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THE UTILIZATION OF SOME FOR LIPID SYNTHESIS IN ADIPOSE TISSUE ISOLATED FROM MATURE PIGS

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บทคัดย่อ

๑. ได้ศึกษาการเปลี่ยนแปลงของ radioactive substrates ได้แก่ U–C^{14} – glucose 2 – C^{14} – pyruvate 1, 5–C^{14} – citrate และ 2–C^{14} -- lactate พร้อมทั้งการ incorporation ของ 1–C^{14} – palmitate และ 2–C^{14} – linoleate ในไขมันของสุกร

๒. ผลของการทดลองแสดงให้เห็นว่า glucose, pyruvate และ lactate ถูกเปลี่ยนไปเป็น lipid ได้ดีกว่า citrate และ acetate การ incorporation ของ substrate ทุกชนิดที่ใช้ในการทดลองเพิ่มขึ้นเมื่อมี glucose อยู่ด้วย อินซูลินเพิ่มการ incorporation ของกรดไขมันอิสระเมื่อมี glucose อยู่ด้วย และมากขึ้นกว่าเมื่อทดลองโดยใช้ glucose เพียงอย่างเดียว

๓. Activity ส่วนใหญ่พบใน triglyceride นอกจากในกรณีของ pyruvate และ linoleate ซึ่งมี activity เท่ากัน พบได้ทั้งใน triglyceride และ non–esterified fatty acid.

INTRODUCTION

Numerous investigations in recent years have been concerned with the metabolism of adipose tissue, especially in experimental animals, as reviewed by Vaughan (1961) and Farvager (1965). The domestic pig has a great capacity to deposit fat in the body (Durham 1966). In pig adipose tissue it has been found that glucose and acetate were readily converted to lipids, but glucose was the chief precursor of glyceride-glycerol (O'Her & Leveille 1968). Subcutaneous adipose tissue appears to have a superior lipogenic capacity to that of mesenteric fat (O'Hea & Leveille 1968). O'Hea and Leveille (1969) also showed that the utilization of various substrates (acetate, pyruvate, lactate, glucose and propionate) for lipid synthesis was variable. Kopelovich & McGrawth (1970) found that glucose significantly increased the incorporation of acetate and lactate into fatty acids, and insulin stimulated the formation of C^{14} labelled fatty acids above levels observed when glucose alone was present. Bally et al. (1960) showed that insulin and glucose increased the incorporation and markedly depressed oxidation of 1-C^{14} -palmitate in rat adipose tissue. Esterification of fatty acids in rat adipose tissue was stimulated by glucose and insulin (Cahill 1963). The influence of glucose and insulin on triglyceride incorporation has also been demonstrated by Schwarz & Botterman (1963). The following experiments were performed to see to what extent

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adipose tissue from mature pigs utilized glucose, acetate, pyruvate, citrate, and lactate for lipid synthesis and to see the influence of glucose and insulin on the esterification of palmitate and linoleate.

MATERIALS AND METHODS.

Castrated pigs of the Danish Landrace breed at about 110-120 kg. live weight, fed ad libitum with pelleted feed containing 12% true protein were used in the experiment. Biopsies were taken from the backfat in the morning about 2 hours after feeding, sliced into thin pieces and incubated with various substrates at 37° C. under 95% O_2 and 5% CO_2 in the metabolic shaker (70 strokes per min.) for 2 hours. The incubation medium consisted of Krebs-Ringer buffer pH. 7.4 ($CaCl_2$ omitted). About 3 uci of radioactive substrates (U-C^{14} glucose, 1-C^{14} - acetate, 2-C^{14} - pyruvate, 1.5-C^{14} - citrate, 2-C^{14} - lactate, 1-C^{14} - palmitate, or 1-C^{14} - linoleate). About 5 umoles of their inactive substrates in the form of sodium salts were added. In cases where the influence of glucose upon the conversion of substrates were investigated, 25 umoles of glucose were added. The sodium salts of fatty acids swere prepared in the form of albumin complex. The 1-C^{14} -palmitate albumin complex was prepared as described by Wlodawwer & Boguslawska (1968), but ethanol was not added to dissolve the acid. The total lipids were extracted with chloroform : methanol (2 : 1) as described by Folch et al. (1957). Phosphate buffer pH 6 was added in order to obtain quantitative extraction of NEFA as outlined by Riis (1968). About 10 mg. of carrier substrates were added to the extraction system to prevent contamination of the extracted lipids with radioactive substrates. The percentages of total lipids were measured by gravimetric method. After the gravimetric determination, about 40 mg. of total lipids were dissolved in 10 ml. of 0.5% PPO scintillator in toluol and left in drakness overnight for counting. The separation of total lipids was carried out by means of TLC using the solvent system of petroleum ether:diethyl ether:acetic acid (82:18:1). The other portions of total lipids (ca: 30 mg.) were methylated as described by Appelguist (1968). The methyl esters were cleaned from contaminants by means of TLC using a solvent systems of petroleum ether:diethyl ether : acetic acid in the proportion 90 : 10 : 1 as described by Appelquist (1967). Half of the methyl esters was used for determination of radioactivity. The efficiency of the counting was determined for each sample by means of an internal standard of 1-C^{14} -palmitate in 100 ul toluene The radioactivity measurements in this experiment were performed on an automatic Beckman LS-100 liquid scintillation counter.

The rest of the methyl esters was kept for determination of the specific activity of the individual fatty acids by means of radiogaschromatography. However, the results from these determinations will not be included in this paper.

RESULTS

Table 1 summarizes the result showing the incorporation of U-C^{14} - glucose, I1-C^{14} -acetate, 2-C^{14} -pyruvate, 1.5-C^{14} -citrate, and 2-C^{14} -lactate into the adipose tissue when these substrates were incubated alone in the presence of glucose. The enhancement of glucose on the incorporation of individual substrates can be observed. The in- corporation of citrate is relatively low among the substrates used.

Table 2 shows the activities of methyl esters of fatty acids which were separated and cleaned by thin-layer chromatography. The activities found in the esters are mostly between 50 and 60 per cent of the activities found in total lipids. In Table 3 the results showing the incorporation of 1-C^{14} palmitate and 1-C^{14} -linoleate are summarized. It can be seen that addition of glucose and insulin increased the incorporation of the fatty acids. However, the statistical analysis to test the relative significant differences between the incorporation of substrates with and without addition of glucose and insulin failed except in the cases of acetate and

palmitate. The radioactivities of the different fractions obtained by thin-layer chromatography of total lipids are shown in Tabe 4. Most of the total activity is found in the triglycerides (TG) fraction.

The activities in diglyceride (DG) and other fractions are relatively low except for pyruvate and palmitate in the absence of glucose where equal activities are found in TG and non-esterified fatty acid (NEFA) fractions. The activity from linoleate is predominant in NEFA and is not influence by glucose or glucose in the presence of insulin.

DISCUSSION:

The results obtained in Table 1 seem to indicate that glucose enhanced the incorporation of every precursor used into lipids. The increased incorporation of acetate, lactate, and pyruvate by glucose have been demonstrated by a number of investigators (Kopelovich & McGrawth, 1970; Schmidt & Katz, 1969; Halperin & Robinson, 1970). This effect indicates the significant increase in NADPH formation from HMP shunt by adipose tissue in vitro. It can be seen that glucose, lactate, and pyruvate are better incorporated into lipid than acetate and citrate. The low incorporation of acetate and high incorporation of glucose into lipids is probably due to a high content of glucose in the diet normally fed to pigs as discussed by O'Hea & Leveille (1969) and the low activity of acetylcoenzyme A carboxylase in adipose tissue (Hanson& baillard, 1967). The low conversion of citrate to lipids probably suggested that citrate may be utilized for other purposes.

Table 2 shows the activity of methyl esters obtained from different substrates. The activities found in methyl esters are about 50% of activities found in total lipids. Therefore, about 50% of substrates incorporated were converted to fatty acids.

Table 3 shows the influence of glucose and glucose plus insulin upon the incorporation of palmitate and linoleate into adipose tissue. Influences of glucose and insulin upon fatty acid incorporation have been reported by Cahill (1963) and Bally et al. (1960). The results reported here seem to indicate a slightly increased incorporation of these two acids. Furthermore, it is interesting to see the incorporation of individual substrate into different lipids, especially the glyceride fractions.

Table 4 shows the incorporation of activity into different fractions of total lipids after separation by thin-layer chromatography. Activities from glucose, acetate, lactate citrate, and palmitate are mostly found in TG fraction,whereas the activities from pyruvate and palmitate in the absence of glucose are found equally in TG and NEFA. Activity from linoleate is predominant in NEFA. This is very likely because glucose is essential for the esterification of pyruvate and palmitate but has no effect on linoleate. It is well known that palmitate is a major constituent in pig adipose tissue. This evidence may probably show that the esterification of palmitate is higher than linoleate in pig adipose tissue.

SUMMARY

1. The conversion of U-C^{14} glucose, 1-C^{14} -acetate, 2-C^{14} pyruvate, 1,5-C^{14} -citrate, and 2-C^{14} lactate and the incorporation of 1-C^{14} palmitate and 2-C^{14} linoleate in pig adipose tissue was investigated.

2. The results show that glucose, pyruvate, and lactate were better incorporated into lipids than citrate and acetate, and the incorporation of every substrate used was enhanced by glucose. Insulin, in the presence of glucose, slightly increased incorporation of fatty acids above levels obserbed when glucose alone was added.

3. Most activities incorporated were found in triglyceride except in cases of pyruvate and linoleate where equal activities were found in triglyceride and non-esterified fatty acid fractions.

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Table 1. Conversion of different substartes into total lipid by adipose tissue.

Substrate	Glucose	mg Insulin	mg substrate converted to lipids			X	S	Sd	t
			NI	N II	N III				
Glucose		—	0.306	0.703	0.648	0.552	0.202		
Acetate	—	—	0.040	0.080	0.049	0.056	0.021	0.013	3.985 X)
Acetate	+	—	0.118	0.109	0.099	0.109	0.010		
Pyruvate	—	—	0.091	0.105	0.016	0.070	0.048	0.076	1.352
Pyruvate	+	—	0.208	0.274	0.036	0.173	0.123		
Citrate	—	—	0.013	0.015	0.015	0.014	—	—	—
Citrate	+	—	0.016	0.016	0.019	0.017	—		
Lactate	—	—	0.238	0.212	0.100	0.183	0.073	0.046	1.790
Lactate	+	—	0.274	0.292	0.228	0.265	0.030		

X) significant difference at 5% level ($t > 2.776$)

Table 2. Conversion of different substrates into methylesters.

Substrate	Glucose	mg substrate converted to methylester			X	S	Sd	t	% activity of total lipid
		N I	N I						
Glucose		0.198	0.376	0.287	0.126				57
Acetate	—	0.022	0.054	0.038	0.029	0.022	0.991		63
Acetate	+	0.052	0.068	0.060	0.013				53
Pyruvate	—	0.050	0.064	0.057	0.010	0.037	2.207		58
Pyruvate	+	0.102	0.174	0.138	0.051				57
Citrate	—	0.006	0.010	0.008	—	0.011	1.000		57
Citrate	+	0.008	0.003	0.006	0.016				38
Lactate	—	0.142	0.134	0.138	0.006	0.014	2.132		50
Lectate	+	0.154	0.181	0.167	0.018				59

Table 3. The incorporation of palmitate and linoleate into lipids.

Substrate	Glucose	Insulin (mU)	mg substrate incorporated to lipids			X	S	Sd	t
			N I	N II	N III				
Palmitate	—	—	0.238	0.222	0.222	0.227	0.009	0.030	3.153 X)
,,	+	—	0.358	0.339	0.263	0.320	0.050	0.060	0.828
,,	+	+	0.471	0.355	0.283	0.370	0.095		
Linoleate	—	500	7.131	5.369	6.459	6.332	0.892	0.544	1.156
,,	+	—	7.114	6.609	7.160	6.961	0.306	0.224	0.305
,,	+	500	7.633	7.981	6.542	7.385	0.238		

X) Significant difference at 5% level ($t > 2.776$)

Table 4. The incorporation of activity into the individual fractions of total lipids (% of total activity) (Results obtained from 2 pigs).

Substrate	Glucose	Insulin	TG	DG	NEFA	PL+MG	Cholesterol.
Glucose		—	76.2 82.3	17.2 13.2	0.7 0.2	2.3 2.6	2.4 2.7
Acetate	—	—	75.8 85.1	12.4 5.7	9.2 1.6	1.6 2.3	0.9 5.7
Acetate	+	—	79.2 78.0	15.6 17.2	— 1.0	1.5 2.4	3.6 1.4
Pyruvate	—	—	46.1 45.3	8.9 9.1	41.9 41.2	2.5 4.0	0.6 0.5
Pyruvate	+	—	78.3 72.3	17.0 17.9	0.7 3.1	1.8 2.9	2.2 3.9
Citrate	—	—	100.0 92.9	— 7.1	— —	— —	— —
Citrate	+	—	94.7 87.5	5.3 12.5	— —	— —	— —
Lactate	—	—	79.6 77.5	13.5 15.7	1.6 1.6	3.3 3.6	2.0 1.6
Lactate	+	—	80.5 75.5	15.1 15.3	0.8 2.4	2.1 3.2	2.0 3.6
Palmitate	—	—	48.0 51.9	2.1 8.2	39.6 34.0	2.1 2.1	7.9 3.8
Palmitate	+	—	74.5 71.7	3.0 15.5	6.8 4.8	1.4 1.3	14.1 7.7
Palmitate	+	+	74.6 70.7	3.1 15.1	3.1 6.2	1.5 1.2	17.6 6.8
Linoleate	—	—	2.5 2.1	10.1 7.8	72.9 68.6	5.5 6.3	8.9 15.1
Linoleate	+	—	5.6 6.9	14.0 10.9	64.7 65.0	8.0 5.9	8.6 11.2
Linoleate	+	+	8.7 8.4	12.4 17.5	59.7 49.7	8.1 11.1	11.0 13.7

รังสีวินิจฉัย: pyometra

The terms " atypical mycobacteria " .....