Inverse Relationship between Adipose Tissue and Skeletal Muscle in the Uptake of Injected Triglyceride Fatty Acid of Chyle Lipoproteins by Diabetic Rats

Fasting rats for a period of time (compared to feeding rats glucose solutions) decreases the uptake of i.v. injected ^{14}C labeled triglyceride fatty acid (TGFA) of very low density chyle lipoproteins by adipose tissue and increases the oxidation of the fatty acid to expired CO_2[1,2]. Both the uptake and oxidation of ^{14}C labeled TGFA of low density lipoproteins by the perfused rabbit heart, on the other hand, are increased by a period of fasting[3]. The present investigation reveals an inverse relationship between adipose tissue and skeletal muscle in the removal of i.v. injected ^{14}C labeled TGFA of chyle lipoproteins from the circulation of alloxan diabetic rats. This study, in which it was found that depressing the utilization of glucose by insulin withdrawal markedly increased the proportion of the labeled fatty acid incorporated into skeletal muscle and decreased the proportion incorporated into adipose tissue while increasing the oxidation of the fatty acid to expired CO_2, focuses attention on the possible role of skeletal muscle in the utilization of dietary (chyle) TGFA.

Diabetes was induced in male Long–Evans rats (170–210 g) by i.v. injection (45 mg/kg) of recrystallized alloxan monohydrate (Eastman Kodak, Rochester, N.Y.) 3 weeks before the rats were used. Untreated chronic alloxan diabetic rats have little visible abdominal depot fat. All rats selected for study, therefore, were maintained on daily s.c. injections of 40 U/kg of protamine-zinc insulin (Eli Lilly & Co., Indianapolis, Ind.) for 8 days, during which time they were fed ad libitum a 60% glucose diet[4]. Thereafter the rats were separated into 2 groups. Each rat of one group continued to receive daily injections of the protamine-zinc insulin for 3 days and on the 12th day received 10 U/kg of regular insulin (Lilly) 2 h before the start of the experiment. Each rat of the other group received 10 U/kg of regular insulin for the next 2 days only. In this manner 2 groups of rats were obtained similar in body weight and in the amount of abdominal depot fat they contained, one acutely insulin deficient with blood sugars[5] in excess of 400 mg/100 ml, the other, insulin maintained, manifesting no elevation in blood sugar.

Very low density chyle lipoproteins, labeled principally in the triglyceride moiety with (1 ^{14}C) palmitic acid, were prepared as previously described[6]. Each rat (3 rats per group) was injected with 1 ml of this preparation into the leg or tail vein while under light ether anesthesia and placed in metabolism cages[7] designed for the collection of expired CO_2. Ten min or 2 h later they were exsanguinated by heart puncture and the gastrocnemius muscle and all of the abdominal fat depots (epididymal, perirenal, omental and mesenteric) were removed, rinsed with ice cold saline, blotted dry and weighed. Approximately 1 g of skeletal muscle was taken from each animal. The grams of abdominal fat removed were: 9.58 (9.42–9.79) and 7.46 (7.41–7.52), respectively, from the insulin maintained and insulin deficient at 10 min, and 9.41 (9.23–9.59) and 8.26 (6.75–9.58), respectively, from the insulin maintained and insulin deficient at 2 h. Tissue lipids were extracted[8] and assayed for ^{14}C[6]. Expired CO_2 was assayed as previously described[9].

Since appreciable amounts of ^{14}C were present in plasma (up to about 40% of the injected dose) 10 min after injection of the chyle preparation, tissue lipid ^{14}C was corrected for ^{14}C in included plasma, using values reported by DEWEY[10] for plasma content of tissues. The lipid ^{14}C content of plasma at 10 min after injection was about 3 times higher in the acutely insulin deficient rats (whose plasma was decidedly lipemic) than in those maintained on insulin. The distribution of ^{14}C in the abdominal fat depots and skeletal muscle, therefore, is expressed in the Table as a percentage of the ^{14}C removed from the blood stream rather than as a percentage of the injected ^{14}C. In calculating the ^{14}C content of skeletal muscle, total body skeletal muscle was estimated as 45% of the body weight[11]. At both the 10-min and 2-h intervals a much higher proportion of the lipid ^{14}C removed from the blood stream was recovered in skeletal muscle and a much lower proportion in the abdominal fat depots in the acutely insulin deficient than in the insulin maintained rats. The Table also shows that over the 2-h period the insulin deficient group converted several times as much of the injected labeled TGFA to expired CO_2 as did the insulin maintained group.

Several studies have shown that the activity of lipoprotein lipase, an enzyme that specifically hydrolyzes triglycerides present in chylomicrons and low density lipoproteins, increases in adipose tissue, heart and mammary gland when the uptake of TGFA of low density lipoproteins by these tissues is also increased[1,3,12–19]. These observations have led to the view that lipoprotein lipase plays an essential role in the uptake of low density lipoprotein TGFA. The wide distribution if lipoprotein lipase throughout the body[20] suggests that a mechanism for the direct uptake of low density lipoprotein TGFA may be widespread. Our observation that in acutely insulin deficient rats an increased oxidation of the injected ^{14}C labeled chyle TGFA was associated with a striking increase in the proportion of lipid ^{14}C recovered in skeletal muscle as early as 10 min after injection raises the possibility that an increased demand for fatty acid as a substrate for energy production in the insulin deficient state might result in a greater direct uptake of TGFA of very low density chyle lipoproteins. Such a suggestion must, however, be tentative since the extent to which recycling of labeled fatty acid may have contributed to

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the observed tissue distribution of ^{14}C even during the first 10 min after injection of the chyle lipoproteins cannot be assessed from our data[21].

^{14}C Distribution in alloxan diabetic rats

10 min		2 h	
Insulin maintained	Insulin deficient	Insulin maintained	Insulin deficient
% ^{14}C removed from plasma found in abdominal fat and skeletal muscle:			
Fat			
13.5 ± 1.8	5.3 ± 0.6	14.3 ± 1.2	3.5 ± 0.4
Muscle			
9.6 ± 1.7	19.1 ± 2.3	7.7 ± 0.4	20.8 ± 2.0
% of injected ^{14}C in expired CO_2:			
0.1 ± 0	0.3 ± 0	2.6 ± 0.5	19.3 ± 1.2

Values are means ± S.E.

Résumé. Chez des rats diabétiques, l'arrêt du traitement insulinique est suivi (a) d'une augmentation de la proportion des acides gras triglycériques du chyle marqués du ^{14}C prelevée dans le sang par les muscles du squelette après injection des triglycériques, (b) d'une diminution de la proportion prelevée par les depots gras abdominaux, et (c) d'une augmentation de leur oxydation en CO_2 expiré.

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The Effect of Chlorpromazine on the Adrenal Gland in Rats with Brain Stem Lesions

It is well known that the central nervous system plays an important role in the control of pituitary ACTH secretion. Several data have been published indicating that this control is exercised through the hypothalamus[1] and that the mesencephalic reticular formation (MRF) plays also a role in this mechanism[2,3].

Since it has also been held that chlorpromazine (CPZ) has an inhibiting action on the MRF[4,5] we have tried in the present work to establish the action of lesions in the MRF and simultaneous administration of CPZ, on the pituitary-adrenal axis, using the ascorbic acid depletion of the adrenals as a functional test[6].

Four groups of white male rats weighing between 180 and 220 g were used (Table). The first group included absolute controls and was injected with saline s.c. during 15 days. Those of the second group received CPZ s.c. in daily doses of 5 mg/kg during 15 days. On those of the third and fourth groups, electrolytic lesions were performed bilaterally in the MRF with a stereotaxic apparatus[2] under pentobarbital anaesthesia and using an intensity of 3 mA for 10 sec. After these localized lesions no changes in the behaviour and sleep-wakefulness cycles of the animals were detected. Two weeks after this operation the animals of the third group started receiving daily injections of saline, while those of the fourth group received daily injections of CPZ 5 mg/kg during the same period.

All animals receiving CPZ, in which indifference to the environment and to the observer was apparent, were fed through a gastric catheter to avoid the side effects and unspecific stress of starvation.

24 h after the last injection all the animals were killed by bleeding under pentobarbital anaesthesia, and the adrenal glands removed and weighed in a torsion balance. After this, the right glands were fixed in formalin-calcium for histological study (results will be published elsewhere), and the left ones were used for ascorbic acid determination[7].

In groups III and IV the actual site and size of central nervous system lesions were established through the study of serial sections. Lesions were found in the periaqueductal grey matter between the nuclei of the III and IV cranial nerves and its adjoining reticular formation.

As seen in the Table, body weight of the animals in the 4 groups did not vary significantly, indicating that the experimental procedures did not modify their nutritional state.

No significant differences in adrenal weight were detected between the animals of the 4 groups. Nevertheless, adrenal ascorbic acid content showed meaningful variations; thus, CPZ administration (group II) produced a significant increase of it, while after MRF lesions (group III) a highly significant depletion of the ascorbic acid content of adrenal glands was observed.

In a control group (not included in the Table), lesions performed in other areas of the mesencephalon including the medial and lateral lemnisci were not effective in modifying the ascorbic acid content of the adrenals.

The administration of CPZ during a period of 15 days was incapable of restoring the ascorbic acid depletion produced by MRF lesions in group IV.

Our results indicate that the lesions at the MRF level and the administration of CPZ have both a considerable

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