weight determined by the difference.

The incubation medium was Krebs-bicarbonate buffer (KRB) containing 2% albumin (Armour, Bovine Serum Albumin powder, Fraction V) purified of free fatty acids according to GOODMAN [28], with a final concentration of free fatty acids always below 50 μval/l.

The binding of palmitic acid to albumin was effected in two different ways:

1. at 40° C by adding drop by drop, a hot solution of potassium palmitate (prepared by precipitating it from an alcoholic solution of palmitic acid by the addition of KOH) to a solution of albumin in the buffer, kept at a temperature of 40° C.

2. or at 4° C by slow rotation over a period of 48 h of a balloon flask containing the 2% albumin solution on a thin bed of palmitic acid, obtained by evaporating under vacuum a solution of palmitic acid (Fluka) in ethanol.

The levels of palmitate in these preparations were measured by titration [16].

All the incubation media were dialyzed twice at 4° C against 20 volumes of buffer (immediately before the experiment). Dialysis tubing was previously treated by boiling in distilled water for a period of two hours.

Insulin (crystalline bovine insulin, Burroughs Wellcome and Co.), 1 U/ml, which was conserved at — 20° C in a slightly acid solution, was diluted with the incubation medium.

Three types of labelled glucose were used: glucose-U-^{14}C, glucose-1-^{14}C, glucose-6-^{14}C (Radiochemical Centre, Amersham). About 1 μc of radioactivity was added to each incubation flask.

After two hours of incubation, in an atmosphere of 95% O_2 + 5% CO_2, 0.4 ml of hyamine hydroxide (1 M) in methanol was added to a cup suspended inside the flask, 0.5 ml of 3N H_2SO_4 was added to the medium. The flasks were shaken slowly for two hours, and the radioactive CO_2 absorbed was measured.

After rinsing the tissues twice with 0.9% saline, the lipids were extracted and saponified according to FLATT and BALL [21]. The fatty acids, extracted with petroleum ether and washed [21], were dried under vacuum, weighed and then dissolved in 3 ml of heptane. The radioactivity in 2 aliquots, each of 0.1 ml, was measured.

The aqueous phase, after extraction of the fatty acids, contained the glycerol produced by the saponification of the lipids. The radioactivity of 2 aliquots, each of 0.5 ml, of this solution was measured without further purification.

The glycogen, prepared from tissue from which the fat had already been removed, was dissolved at 100° C in 1 ml of 30% KOH, precipitated at — 20° C after addition of 1.3 ml of ethanol (95%), centrifuged, and the supernatant carefully eliminated. The precipitate was then dissolved in 2 ml of distilled water. Radioactivity was counted in two 0.5 ml aliquots. The total glycogen was measured in duplicate by the anthrone method in comparison with a glycogen standard.

The complete incubation medium combined with the saline from the two rinsings of the tissue was deproteinized by the addition of 1 volume of 0.66 M $HClO_4$, and the total volume adjusted in graduated tubes. Glucose was measured by the o-toluidine method.

Radioactivity was measured in a Nuclear-Chicago Unilux 1 scintillation counter. For CO_2, fatty acids and glycerol, a mixture of PPO, POPOP, ethanol and toluene was used. To this liquid was added Cab-O-Sil (Thixotropic Gel Powder, Packard) in quantities sufficient to stabilize the aqueous suspension obtained with the glycerol solution.

The radioactivity of glycogen was measured with Bray's scintillation medium [7]. The correction for quenching was carried out for each series of measurements. The quenching effect was determined by measuring under the same conditions, the radioactivity of two aliquots, each of 0.1 ml, of radioactive incubation medium of the experiment diluted 1:10. It was verified that the addition of different fractions of radioactivity caused a diminution of less than 2% in the counts/min. For the fatty acids, the percentage of quenching increased when the quantity of fatty acids per counting flask surpassed 20—25 mg.

All statistics were calculated according to Student's *t-test*.

Results

In all experiments the effects of insulin and palmitate, alone or in combination, on glucose-U-^{14}C metabolism have been compared,

In the first experiments shown in Table 1, a preparation of the albumin-palmitate complex prepared at 40° C was used (see methods). No significant effect of this preparation on glucose metabolism was noted. The absence of the effect of increased glucose-U-^{14}C incorporation into the glyceride-glycerol probably indicates that adipose tissue is not capable of esterifying in measurable quantities the palmitate complex prepared in this manner.

The preparations effected at 4° C (see methods), of which the maximum level of free fatty acids was variable and always inferior to that of hot preparations, changed significantly glucose-U-^{14}C metabolism (Table 1). In the presence of quite high concentrations of glucose, there was an increase in the incorporation of glucose into glyceride-glycerol, which indicates an esterification of palmitic acid by adipose tissue in both the presence and absence of insulin. An inhibition of fatty acid synthesis was never observed. On the contrary, in both the presence and absence of insulin, lipogenesis from glucose was increased in the presence of palmitate, and this effect was accompanied by a stimulation of the oxidation of glucose to CO_2. However, the synthesis of glycogen was not significantly changed by palmitate.

With low concentrations of glucose (0.5 mg/ml) the stimulation of glucose metabolism by palmitate was also seen.

When adipose tissue was incubated in the presence of two concentrations of palmitate, the increase in glucose metabolism due to palmitate was directly proportional to the level of palmitate in the incubation medium (Table 2). In certain experiments of this series the palmitate produced an increase in glycogen synthesis. This effect was not found to be reproducible in all experiments, and therefore will not be taken into consideration.

When adipose tissue was incubated with non-purified albumin, the content of FFA, although low (107 μval/l; control, 25 μval/l), was sufficient to exercise a remarkable stimulation of glucose metabolism (exp 2, Table 2). In comparing the incubation medium containing gelatin as the base with that containing one of non-purified albumin, the same was observed, but only in the presence of insulin (exp. 4, Table 2).

Some experiments were carried out with two differently labelled substrates: glucose-1-^{14}C and glucose-6-^{14}C being compared in the same experiment (Table 3). Palmitate increased the incorporation of the 1st and the 6th carbons of glucose in the glyceride-glycerol fatty acids and CO_2. Even when the oxidation of the 6th carbon was increased by the palmitate, it remained low in absolute value.

The 6th carbon of glucose being oxidized exclusively in the Krebs cycle, the relative incorporation of this labelled carbon in CO_2 and fatty acids is an indication of the relative importance of the 2 metabolic paths: the Krebs cycle and fatty acid neosynthesis, which are the two paths of acetyl-CoA utilization [40]. These calculations were made from the results of experiments 1 and 3 of Table 3. For each of the two parameters (CO_2 and FA), 3 measurements of the incorporation of glucose-6-^{14}C were made per experiment The ratios and the corresponding means were calculated by combining these measurements according to all the possibilities, 9 at the maximum (Table 4). The unexpected result is that the relative importance of fatty acid synthesis, compared with that of the Krebs cycle, was increased slightly, but significantly, by palmitate. This effect occurred in both the presence and the absence of insulin in the incubation medium.

Discussion

Table 1 shows that the effect of palmitate *in vitro* on the metabolism of glucose in adipose tissue depends upon the way the albumin-palmitate complex is prepared: the preparations made by adding warm potassium palmitate to a solution of albumin bring about no significant change, whereas those prepared in the cold, by prolonged contact of albumin solution with a thin layer of palmitic acid are "active", giving definite and reproducible results. KESSLER, DEMENY and SOBOTKA [39] have prepared the albumin-palmitate-1-^{14}C complex from both the acid and salt forms. With the preparation from the acid form, the uptake *in vitro* and turnover rate *in vivo* of palmitate are 2.5 and 2 times greater respectively than those with the second preparation. GOODMAN has established the existence of 3 types of binding between palmitate and serum albumin, with variable dissociation coefficients. One can suppose like KESSLER et al. [39], that the different preparations of the albumin-palmitate complex possess different binding characteristics favouring to a greater or lesser extent the exchange with adipose tissue, and also the secondary metabolic effects of that exchange.

The magnitude of the effects obtained with the "active" preparations of palmitate is related to the level of palmitate in the medium (Table 2).§

Non-purified bovine albumin still contains a measurable quantity of FFA which can influence the metabolism of glucose in a manner comparable with preparations of the "active" complex (Table 2), and one can suppose that the FFA bound to albumin in plasma is likely to have similar effects on adipose tissue. However, one cannot exclude the possibility that GOODMAN's method for purifying albumin could remove, along with FFA, other impurities that might be responsible for the effects observed with non-purified albumin.

The conversion of glucose to glyceride-glycerol could constitute in certain conditions an indirect measure of esterification. That this is increased in the presence of FFA is, of course, expected.

Together with this effect, there is moreover, a more extensive metabolic stimulation, which also influences the paths of glucose oxidation and the synthesis of fatty acids; an increase in the synthesis of glycogen has

Table 1. *Influence of different preparations of palmitate on the glucose metabolism by the rat epididymal fat tissue, with or without insulin*

Krebs-Ringer-Bicarbonate, albumin 2%, albumin-palmitate complex prepared at 40°C or at 4°C (see methods); in the medium control, FFA < 50 μval/l; when added, insulin 125 μU/ml; glucose-U-^{14}C.
Means ± standard deviation; results expressed in mμ moles glucose/mg fresh tissue. 2 h.
Rats: 170–180 g, diet rich in glucose (see methods)

Added to buffer	T° [a]	glucose mg/ml	No. of measures	^{14}C-glucose incorporated into				
				CO_2	Fatty acid	Gl.-Glycerol	Glycogen	Total glycogen[b]
—		2	6	1.408 ± 0.22	0.257 ± 0.09	1.74 ± 0.22	0.035 ± 0.009	0.255 ± 0.060
insulin				3.84 ± 0.33	2.33 ± 0.20	1.96 ± 0.08	0.370 ± 0.064	0.810 ± 0.11
palmitate (1100 μval/l)	40°C			1.190 ± 0.33 n.s.	0.266 ± 0.05 n.s.	1.59 ± 0.15 n.s.	0.0405 ± 0.015 n.s.	0.270 ± 0.103 n.s.
ins. + palmitate				3.69 ± 0.60 n.s.	2.10 ± 0.46 n.s.	2.14 ± 0.15 n.s.	0.341 ± 0.017 n.s.	0.816 ± 0.137 n.s.
—		3.5	6	1.11 ± 0.12	0.189 ± 0.008	1.31 ± 0.004	0.061 ± 0.013	0.370 ± 0.111
insulin				3.42 ± 0.26	2.55 ± 0.23	1.49 ± 0.21	0.525 ± 0.163	0.969 ± 0.185
palmitate (437 μval/l)	4°C			2.33 ± 0.10[c]	0.654 ± 0.103[c]	2.23 ± 0.28[c]	0.045 ± 0.026 n.s.	0.370 ± 0.129 n.s.
ins. + palmitate				6.20 ± 0.67[d]	4.34 ± 0.23[d]	2.20 ± 0.22[d]	0.440 ± 0.055 n.s.	0.765 ± 0.068 n.s.
—		0.5	3	1.12 ± 0.14	0.648 ± 0.043	0.915 ± 0.089	0.050 ± 0.008	0.44 ± 0.05
insulin				4.30 ± 0.42	4.00 ± 0.22	1.19 ± 0.16	0.146 ± 0.035	0.588 ± 0.061
palmitate (552 μval/l)	4°C			1.76 ± 0.10[d]	0.803 ± 0.045[e]	1.21 ± 0.15[e]	0.059 ± 0.004 n.s.	0.303 ± 0.37 n.s.
ins. + palmitate				5.11 ± 0.17[e]	4.93 ± 0.26[e]	1.74 ± 0.20[e]	0.157 ± 0.036 n.s.	0.633 ± 0.052 n.s.
—		5	3	1.95 ± 0.26	1.02 ± 0.04	1.04 ± 0.13	0.079 ± 0.013	0.551 ± 0.033
insulin				8.03 ± 0.38	10.6 ± 0.7	2.13 ± 0.16	1.30 ± 0.10	2.40 ± 0.24
palmitate (437 μval/l)	4°C			3.05 ± 0.14[d]	2.25 ± 0.38[d]	1.98 ± 0.25[e]	0.093 ± 0.010 n.s.	0.491 ± 0.041 n.s.
ins. + palmitate				11.4 ± 0.10[c]	15.1 ± 0.7[d]	2.81 ± 0.13[e]	1.60 ± 0.26 n.s.	2.82 ± 0.33 n.s.

[a] Temperature of the preparation of the complex albumin-palmitate (see text)
[b] mμ mole glucose/mg fresh tissue, after complete hydrolysis of glycogen
[c] $p < 0.001$
[d] $p < 0.01$
[e] $p < 0.05$
n.s. = non-significant

also been noted, but this was not observed in all experiments, and will not be further considered in the discussion. Similar effects were observed using animals placed in particular nutritional conditions: fasting 2 or 3 days or refeeding after fasting (unpublished results.)

levels of palmitic acid in these experiments were very high: 4500 μval/l compared with 650 in the control medium.

On the basis of the results of LEBOEUF and CAHILL [41] and with what occurs in the liver, it has been concluded that there is in adipose tissue *in vitro* an inhibition of lipogenesis in the presence of FFA [70]. In no case do our results indicate inhibition; on the contrary, the inverse phenomenon is found, i.e. a stimulation of biosynthesis of fatty acids by tissue incubated with palmitate. A partial explanation of these apparently

Table 2. *Measures of the glucose metabolism by the rat epididymal fat tissue in the presence of increasing concentrations of palmitate*

Means ± standard deviation of 4 values
The conditions of incubation are similar to those mentioned in Table 1. glucose 3.5 mg/ml, + glucose U-^{14}C

Insulin μU/ml	Palmitate μval/l	^{14}C-glucose incorporated into CO_2	Fatty acid	Gl-Glycerol
—	—	1.40 ± 0.15	0.505 ± 0.030	1.16 ± 0.10
	552	2.15 ± 0.13[a]	1.23 ± 0.14[b]	1.62 ± 0.12[c]
	818	3.64 ± 0.25[a, d]	1.62 ± 0.35[c]	2.02 ± 0.16[c]
125	—	5.47 ± 0.43	5.42 ± 0.43	1.29 ± 0.04
	552	8.31 ± 0.65[a]	7.96 ± 0.20[c]	2.22 ± 0.16[b]
	818	13.3 ± 1.4[a]	12.1 ± 1.0[c]	3.56 ± 0.19[b]
—	—	2.14 ± 0.17	1.16 ± 0.13	1.00 ± 0.11
	570	4.74 ± 0.45[b]	3.02 ± 0.11[b]	1.70 ± 0.14[a]
	1.128	4.99 ± 0.26 n.s.	3.61 ± 0.18[c]	2.00 ± 0.17 n.s.
	107[e]	3.56 ± 0.17[b]	1.97 ± 0.13[a]	1.44 ± 0.14[c]
125	—	10.2 ± 0.12	10.6 ± 0.5	1.65 ± 0.12
	570	14.2 ± 0.4[a]	16.9 ± 0.8[b]	2.69 ± 0.10[b]
	1.128	19.5 ± 0.7[b]	20.9 ± 0.9[c]	3.45 ± 0.17[a]
	107[e e]	13.9 ± 0.6[a]	16.05 ± 0.94[b]	2.23 ± 0.11[a]
—	—[f]	2.47 ± 0.56	1.18 ± 0.11	1.22 ± 0.15
	114[e]	2.21 ± 0.05 n.s.	1.23 ± 0.24 n.s.	1.51 ± 0.16 n.s.
125	—	7.07 ± 0.67	6.86 ± 0.76	1.19 ± 0.05
	114[e]	13.6 ± 1.3[b]	15.5 ± 2.0[b]	1.70 ± 0.08[b]

[a] $p < 0.01$ [b] $p < 0.001$ [c] $p < 0.05$ [d] compared with the mean concentration of palmitate
[e] non-purified albumin 2% [f] gelatin 2%, instead of albumin, FFA < 20 μval/l n.s. = non-significant

Table 3. *Influence of palmitate on the 1-^{14}C-glucose and 6-^{14}C-glucose*

The conditions of incubation are similar

Added to medium	CO_2		Fatty acid	
	1-^{14}C-glucose	6-^{14}C-glucose	1-^{14}C-glucose	6-^{14}C-glucose
—	2.31 ± 0.06	0.897 ± 0.080	1. 49 ± 0.09	2.26 ± 0.07
insulin	10.4 ± 0.5	1.09 ± 0.07	6.87 ± 0.19	13.1 ± 0.6
palmitate (850 μval/l)	3.81 ± 0.21[a]	1.34 ± 0.11[b]	2.24 ± 0.09[a]	4.00 ± 0.06[a]
ins. + palmitate (id.)	14.6 ± 0.4[a]	1.28 ± 0.07[c]	10.1 ± 0.5[a]	21.2 ± 2.1[b]
—	1.60 ± 0.12	0.995 ± 0.138		
insulin	9.92 ± 0.4	1.126 ± 0.165		
palmitate (818 μval/l)	4.39 ± 0.56[b]	1.56 ± 0.21[c]		
ins. + palmitate	15.5 ± 0.9[a]	1.71 ± 0.14[b]		
—	1.54 ± 0.17	0.94 ± 0.03	1.260 ± 0.067	2.05 ± 0.19
insulin	7.03 ± 0.81	1.05 ± 0.07	5.62 ± 0.32	10.6 ± 0.3
palmitate (748 μval/l)	3.81 ± 0.11[a]	1.20 ± 0.04[c]	2.13 ± 0.21[b]	4.06 ± 0.21[b]
	13.4 ± 0.4[a]	1.24 ± 0.05[c]	9.16 ± 0.26[a]	15.0 ± 0.8[a]

[a] $p < 0.001$ [b] $p < 0.01$ [c] $p < 0.05$

These results are in part different from those of LEBOEUF and CAHILL [41]; on the one hand, they observed an increase, in the presence of FFA, of glucose uptake and glyceride-glycerol synthesis; and on the other, an inhibition, in the presence of insulin, of glucose conversion to glycogen and fatty acids. The

paradoxical results may be found in the generally accepted concept of compartmentation of tissue FFA in different and relatively independent pools [66, 38, 15, 4, 58].

WINAND, FURNELLE and CHRISTOPHE [69] have shown with epididymal adipose tissue of mouse *in vitro* that there are 3 pools: the FFA synthesized *in vitro*, the FFA coming from lipolysis and the FFA taken up from the medium. The FFA of the third pool do not inhibit the incorporation of newly formed fatty acids into lipids. Moreover, the tissue FFA play no role whatever in the assimilation and esterification of extratissular FFA [38, 15].

In a medium rich in FFA, particular oxidation of the sixth carbon of glucose has been mentioned [20, 10]. Our results obtained using as substrate glucose labelled in positions 1 and 6 do not correspond with this observation. If palmitate stimulates the incorporation of both the 1st and 6th carbon into CO_2, the oxidation of the 6th carbon is of little importance.

The oxidation of the 6th carbon is exclusively reserved for the Krebs cycle and is little stimulated by insulin or palmitate (Table 3). By comparing the incorporation of the 6th carbon into CO_2 and fatty acids, one can estimate the relative rate of the Krebs cycle and fatty acid synthesis (Table 4). Insulin channels acetyl-CoA in the direction of lipogenesis, thus restraining its utilization by the Krebs cycle. The action of palmitate, though smaller, acts in the same way, and accelerates the synthesis of fatty acids more than that of the Krebs cycle. The percentage of glucose utilization by the pentose cycle was calculated from the incorporation of the first and sixth carbons of glucose into CO_2 and fatty acids, following the methods of KATZ et al. [36], and it appeared to be unmodified or slightly raised by the presence of palmitate. This is in agreement with the above mentioned stimulation of the lipogenetic pathway.

In other papers a particualr importance was given to citrate, the level of which influences the activity of several important enzymes of glucose metabolism. In the perfused heart or in the diaphragm incubated in a medium rich in fatty acids, GARLAND and RANDLE [26] have measured an increase of the citrate level, caused by an excess of acetyl-CoA due to a fast oxidation of fatty acids of the medium or of the perfusate. By the inhibition of phosphofructokinase, citrate might contribute in the muscle to the slowing down of glycolysis observed in the presence of FFA [26].

In the normal adipose tissue incubated in the presence of glucose, the oxidation of exogenous fatty acids is of little importance [5, 60] and would be unlikely to furnish a sufficient source of acetyl-CoA and citrate. DENTON et al. [12] have observed an increase in the citrate level in adipose tissue under the influence of adrenaline or alloxan diabetes. Moreover, even if it were demonstrated that citrate accelerates *in vitro* the acetyl-CoA-carboxylase of the liver [48] and of the adipose tissue [46], citrate is also an inhibitor of the phosphofructokinase extracted from adipose tissue [13]; and these two effects would have an inverse relationship in the neosynthesis of fatty acids in this tissue. The physiological effect of citrate thus becomes difficult to establish.

In normal adipose tissue, practically all the FFA taken up from the medium are esterified [60], and the degree of esterification is related to the concentration of FFA in the incubation medium [5].

Before esterification the FFA must be activated by CoA to give acyl-CoA, and this process needs energy in the form of ATP. The source of ATP is in cellular respiration by oxidative phosphorylation of reduced coenzymes, at the mitochondrial level. BALL and JUNGAS [2] have established experimentally a proportional relation between the quantity of fatty acid esterified and the oxygen consumed by adipose tissue.

metabolism by the rat epididymal fat tissue

to those mentioned in Table 2.

Gl-Glycerol		Glycogen	
1-^{14}C-glucose	6-^{14}C-glucose	1-^{14}C-glucose	6-^{14}C-glucose
0.798 ± 0.043	0.776 ± 0.053	0.076 ± 0.009	0.076 ± 0.008
1.09 ± 0.06	1.37 ± 0.21	1.32 ± 0.15	1.36 ± 0.14
0.966 ± 0.056^{c}	1.16 ± 0.07^{b}	0.076 ± 0.012 n.s.	0.080 ± 0.010 n.s.
1.58 ± 0.10^{b}	2.21 ± 0.05^{b}	1.63 ± 0.13 n.s.	1.50 ± 0.15 n.s.
1.29 ± 0.17	1.45 ± 0.09	0.068 ± 0.027	0.061 ± 0.013
1.37 ± 0.07	1.82 ± 0.07	0.863 ± 0.014	0.865 ± 0.115
2.000 ± 0.09^{b}	1.96 ± 0.12^{c}	0.106 ± 0.045 n.s.	0.111 ± 0.025 n.s.
2.58 ± 0.16^{a}	2.66 ± 0.20^{b}	1.012 ± 0.09 n.s.	1.09 ± 0.22 n.s.
0.913 ± 0.057	1.04 ± 0.09	0.073 ± 0.003	0.065 ± 0.016
1.07 ± 0.05	1.32 ± 0.04	0.854 ± 0.084	0.899 ± 0.071
1.49 ± 0.11^{a}	1.42 ± 0.03^{b}	0.076 ± 0.004 n.s.	0.081 ± 0.011 n.s.
1.63 ± 0.03^{a}	2.035 ± 0.05^{a}	1.09 ± 0.06 n.s.	1.07 ± 0.08 n.s.

What is the origin of the reduced coenzymes, the oxidation of which furnishes energy ? The oxidation of fatty acids, being low in normal adipose tissue, cannot constitute, as in muscle, an important source of reduced coenzymes. During the reactions which lead to the synthesis of fatty acids from glucose, the adipose tissue produces and consumes reduced coenzymes. FLATT and BALL [21] have calculated the balance, and have shown that adipose tissue produces an excess of reduced coenzymes, the quantity of which corresponds very well to the uptake of oxygen measured during this process. These authors then put forward the hypothesis that "one controlling factor in the synthesis of fatty acids from glucose may be . . . the rate at which high energy phosphate is needed by the cell."

In the light of this hypothesis, the effect on glucose metabolism produced *in vitro* by the FFA, becomes clearer. In this particular case, the adipose cell uses energy while it esterifies the fatty acid of the medium. The energy used by this process of esterification brings about an acceleration of glucose metabolism, in particular in the direction of fatty acid synthesis, thus producing an increase in the production of reduced coenzymes. The excess, not used in reduction reactions, is oxidized by molecular O_2, thus restoring the ATP reserves.

In conclusion the results of this study have shown that *in vitro*, the FFA do not diminish the utilization of glucose by adipose tissue, and, in particular, exercise no inhibiting action on the synthesis of fatty acids from glucose. It can be shown, on the contrary, that there is a certain stimulation of glucose metabolism by the FFA, explicable on an energetic basis.

These observations do not exclude the hypothesis of an inhibition of lipogenesis by high levels of intracellular FFA [43 and 44], even though this effect has not yet been directly demonstrated. Measurements of FFA, which are yet to be published, allowed us to establish the absence of a relation between the levels of FFA in the tissue and their concentration in the medium.

The interpretation of the results of this study on a physiological level, leads to the idea that, in all cases where the serum levels of FFA have been increased *in vivo* by an exterior process without a direct influence on lipolysis (infusion of an emulsion of triglycerides [18], ingestion of a meal rich in fat followed by an injection of heparin [59, 64]) the adipose tissue more than likely plays no part in the decrease of peripheral utilization of glucose.

In experimental situations where the serum FFA levels are acutely increased by infusions of lipolytic hormone, such as growth hormone or norepinephrine [53, 31, 63, 50], the abnormal evolution of the glycaemia does not seem to have any relation to a diminished utilization of glucose by the adipose tissue. Indeed, the uptake of glucose *in vitro* is increased by growth hormone [34, 42], and by adrenaline [10, 67] which accelerates particularly lipogenesis [21]. The tissular levels of FFA, elevated in the presence of adrenaline alone, are rapidly reduced to normal values by glucose and insulin together [35]. A decrease in glucose tolerance simultaneous with an increased serum level of FFA, is also observed during fasting, during deprivation of carbohydrates [45, 14, 30], and in the alloxan diabetic state; and, in these conditions, the utilization of glucose *in vitro* by adipose tissue is seriously diminished [57, 38, 42, 71, 33].

The cause of this metabolic inhibition may not be in direct relation with elevated FFA, levels of tissue but could be looked for in metabolic adaptation mechanisms of a more complex nature, probably related to insulin insufficiency [32], which could bring about under these conditions a decrease in the activity of certain important enzymes for the utilization of glucose [52, 3, 72, 29, 40].

Table 4. *Influence of palmitate on the percentage utilization of acetyl-CoA in the Krebs cycle and the fatty acid synthesis calculated from results of Table 3*

100% = carbon incorporated in CO_2 + carbon incorporated in fatty acids.
means ± S.E.M.

Added to buffer	Percentage utilization of acetyl-CoA by	
	Krebs cycle	fatty acid synthesis
	28.90 ± 0.370 (6)[a]	71.08 ± 0.360 (6)
insulin	8.35 ± 0.158 (9)	91.63 ± 0.158
palmitate (850 μval/l)	25.13 ± 0.451 (9)[b]	74.85 ± 0.444[b]
insulin + palmitate (id.)	5.72 ± 0.150 (9)[b]	94.27 ± 0.159[b]
	31.62 ± 0.597 (9)	68.37 ± 0.599 (9)
insulin	9.00 ± 0.180 (9)	91.00 ± 0.180 (9)
palmitate (748 μval/l)	26.47 ± 0.480 (9)[b]	73.5 ± 0.471 (9)[b]
insulin + palmitate (id.)	7.65 ± 0.130 (9)[b]	92.34 ± 0.126 (9)[b]

[a] number of values (see text)
[b] $p < 0.001$

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