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In Vivo and In Vitro Metabolism in Hypothalamic Obesity*

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Summary. U-^{14}C-Glucose was injected into weanling rats two weeks after electrolytic destruction of the ventromedial hypothalamic nuclei. Incorporation of radioactivity into plasma lipids as well as liver, adipose tissue, diaphragm and carcass glycogen, total lipid and saponifiable fatty acids was measured. On a fat free as well as on a chow diet, rats with lesions incorporated more radioativity into all adipose tissue components and into liver fatty acids but not into liver glycogen. On the fat containing diet (chow) radioactivity of plasma lipid was increased and that of liver total lipid unchanged, whereas on a fat-free diet incorporation into plasma lipid was not increased while that into liver lipid was. Diaphragm total lipid and fatty acid radioactivity was increased while that of diaphragm glycogen was not. Carcass lipid, fatty acid and glycogen radioactivity were increased. — Diaphragm was also incubated *in vitro* with U-^{14}C-Glucose or 1-^{14}C-Palmitate. Glucose incorporation into total lipid and fatty acid was increased whereas oxidation and incorporation into glycogen were not. Palmitate oxidation and incorporation into phospholipid were decreased while incorporation into triglyceride was increased. — Results have been discussed in the light of similar changes previously noted with adipose tissue *in vitro*.

Métabolisme dans l'obésité hypothalamique in vivo et in vitro

Résumé. Du glucose U-^{14}C a été injecté à des rats sevrés deux semaines après la destruction électrolytique des noyaux ventromédiaux de l'hypothalamus. Nous avons mesuré l'incorporation de la radioactivité dans les lipides du plasma de même que dans le foie, le tissu adipeux, le glycogène du diaphragme et du corps, les lipides totaux et les acides gras saponifiables. Au cours d'un régime avec ou sans graisse, les rats avec lésions incorporaient davantage de radioactivité dans tous les composés du tissu adipeux et dans les acides gras du foie, mais non dans le glycogène du foie. Pendant le régime avec graisse, la radioactivité des lipides du plasma était augmentée et celle des lipides totaux du foie restait constante, tandis que pendant le régime sans graisse, l'incorporation dans les lipides du plasma n'était pas augmentée, contrairement à l'incorporation dans les lipides du foie. La radioactivité des lipides totaux et des acides gras du diaphragme était augmentée tandis que celle du glycogène du diaphragme ne l'était pas. La radioactivité des lipides, des acides gras et du glycogène du corps était augmentée. — Le diaphragme a été également incubé *in vitro* avec le glucose U-^{14}C ou le palmitate 1-^{14}C. L'incorporation du glucose dans les lipides totaux et les acides gras était augmentée tandis que son oxydation et son incorporation dans le glycogène ne l'étaient pas. L'oxydation et l'incorporation du palmitate dans les phospholipides étaient réduites, tandis que son incorporation dans les triglycérides était augmentée. — Les résultats ont été discutés dans le sens de changements similaires préalablement notés avec le tissu adipeux *in vitro*.

Der in vivo und in vitro Metabolismus bei hypothalamisch bedingter Fettsucht

Zusammenfassung. Zwei Wochen nach elektrolytischer Zerstörung der ventromedialen Nuclei des Hypothalamus wurde entwöhnten Ratten U-^{14}C-markierte Glucose eingespritzt. Es wurde die Inkorporation der Radioaktivität in Plasmalipide als auch in die Leber, das Fettgewebe, das Glykogen des Diaphragmas und des Körpers, die Gesamtlipide und die verseifbaren Fettsäuren gemessen. Bei fettfreier als auch bei fetthaltiger Diät inkorporierten Ratten mit Schäden im Hypothalamus mehr Radioaktivität in alle Fettgewebskomponenten und in Leberfettsäuren, aber nicht in Leberglykogen. Bei der fetthaltigen Diät war die Radioaktivität der Plasmalipide erhöht und die der gesamten Leberlipide unverändert geblieben. Bei einer fettfreien Ernährung war die Inkorporation in Plasmalipide nicht erhöht, während jene in Leberlipide angestiegen war. Die Radioaktivität der Gesamtlipide des Diaphragmas und der Fettsäuren war erhöht, diejenige des Diaphragmaglykogens jedoch nicht. Lipid-, Fettsäuren- und Glykogenradioaktivität des Körpers waren erhöht. — Das Diaphragma wurde ebenfalls *in vitro* mit U-^{14}C-Glucose oder 1-^{14}C-Palmitat inkubiert. Die Glucoseinkorporation in Gesamtlipide und Fettsäuren war erhöht, dagegen nicht die Oxidation und Inkorporation in Glykogen. Die Palmitatoxidation und -inkorporation in Phospholipide war vermindert aber die Inkorporation in Triglyceride erhöht. — Die Ergebnisse werden im Lichte ähnlicher Veränderungen, die früher an Fettgeweben *in vitro* festgestellt wurden, diskutiert.

Key words: Hypothalamus, obesity, adipose tissue, lipogenesis, insulin, hyperlipemia.

Previous work from these laboratories [1, 2, 3] has shown that weanling rats with lesions of the ventromedial hypothalamus have obesity, hyperinsulinemia and hypertriglyceridemia in spite of normal food intake and normoglycemia. Adipose tissue from these rats exhibits increased glucose oxidation and lipogenesis but decreased fatty acid oxidation *in vitro*. Although the obesity, hyperinsulinemia and changes in adipose tissue metabolism persist on a

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fat-free diet, the hypertriglyceridemia disappears [2].

The present study was undertaken to investigate the following questions concerning metabolic abnormalities in these animals: 1. Does muscle show changes of *in vitro* metabolism similar to those of adipose tissue? 2. Is *in vivo* adipose tissue and muscle metabolism similar to that seen *in vitro*? 3. Can the concomitant findings of hyperinsulinemia, normoglycemia and increased adipose tissue glucose utilization be explained by insulin resistance or decreased glucose utilization by other tissues? 4. What are the effects of a fat-free diet on *in vivo* glucose utilization?

Materials and Methods

Male weanling rats were obtained from Sprague-Dawley, individually caged at 23° C in a light cycled environment (6 AM — 6 PM light, 6 PM — 6 AM dark) and given water and Purina Rat Chow *ad libitum*. When the animals weighed 70—80 g they were anesthetized with hexobarbital (14 mg/100 g), weighed and measured. Electrodes were placed in the ventromedial hypothalamic nuclei according to previously established coordinates [4] and withdrawn either after passage of 1.5 m amps current for 10 seconds (VMN) or without passage of current (Sham). Food intake was measured thereafter until sacrifice two weeks later.

In vitro studies on diaphragm: Rats were sacrificed by decapitation and the diaphragms quickly removed, bisected and weighed. One hemidiaphragm was incubated for 2 h with U-^{14}C-glucose (150 mg%) and the other with 1-^{14}C-palmitate (125 μM) for measurement of $^{14}CO_2$ production. Tissues were then homogenized in water and analyzed for protein content, for incorporation of U-^{14}C-glucose into total lipid, saponifiable fatty acid and glycogen, or for incorporation of 1-^{14}C-palmitate into various lipid fractions. Details of incubation and analysis were as previously described [1, 2].

In vivo studies with U-^{14}C-glucose: Two separate studies were performed, one using animals fed a Purina chow diet (3.44 calories/g; 6% animal fat; 20% protein; 56% carbohydrate by weight) and the other using animals fed a fat free diet (Nutritional Biochemicals Co. "Fat-Free Test Diet": 3.22 calories/g; 20% protein; 59% carbohydrate by weight). Rats were injected intra-peritoneally with 2.5 micro-curies of U-^{14}C-glucose (New England Nuclear, 5 μC/μmole) and sacrificed by decapitation 30 min later. Incorporation of radioactivity into lipid and glycogen of selected tissues was measured as previously described [1, 2]. Heparinized blood was obtained at sacrifice and an aliquot extracted and analyzed for triglyceride content [5] and lipid radioactivity. To determine specific activity of plasma glucose an aliquot of plasma was deproteinized with barium and zinc. Glucose was measured on an aliquot of the supernatant (Glucose oxidase, Worthington). The remaining supernatant was added to mixed bed resin (Bio-Rad AG501-X8) which had previously been equilibrated with a solution of glucose in distilled water (200 mg/ml). The mixture was shaken at room temperature for 60 min and the supernatant removed for counting. Standards of ^{14}C-glucose were similarly treated to permit correction for loss of glucose in this procedure. Recovery of radioactive glucose averaged 60—80% by this method. Frozen carcasses were lightly thawed and ground in a meat grinder. The ground carcass was then homogenized in 3 volumes of water and aliquots taken for analysis of glucose incorporation into lipid and glycogen as previously described [1, 2]. Data was corrected for the weight of each rat so as to permit calculations to be made on the basis of 2.5 microcuries injected per 100 g rat weight.

Results

Table 1 shows results with diaphragm *in vitro*. Compared to results in sham-operated animals, glucose incorporation into total lipid and saponifiable fatty acids was greater in diaphragms from VMN rats whereas oxidation and incorporation into glycogen was unchanged. Of the total lipid radioactivity, saponifiable fatty acid comprised 33% in sham-operated controls whereas in VMN animals, this figure was 53%. Palmitate oxidation and incorporation of radioactivity into phospholipid were depressed in VMN diaphragm, whereas incorporation into tissue triglyceride was increased.

The results obtained after *in vivo* injection of tracer amounts of U-^{14}C-glucose into sham or VMN rats fed a lab chow diet (6% animal fat) are shown in Table 2. VMN rats showed increased plasma triglyceride content as well as greater than normal incorporation of radioactivity into plasma and adipose tissue lipids, liver and adipose tissue saponifiable fatty acids, and adipose tissue glycogen. In both tissues saponifiable fatty acid labelling was most markedly stimulated. In normals this fraction accounted for 7 and 22% of total lipid radioactivity of liver and adipose tissue respectively. In VMN rats, in contrast, these figures are 36 and 73% respectively. Results of *in vivo* injection of U-^{14}C-glucose into rats maintained on a fat-free diet are shown in Table 3. Adipose tissue of VMN rats again showed greater radioactivity in total lipid, saponifiable fatty acids and glycogen. Diaphragm and liver lipid and saponifiable fatty acid labelling was likewise increased, whereas glycogen was not. Carcass radioactivity changes resembled those of adipose tissue. Plasma glucose content and specific activity and plasma triglyceride levels and radioactivity were not different in the two groups.

As noted in the other experiments the labelling of the saponifiable fatty fraction was selectively enhanced. VMN destruction was associated with 20 fold increase in labelling of this fraction in adipose tissue and liver

while total lipid labelling increased 9 and 2.5 times respectively. In diaphragm the increases were 8 fold into saponifiable fatty acid and 3 fold into total lipid.

Discussion

The data obtained using isolated diaphragms *in vitro* indicate that VMN rat muscle utilizes glucose more rapidly than normal for lipogenic pathways much as does adipose tissue from these animals [1]. On the other hand glucose oxidation and incorporation into glycogen are similar in VMN and control rat diaphragms. These data are not consistent with the possibility that decreased glucose utilization by tissues other than liver and adipose tissue may account for coexistent hyperinsulinemia and normoglycemia. Previous studies on adipose tissue in experimental obesity [1, 2] have suggested that increased glucose utilization is not the result of hyperinsulinemia alone. This hypothesis is strengthened by the present findings since glycogen labelling is not increased in VMN diaphragm *in vitro* or *in vivo*, whereas this pathway is known to be exquisitely sensitive to insulin in normal animals [6].

The finding of decreased fatty acid oxidation by VMN diaphragm *in vitro* is consistent with previous data on adipose tissue [1, 2]. The increased glucose oxidation and decreased fatty acid oxidation seen in both muscle and adipose tissue could produce an elevated respiratory quotient and a decreased oxygen consumption. The latter, which was described by Han in VMN animals [7] can therefore be explained on the basis of changes noted in the pattern of *in vitro* substrate oxidation.

Table 1. *In vitro metabolism of U-^{14}C-glucose and 1-^{14}C-palmitate by VMN rat diaphragm*

	Sham (6)	VMN (7)	$P <$
U-^{14}C-glucose incorporated (DPM/mg protein)			
CO_2	1627 ± 176	2086 ± 170	NS
Total lipid	192 ± 10	447 ± 62	0.001
Saponifiable fatty acids	63 ± 7	237 ± 46	0.001
Glycogen	1128 ± 98	1009 ± 120	NS
1-^{14}C-palmitate incorporated (DPM/mg protein)			
CO_2	2463 ± 108	2132 ± 135	0.05
Phospholipid	1218 ± 49	843 ± 85	0.001
Diglyceride	1464 ± 92	1683 ± 142	NS
Triglyceride	3241 ± 398	5754 ± 432	0.001

Results expressed as mean ± S.E.M.
( ): # of animals in each group.
NS: not statistically significant ($p > 0.05$).

Table 2. *Disposition of U-^{14}C-glucose 30 min after injection into VMN and Sham rats fed a chow diet containing 6% fat*

	Sham (7)	VMN (6)	P <
Adipose tissue (DPM/mg protein)			
Lipid	505 ± 302	11472 ± 2447	0.001
Saponifiable fatty acids	113 ± 35	8409 ± 2250	0.001
Glycogen	22 ± 8	241 ± 50	0.001
Liver (DPM/mg tissue)			
Lipid	4.5 ± 0.4	5.9 ± 0.9	NS
Saponifiable fatty acids	0.33 ± 0.10	2.1 ± 0.5	0.05
Glycogen	28 ± 13	28 ± 6	NS
Plasma			
Triglyceride mg/100 ml	28 ± 2	75 ± 12	0.01
Lipid DPM/ml	95 ± 3	236 ± 48	0.02
Glucose mg/100 ml	148 ± 45	143 ± 95	NS

Results expressed as mean ± S.E.M.
( ): # of animals in each group.
NS: not statistically significant ($p > 0.05$).

The increased incorporation of fatty acid into muscle triglyceride is consistent with the fat accumulation and preservation seen in the whole animal. The increased triglyceride synthesis may contribute to decreased phospholipid labelling by removing diglyceride which acts as substrate. On the other hand, decreased labelling of diaphragm phospholipid with 1-^{14}C-palmitate may reflect decreased turnover of muscle protoplasm and membranes. Such a suggestion is in

line with the decrease in muscle mass noted previously in VMN rats which have normal body weight in spite of increased carcass fat content [1, 2, 8, 9].

The increased labelling of plasma lipids in VMN rats by intraperitoneally injected U-^{14}C-glucose was apparent in rats ingesting the 6% fat diet but not in rats on a fat-free diet. The plasma triglyceride values parallel these results and are in accord with the previously noted absence of hypertriglyceridemia in VMN rats fed a fat-free diet [2]. It is not possible, however, to differentiate increased synthesis of plasma triglycerides from decreased clearance.

We cannot presently explain the difference between *in vivo* and *in vitro* findings, but greater physiological relevance must be attached to the former.

One of the most striking findings in these and other experiments [1, 2] is the selective enhancement of glucose incorporation into saponifiable fatty acids. This specific stimulation of fatty acid synthesis would divert great quantities of carbohydrate into lipid storage and may be the major factor in the development of obesity in weanling VMN rats.

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Table 3. *Disposition of U-^{14}C-glucose 30 min after injection into VMN and Sham rats fed a fat-free diet*

	Sham (8)	VMN (8)	P <
Adipose tissue (DPM/mg protein)			
Lipid	398 ±221	3646 ±1721	0.01
Saponifiable fatty acids	119 ± 71	2485 ±1492	0.02
Glycogen	72 ± 13	184 ± 48	0.05
Liver (DPM/mg tissue)			
Lipid	3.4 ± 0.8	9 ± 2	0.02
Saponifiable fatty acids	0.28± 0.10	5.4± 1.8	0.01
Glycogen	8.0 ± 1.4	12.8± 2.2	NS
Diaphragm (DPM/mg protein)			
Lipid	4.1 ± 0.7	13 ± 2	0.01
Saponifiable fatty acids	0.85± 0.23	6.4± 1.6	0.01
Glycogen	3.7 ± 0.6	6.0± 1.5	NS
Plasma			
Triglyceride mg/100 ml	28 ± 4	37 ± 5	NS
Lipid DPM/ml	188 ± 41	331 ± 60	NS
Glucose mg/100 ml	90 ± 2	103 ± 6	NS
Glucose DPM/ml	81 ± 10	64 ± 11	NS
Glucose DPM/mg	89 ± 10	64 ± 14	NS
Carcass (DPM/g)			
Lipid	694 ±124	6382 ±1904	0.01
Saponifiable fatty acids	328 ± 72	4443 ±1508	0.02
Glycogen	832 ±115	1640 ± 130	0.01

Results expressed as mean ± S.E.M.
( ): # of animals in each group.
NS: (not statistically significant ($p > 0.05$).

The studies using intraperitoneally injected U-^{14}C-glucose strongly support our findings of increased adipose tissue [1, 2] and diaphragm lipogenesis *in vitro* (Table 1). The present data indicate that *in vivo* lipogenesis is stimulated after VMN destruction and that total glucose utilization is increased in adipose tissue, liver, muscle or carcass. Adipose tissue both *in vivo* and *in vitro* appears to be the tissue most profoundly altered by VMN destruction. In liver, total lipid labelling was increased *in vivo* only on a fat-free diet where lipogenesis is maximal [10]. However, incorporation into liver saponifiable fatty acids was increased on both diets but only minimally if the diet contained a normal amount of fat. Glucose incorporation *in vivo* into liver and diaphragm glycogen was not increased in VMN rats, which is similar to findings with adipose tissue [2] and diaphragm *in vitro*. In contrast, however, adipose tissue glycogen labelling *in vivo* was increased in VMN rats.

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