THE SYNTHESIS OF FATTY ACIDS AND GLYCOGEN BY ADIPOSE TISSUE *IN VITRO*

by

G. ROSE AND B. SHAPIRO

Department of Biochemistry, Hebrew University Hadassah Medical School, Jerusalem (Israel)

After depletion of the fat stores in adipose tissue by a prolonged fast, a rapid accumulation of fat starts 1–2 days after refeeding. Generally, fat accumulation is preceded by a deposition of glycogen[1], which disappears when fat formation is at its maximum. The glycogen-containing tissue exhibits a respiratory quotient exceeding 1, as against a quotient of 0.6–0.7 in the tissue of starved animals[2]. Hence it was suggested by WERTHEIMER that glycogen serves as a precursor of fat synthesis in adipose tissue[3].

There exists conclusive evidence that a considerable part of the fat accumulated in adipose tissue is synthesised within the tissue itself, and is not merely deposited there after being produced by other organs. SHAPIRO AND WERTHEIMER[4] carried out experiments, in which adipose tissue was incubated *in vitro* in deuterium oxide enriched serum. Fatty acids isolated from the incubated tissue were found to contain an excess of stably bound deuterium. Recently it was shown by several authors that adipose tissue is capable of introducing ^{14}C-labelled acetate and glucose into long chain fatty acids *in vitro*[5–8]. In our previous publication[5] we reported that, similar to liver[9], the synthetic capacity of adipose tissue is markedly diminished by starvation and can be renewed by a short period of refeeding.

In the present communication, experiments are described, which were undertaken in order to study the factors involved in fatty acid synthesis in adipose tissue and the "block" caused by starvation. For this purpose the medium used in our previous experiments, *i.e. serum*, had to be exchanged for a better defined medium. The choice for these experiments was a serum albumin solution in isotonic buffers.

EXPERIMENTAL

Male albino rats of local strain (attaining a mature weight of 250 g in 6–7 months) weighing between 100 and 110 g, were fasted for 5–6 days to approximately 30–35% weight loss, and divided into two groups, (1) refed for 24 h (2) not refed. The animals were killed under sodium pentobarbital anaesthesia and subsequent bleeding by cutting the abdominal aorta and vena cava simultaneously. The mesenteria were removed, washed in ice-cold physiological saline, cleaned quickly of blood, muscle, and adjoining tissue, weighed, and incubated with constant shaking in a waterbath at 37° C for 3½ h. Mesenterium was chosen throughout these experiments since it represents a fairly thin, uniform layer of adipose tissue. The incubation was carried out in specially constructed flasks of about 10 ml volume fitted with an aeration device. The incubating medium consisted of a 5% solution of serum albumin in Krebs-Ringer-phosphate, enriched with appropriate amounts of radioactive precursors. The latter constituted in the separate experiments

References p. 510.

uniformly labelled glucose, 2-^{14}C labelled pyruvate, (Na salt) and 1-^{14}C labelled acetate (Na salt) obtained from the Radio-chemical Centre, Amersham, England.

After the incubation, a stream of CO_2-free air was passed through the incubation mixture to carry the radioactive $^{14}CO_2$ into a suction tube containing 3 ml of a 1 % solution of $Ba(OH)_2$. To free "adhering" $^{14}CO_2$ from the tissue and the medium the reaction mixtures were acidified by adding a drop of N/1 H_2SO_4. The radioactive $Ba^{14}CO_3$ was filtered onto a filter paper disc by means of the Tracerlab E.19 precipitation apparatus and analysed for its ^{14}C content.

The tissues were washed free of the radioactive medium and saponified by boiling with ½ ml of 60 % solution of KOH for 3 hours. Long-chain fatty acids and glycogen were isolated by the procedures described previously[5]. Utilisation of the substrate by the tissue was measured by the determination of radioactivity and respective substrate content of the medium before and after the incubation. Glucose was measured according to SOMOGYI[10], acetate by micro-titration with NaOH after acidification with H_2SO_4 and steam distillation. Pyruvate was measured according to FRIEDEMAN AND HAUGEN[11].

All determinations of radioactivity were made as described previously[5].

RESULTS

As can be seen from the results reported in Table I, fatty acid synthesis was markedly depressed by prolonged fasting, but only a short period of refeeding (24 h) sufficed to re-establish the synthetic capacity of the tissue. The amount of fat present in the tissue was of importance since fatty acid synthesis was found to be greatly diminished in very fat tissues. Differences in the fat content of the mesenteria of our animals, despite standard conditions of fasting and refeeding, caused considerable deviations in our results.

TABLE I

THE EFFECTS OF STARVATION AND REFEEDING ON THE SYNTHESISING ACTIVITY OF ADIPOSE TISSUE

	Substrate utilisation		*$^{14}CO_2$ formation*		*Fatty acid synthesis*		*Glycogen formation*	
	mg/ml	*No. of expts.*	*% initial count*	*No. of expts.*	*% initial count*	*No. of expts.*	*% initial count*	*No. of expts.*
a. Uniformly labelled glucose as the precursor: (Initial concentration 1.5–5 mg/ml)								
Starved/ refed rats	0.43	8	8.43	8	2.42	7	1.53	11
Starved rats	0.28	6	0.99	5	0.16	4	6.81	7
b. 1-^{14}C-labelled acetate as the precursor: (Initial concentration 1.5–2 mg/ml)								
Starved/ refed rats	negligible	6	2.28	16	0.81	14		
Starved rats	negligible	4	2.61	5	negligible	5		
c. 2-^{14}C-labelled pyruvate as the precursor: (Initial concentration 1 mg/ml)								
Starved/ refed rats	0.77	7	14.90	6	6.09	6	negligible	
Starved rats	0.79	4	12.60	4	1.38	4	negligible	

Results are expressed as the mean of the mentioned number of experiments calculated per 100 mg fresh tissue.

Synthesis of fatty acids from labelled glucose, acetate, and pyruvate

Fatty acid synthesis in mesenteric adipose tissue occurred with all three of the precursors, and was greatly increased in the refed group of rats as compared to the starved group. Synthesis was found to be highest with pyruvate as the substrate,

References p. 510.

followed by glucose, and was much lower with acetate. With the latter as the precursor and no additional substrate the amount of fatty acids synthesised by the tissues of the starved rats was found to be negligible.

$^{14}CO_2$ formation

As shown by the results reported in Table I, appreciable amount of ^{14}C-carbon was incorporated into the respired CO_2 by the tissues of both groups of rats. No significant difference between the two groups in $^{14}CO_2$ production occurred with labelled acetate or pyruvate as the precursor. With labelled glucose, $^{14}CO_2$ production in the starved rats was depressed to very low values.

Glycogen synthesis

Glycogen was found to be synthesised from uniformly labelled glucose by mesenteric adipose tissue of both starved/refed and starved rats. The amount of ^{14}C-carbon incorporated into glycogen was found to be even greater in the starved group. However experiments repeated later on homogenates of mesenteric fat tissue with radioglucose enriched medium showed little or no difference in synthesis of glycogen between the two groups of rats.

The glycogen synthesis in the experiments employing radio-pyruvate as the precursor was negligible in all cases.

Utilisation of substrates

(1) Experiments with radioacetate:

No significant changes in substrate concentration were found in the medium before and after the incubation. There was small decrease in radioactivity in the medium, which could be accounted for by the appearance of ^{14}C-carbon in respiratory CO_2 and synthesised fatty acids.

(2) Experiments with radioglucose:

As illustrated in Table I, glucose utilisation by the tissues of the starved rats was lower by about one-third than the glucose utilisation in the starved/refed rats. It was observed that the decrease of radioactivity in the medium was much less than the actual glucose utilisation, pointing to the conversion of glucose to some water-soluble metabolite.

(3) Experiments with radiopyruvate:

An appreciable degree of pyruvate utilisation was observed in these experiments which appeared to be the same for the starved, as well as for the starved/refed group of rats. The amount of pyruvate utilised was about twice the amount of radioactivity which disappeared from the medium, indicating a conversion of pyruvate to some radioactive metabolite, as in the case of glucose.

Effect of additional substrates

In order to overcome the "block" in fatty acid synthesis in starved animals, the effect of additional non-labelled substrates to the radioactive acetate-enriched incubation medium was tested. The results of these tests are summarised in Table II. Of the substances tested only glucose gave a positive result, raising the amount of fatty acid synthesised from the isotopic precursor by the tissue of starved rats from a negligible to a significant value. Tissue from refed rats showed no significant

References p. 510.

increase in fatty acid synthesis under these conditions. α-Ketoglutarate had no effect whatsoever on either the formation of $^{14}CO_2$ or fatty acid synthesis. Sodium succinate, shown by FELLER[6] and FARVAGER AND GERLACH[8] to increase ^{14}C-incorporation into fatty acids, synthesised by adipose tissue slices in the presence of glucose, showed no stimulating effect in our experiments, when tested in the absence of additional glucose.

TABLE II

THE EFFECT OF ADDITIONAL SUBSTRATES ON $^{14}CO_2$ PRODUCTION AND FATTY ACID SYNTHESIS IN ADIPOSE TISSUE

Labelled substrate (0.015 M)	*Unlabelled substrate (0.01 M)*	*% ^{14}C-recovered in*	
		Respiratory CO_2	*Fatty acids (per 100 mg fresh tissue)*
	Starved rats		
1-^{14}C Acetate	None	1.70	negligible
	Glucose	1.07	0.62
	None	1.52	negligible
	Glucose	2.82	0.33
	None	1.77	negligible
	α-Ketoglutarate	2.04	negligible
	None	3.83	negligible
	α-Ketoglutarate	2.39	negligible
	None	1.51	negligible
	Succinate	2.49	negligible
	Starved/refed rats		
1-^{14}C Acetate	None	1.18	0.59
	Glucose	1.47	0.91
	None	1.42	negligible
	Glucose	1.14	0.23
	None	1.53	0.31
	α-Ketoglutarate	1.59	0.26
	None	2.18	negligible
	α-Ketoglutarate	1.44	negligible
	None	0.85	0.86
	Succinate	0.73	0.21
	None	8.45	1.98
	Succinate	7.02	1.63

Each incubation flask contained two halves of mesenterium of a pair of rats, one containing additional nonlabelled substrate, the other an equivalent volume of isotonic saline. Total volume 1.0 ml.

One of the characteristic differences between the starved and starved/refed rats is the appearance of glycogen inside the adipose tissue cell in the latter group. Yet glycogen when added to the incubation medium containing starved rat mesenterium did not enhance fat synthesis, probably because of its large molecular size, which would make penetration into the fat cell impossible. Experiments with homogenates of adipose tissue were not conclusive since upon homogenisation, the fat synthesising capacity was completely lost.

Effect of malonate

Malonate, shown by POPJAK AND TIETZ[12] to have a stimulating effect on fatty acid synthesis by cell-free extracts of rat mammary gland, was added to the incu-

References p. 510.

bating medium, and the results are summarised in Table III. Addition of malonate was found to decrease the ^{14}C-incorporation into respiratory CO_2 in both groups of rats, with pyruvate as well as with acetate as the labelled precursor. Fat synthesis was correspondingly depressed in both groups in the pyruvate experiments, but slightly increased in the acetate experiments with refed rats.

TABLE III

THE EFFECT OF MALONATE ON THE $^{14}CO_2$ PRODUCTION AND FATTY ACID SYNTHESIS IN ADIPOSE TISSUE

Precursor	*Condition of rat*	*Malonate (0.05 M)*	*% ^{14}C-incorporation into*	
			CO_2	*Fatty acids*
2-^{14}C pyruvate (0.01 *M*)	starved		15.3	2.59
	starved	+	11.3	1.82
	starved	—	16.9	2.60
	starved	+	6.24	1.24
	refed	—	14.8	11.85
	refed	+	6.52	9.68
	refed	—	21.85	3.99
	refed	+	14.17	2.87
	refed	—	6.69	5.61
	refed	+	8.38	13.15
1-^{14}C acetate (0.02 *M*)	refed	—	0.52	0.17
	refed	+	0.23	0.25
	refed	—	1.25	0.49
	refed	+	0.43	0.86
	refed	—	1.4	0.88
	refed	+	0.2	1.36

Conditions as in Table II.

DISCUSSION

Results presented in this paper demonstrate the comparatively high activity of adipose tissue in fatty acid synthesis. Degradation of the synthesised fatty acids, as described in our previous publication[5], showed, that the ^{14}C-carbon is distributed throughout the fatty acid molecule, and not only confined to the carboxyl carbon, indicating clearly that a complete new synthesis occurred. The rate of synthesis reported in this paper is lower than that found in our previous experiments, where blood serum served as the incubating medium. The activating effect of serum cannot be attributed to the glucose content, since addition of glucose to our Ringer solutions did not increase the activity, except in tissues from starved animals when tested with radioacetate. The effect of serum may be related to its stabilising effect on the oxygen consumption of the tissue, as was found by MIRSKI[2].

From the present and previous measurements it has been calculated that, with glucose as the precursor, adipose tissue is capable of synthesising approximately 1% of its fresh weight of fatty acids per day. Since this figure may not represent optimal conditions, (as much higher rates of synthesis were reported by HAUSBERGER *et al.*[7] in insulin-treated animals) it may be taken as proved, that adipose tissue synthesises a considerable portion of the fat accumulated in it.

The relative activity of the three substrates used as precursors of the synthesised fatty acids was similar to that found by HIRSCH, BARUCH AND CHAIKOFF[13] in mam-

References p. 510.

mary tissue and quite different from that found in liver[9]. Glucose and pyruvate served as much more efficient precursors than acetate. The production of $^{14}CO_2$ from radioacetate was slightly higher than that reported by FELLER[6], but did not exceed 3% of the counts introduced, or about 40 micrograms of acetate converted into CO_2. Since no net uptake of this substrate could be detected, the introduction of ^{14}C-carbon from acetate into CO_2 and fatty acids may be due to an exchange reaction, without any net conversion. With glucose and pyruvate however, the net uptake from the medium was very high and markedly exceeded the disappearance of radioactivity. A radioactive metabolite of these precursors must have accumulated in the medium. The high rate of conversion of 2-^{14}C-pyruvate into $^{14}CO_2$ points to a considerable activity of the enzymes concerned with the metabolism of the 2-carbon unit, presumably the tricarboxylic cycle.

The depressed synthesis of fatty acids after starvation occurred with all three of the precursors tested. With radioacetate, the addition of glucose caused a partial reactivation. The results point to a conclusion similar to that arrived at by HIRSCH *et al.*[13] in their work on mammary tissue. A certain type and rate of glucose metabolism seem to be necessary in order to induce fatty acid synthesis from its precursors. This is evident from the fact that both $^{14}CO_2$ production and the utilization of pyruvate and acetate were not significantly affected by starvation. With glucose, however, utilization was decreased by 30% and $^{14}CO_2$ production by 90%. The block in glucose metabolism in starved rat adipose tissue is not located at the hexokinase step, since the overall utilization is decreased to a much lower extent than the CO_2 production.

This conclusion is corroborated by our finding that the incorporation of ^{14}C-carbon from glucose into glycogen is not diminished by starvation. This would mean that the block in carbo-hydrate metabolism is situated beyond the glucose-phosphate step, or that the pathway of glucose metabolism is changed during starvation and diverted to a metabolism which does not facilitate fat synthesis. These findings may also serve as an explanation for the repeatedly confirmed fact that glycogen accumulates in adipose tissue only after a period of starvation followed by refeeding[14], and disappears gradually when feeding is continued. In the "starved" tissue, complete combustion to CO_2 and conversion to fatty acids is blocked, and precursors of glycogen, *i.e.* glucose phosphates, may accumulate, giving rise to an increased net glycogen production. When feeding is continued, the block is removed and glycogen disappears.

SUMMARY

The incorporation of 1-^{14}C-labelled acetate, pyruvate-2-^{14}C, and uniformly ^{14}C-labelled glucose into fatty acids, CO_2, and glycogen by mesenteric adipose tissue was studied. Fat-depleted tissue, obtained after prolonged starvation, was compared with tissue obtained after refeeding for 24 hours.

"Refed" tissue showed a high activity in fatty acid synthesis with radiopyruvate and radioglucose as precursors and a considerably lower one with radioacetate. With "starved" tissue, fatty acid synthesis was depressed to 1/15th–1/6th its value with all three precursors.

Addition of non-labelled glucose partially reactivated fatty acid synthesis from acetate in "starved" tissue, but had no effect with the other two precursors, as well as with "refed" tissue. Succinate and α-ketoglutarate were without effect.

No net utilization of acetate was detected. Pyruvate utilization was rapid and was equal in starved and refed tissue. Glucose utilisation was depressed by 30% in the "starved" tissue.

$^{14}CO_2$ production was not decreased by starvation when pyruvate or acetate served as substrates, but was inhibited by 90% with glucose.

Incorporation of ^{14}C-carbon of radioglucose into glycogen was found to be unaffected by starvation.

References p. 510.

RÉSUMÉ

Les auteurs ont étudié l'incorporation d'acétate 1-^{14}C, de pyruvate-2-^{14}C et de glucose uniformément marqué par ^{14}C dans les acides gras, le CO_2, et le glycogène par le tissu adipeux du mésentère. Le tissu privé de lipides, obtenu après jeûne prolongé, a été comparé avec le tissu obtenu après redistribution de nourriture pendant 24 h.

Le tissu "nourri de nouveau" synthétise des acides gras activement à partir du radiopyruvate et du radioglucose et beaucoup plus faiblement à partir du radioacétate. La synthèse des acides gras à partir des trois précurseurs par le tissu "à jeun" est réduite à 1/15ème-1/6ème de sa valeur.

L'addition de glucose non marqué réactive en partie la synthèse des acides gras à partir d'acétate dans le tissu "à jeun", mais n'a pas d'effet avec les deux autres précurseurs. Elle n'a pas d'effet non plus sur le tissu "nourri de nouveau". Le succinate et l'α-cétoglutarate sont sans action.

Aucune utilisation nette de l'acétate n'a été décelée. L'utilisation du pyruvate est rapide. Elle est la même dans le tissu "à jeun" et dans le tissu "nourri". L'utilisation du glucose est diminuée de 30% dans le tissu "à jeun".

La production de $^{14}CO_2$ n'est pas diminuée par le jeûne quand on prend comme substrat le pyruvate ou l'acétate, mais elle est inhibée à 90% quand le substrat est le glucose. L'incorporation du ^{14}C du radioglucose dans le glycogène n'est pas affectée par le jeûne.

ZUSAMMENFASSUNG

Die Einverleibung von 1-^{14}C markiertem Azetat, 2-^{14}C-Pyruvat und von gleichmässig mit ^{14}C markiertem Glukose in Fettsäuren, CO_2 und Glycogen durch mesenterische Fettgewebe wurde untersucht. Nach längerem Fasten erhaltenes entfettetes Gewebe wurde mit 24 Stunden nach erneuertem Füttern erhaltenem Gewebe verglichen.

Das letztere Gewebe zeigte mit radioaktivem Pyruvat und radioaktiver Glukose als Vorgänger eine hohe Aktivität der Fettsäuresynthese, während dieselbe mit radioaktivem Azetat bedeutend geringer war. Bei "fastenden" Geweben sank die Fettsäuresynthese mit allen 3 Vorgängern auf 1/15–1/6 ihres Wertes.

Durch Hinzufügung von unmarkierter Glukose wurde die Fettsäuresynthese aus Azetat in "fastenden Geweben" teilweise wiederhergestellt; es wurde jedoch dadurch keinerlei Wirkung mit den anderen zwei Vorgängern, sowie mit nach erneuertem Füttern erhaltenen Geweben erzielt. Succinat und α-Ketoglutarat waren wirkungslos.

Es konnte kein klar ersichtlicher Azetatverbrauch festgestellt werden. Pyruvat wurde schnell und in gleichem Masse durch von "fastenden" und neugefütterten Geweben verbraucht. Der Glukoseverbrauch sank in "fastendem" Gewebe um 30%.

Die $^{14}CO_2$-Erzeugung zeigte keine Verminderung durch Fasten, falls Pyruvat oder Azetat als Substrat benützt wurden; es wurde jedoch mit Glukose eine 90%-ige Hemmung festgestellt.

Fasten hatte keinen Einfluss auf die Einverleibung von ^{14}C-Kohlenstoff aus radioaktiver Glukose in Glycogen.

REFERENCES

1 E. TUERKISCHER AND E. WERTHEIMER, *J. Physiol.*, 104 (1946) 361.
2 A. MIRSKY, *Biochem. J.*, 36 (1942) 232.
3 E. WERTHEIMER, *J. Physiol.*, 103 (1945) 359.
4 B. SHAPIRO AND E. WERTHEIMER, *J. Biol. Chem.*, 173 (1948) 725.
5 G. ROSE, I. STERN AND B. SHAPIRO, *Acta Med. Orient.*, 12 (1953) 187.
6 D. D. FELLER, *J. Biol. Chem.*, 206 (1954) 171.
7 F. X. HAUSBERGER, S. M. MILSTEIN AND R. J. RUTMAN, *J. Biol. Chem.*, 208 (1954) 431.
8 P. FARVARGER AND J. GERLACH, *Helv. Physiol. et Pharmacol. Acta*, 12 (1954) C15.
9 E. J. MASORO, J. L. CHAIKOFF, S. S. CHERNICK AND J. M. FELTS, *J. Biol. Chem.*, 185 (1950) 845.
10 M. SOMOGYI, *J. Biol. Chem.*, 117 (1937) 771.
11 T. E. FRIEDEMANN AND G. E. HAUGEN, *J. Biol. Chem.*, 147 (1943) 415.
12 G. POPJAK AND A. TIETZ, *Biochem. J.*, 57 (1954) XIV.
13 P. F. HIRSCH, H. BARUCH AND J. L. CHAIKOFF, *J. Biol. Chem.*, 210 (1954) 785.
14 E. WERTHEIMER, *Pflügers Arch. ges. Physiol.*, 219 (1928) 190.

Received May 16th, 1955