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Preliminary Communications

An Enzymatic Defect in a Group of Obese Patients*

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The possibility has been raised that a metabolic defect underlies the development of some forms of obesity in man (Mayer, 1957; Tepperman, 1958; Astwood, 1962). However, attempts to define such a lesion in the metabolism of human adipose tissue in vitro has so far proved unsuccessful. Reports have confirmed that lipolysis in adipose tissue of obese patients is unimpaired when stimulated by adrenaline in vivo (Orth and Williams, 1960) or in vitro (Mosinger *et al.*, 1965; Björntorp and Hood, 1966; Galton and Bray, 1966), and therefore makes a block in the mobilization of fatty acids from adipose tissue an unlikely basis for the excessive accumulation of triglycerides in this disease.

A study of lipogenesis in adipose tissue of obese and normal-weight patients has not revealed, so far, any differences in the activity of glucose-6-phosphate dehydrogenase and malic enzyme, which generate essential cofactors for the synthesis of fatty acids (Shrago *et al.*, 1966; and citrate-cleavage enzyme, which is thought to supply an important part of the acetyl coenzyme A necessary for fatty-acid synthesis (Bhaduri and Shre, 1963), is essentially absent in human adipose tissue (Shrago *et al.*, 1966). This suggests that the synthesis of fatty acids in human adipose tissue may not be an important factor in the development of obesity.

Another essential precursor for lipogenesis is α -glycerol phosphate, which is formed from dihydroxyacetone phosphate during glycolysis by the action of a cytoplasmic α -glycerophosphate dehydrogenase. The experiments reported here were designed to examine the source and disposal by oxidation of α -glycerol phosphate in human adipose tissue obtained from obese and normal-weight patients, and to see whether there was an increase in the availability of α -glycerol phosphate for synthesis of triglycerides in adipose tissue of the former. It was also possible to study two overweight patients in whom a hypothalamic lesion was very likely responsible for the obesity, and the metabolic behaviour of their adipose tissue was compared with tissue of obese patients with no other disorder.

MATERIALS AND METHODS

Source of Tissue.—Human subcutaneous adipose tissue was obtained during abdominal operations from 25 patients (9 males,

16 females) whose ages ranged from 15 to 69 years and whose mean body weight was 152 lb. (68.9 kg.). Patients with diabetes and jaundice were excluded. Preoperative treatment involved a fast of eight hours before the procedure, premedication with pentobarbitone sodium and atropine, and general anaesthesia induced by thiopentone and maintained with either cyclopropane with oxygen or halothane with nitrous oxide and oxygen. Adipose tissue from obese patients was removed from the anterior abdominal wall under local anaesthesia (approximately 6 ml. of 1% xylocaine injected intradermally). A summary of the clinical details of these patients is given in Table I. After removal the adipose tissue was washed and either transferred to 3 ml. of albumin-bicarbonate buffer for preparation of isolated adipose cells by a modification of the procedure of Rodbell (1964), or homogenized for approximately one minute in 0.25 M sucrose (1:4) in a glass mortar surrounded by chipped ice with a motor-driven teflon pestle. It was frequently necessary to complete homogenization in a hand mortar-and-pestle when the adipose tissue was fibrous.

TABLE I.—Summary of the Clinical Details of the Obese Patients

No. of patients	..	..	..	11 (2 male, 9 female)
Age range	..	..	..	15–40 years
Mean weight (range)	..	..	..	340 (280–430 lb.); 154.2 (127–195) kg.;
Height range	..	..	..	5 ft. 2 in.–5 ft. 9 in.; 157–175 cm.
Onset of obesity	..	..	..	Early teens, except for one in late twenties

Mitochondria were prepared from the homogenate by a method similar to that of Schneider (1951) by means of a Lourdes high-speed centrifuge (motor head: 16RA) in a cold room (4° C.) or in a refrigerated International Centrifuge (motor head: 856). The supernatant after the first centrifugation at 17,000 $\times g$ was kept for assay of the soluble cytoplasmic enzymes. After washing, the mitochondrial pellet was suspended in 0.03 M phosphate buffer (4:1) and used for the assay of mitochondrial glycerophosphate and succinic dehydrogenases.

Assay Procedures.—Oxygen consumption was measured in conventional Warburg vessels, as previously described (Galton, 1966). Assays of nicotinamide-adenine dinucleotide (NAD)-linked malate dehydrogenase and glycerophosphate dehydrogenase were performed in the cytoplasmic fraction, with the use of an incubation system, as described elsewhere (Galton and Fain, 1966), except that enzyme activity was measured by the change in absorption of nicotinamide nucleotides in a recording spectrophotometer (Bausch and Lomb, spectronic 505) at a wavelength of 340 m μ . The pH of the sample was measured after each assay, and was 9.4 ± 0.5 . The temperature of the cuvette holder was $28 \pm 1^\circ$ C., and the activity of the enzyme was expressed as m μ moles of NADH formed/min./mg. of protein homogenate.

Assay of the NAD-independent glycerophosphate dehydrogenase was performed on a suspension of the mitochondrial fraction according to the method of Ringler and Singer (1962).

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Succinic dehydrogenase was estimated by a modification of the method of Slater and Planterose (1960).

Supernatant and mitochondrial protein was measured by a modification of the biuret method (Ringler and Singer, 1962), crystalline bovine serum albumin being used as a standard.

RESULTS

Malate dehydrogenase has been reported as the most active enzyme in human adipose tissue (Schmidt and Schmidt, 1960). However, under the conditions of the assay in Table II the activity of NAD-linked glycerophosphate dehydrogenase was greater ($P=0.007$). This suggests that substantial amounts of α -glycerol phosphate can be formed from dihydroxyacetone phosphate during glycolysis; and the equilibrium constant of the reaction very much favours the formation of glycerol phosphate (Hohorst, 1960). In homogenates prepared from obese patients the activity of glycerophosphate dehydrogenase is slightly reduced when compared with normal-weight patients ($P=0.05$). In contrast the activity of malate dehydrogenase, which was chosen as a reference enzyme, was similar in the cytoplasmic fractions of obese and slender patients.

The further metabolism of glycerol phosphate in adipose tissue is either to triglyceride by esterification with fatty acids or to enter mitochondria, where it is oxidized by a glycerophosphate dehydrogenase which does not require NAD^+ . Mitochondrial glycerophosphate dehydrogenase is present in human adipose tissue (Table II); however, mitochondria prepared from adipose tissue of obese patients displayed a reduced ability to oxidize glycerol phosphate. Frequently no oxygen consumption was seen for the first 30 minutes of incubation and only a small uptake was observed in the succeeding half-hour. The activity of succinic dehydrogenase, which was selected as a reference enzyme, was similar in mitochondria prepared from obese and slender patients (Table II).

It is difficult to correlate the data obtained from the study of homogenates to the complex conditions existing in the undamaged cell. However, a deficiency of an important mitochondrial dehydrogenase would be expected to lead to a reduced consumption of oxygen by the intact cell. This was observed when the oxygen uptake of isolated adipose cells obtained from tissue of slender and obese patients was measured in a Warburg apparatus (Table II).

Table III shows the oxygen uptake and activities of the mitochondrial and cytoplasmic glycerophosphate dehydro-

genases in two cases of obesity in which the gain in weight was most likely due to a hypothalamic lesion. The evidence for this is very good in one patient (N. S.), where the gain in weight directly followed removal of a dermoid cyst from the third ventricle. In both cases the obesity had been present longer than in their obese controls. The oxygen uptake was greater in the patients with presumed hypothalamic lesions than in controls of obese patients with no other overt disease. Furthermore, the activity of the mitochondrial α -glycerophosphate dehydrogenase was greater than in obese controls, but fell below the mean activity in patients of normal weight. This suggests that the defect in the mitochondrial α -glycerophosphate dehydrogenase is not found in obesity of hypothalamic origin; but the rarity of these cases makes it difficult to evaluate this on a statistical basis.

DISCUSSION

Before assessing the data presented in this paper it is necessary to make several qualifications. Firstly, only fully developed cases of obesity from a homogeneous clinical group were examined. This group was characterized by a severe obesity developing in early life, and it is not known whether the differences described here are present in the initial stages of this and other types of the disease. The reduction in activity of the mitochondrial glycerophosphate dehydrogenase might, for example, be secondary to the development of obesity. Secondly, it is difficult to make comparisons between normal and pathological tissue, since in obesity it has been shown that there is an increase in the total number of adipose cells and that each cell contains more lipid (Hirsch *et al.*, 1966). Since it is metabolic activity that has been studied, more meaningful comparisons are probably those based on tissue nitrogen, which is a measure of protoplasmic mass, than on wet weight. Thirdly, there are differences between the compared groups other than obesity. The adipose tissue was removed under general anaesthesia from the normal-weight group, and under local anaesthesia from the obese group. However, no difference in metabolic activity was found between adipose tissue of two normal-weight patients who had undergone spinal anaesthesia and the two normal-weight patients, adjacent in the series, who had general anaesthesia.

The presence of an active glycerophosphate dehydrogenase in the cytoplasmic fraction of normal human adipose tissue, as demonstrated in Table II, would be expected, since glycerol

TABLE II.—Oxygen Uptake and Activities of Several Mitochondrial and Cytoplasmic Enzymes in Human Adipose Tissue

Group	Oxygen Uptake (μ l./100 μ g. Tissue N ₂ /hr.)	Activities of:			
		Mitochondrial Enzymes		Cytoplasmic Enzymes	
		α -Glycerophosphate Dehydrogenase (μ l. O ₂ /mg. Protein/ 30 min.)	Succinic Dehydrogenase (Δ O.D. ₅₁₀ /mg. Protein/10 min.)	α -Glycerophosphate Dehydrogenase (m μ moles NADH Formed/mg. Protein/min.)	Malate Dehydrogenase (m μ moles NADH Formed/mg. Protein/min.)
Normal weight	1.6 $\pm$ 0.2 (9)	12.1 $\pm$ 1.4 (9)	0.045 $\pm$ 0.01 (5)	36 $\pm$ 4 (7)	18 $\pm$ 1 (7)
Obese	0.72 $\pm$ 0.09 (10)	2.5 $\pm$ 0.7 (7)	0.048 $\pm$ 0.01 (5)	20 $\pm$ 2 (7)	19 $\pm$ 2 (7)
P value (obese v. normal weight)	0.017	0.007	—	0.05	—

Results are means $\pm$ S.E.M., with number of experiments in parentheses. The significance of the difference (P) was calculated by a non-parametric sign test.

TABLE III.—Oxygen Uptake and Activities of Enzymes in Adipose Tissue of Obese Patients

Patient	Age in Years	Sex	Weight		Height		Cranial Pathology	Oxygen Uptake μ l./mmole Triglyceride/hr.)		Mitochondrial α -Glycero- phosphate Dehydrogenase (μ l. O ₂ /mg. Protein/30 min.)	Cytoplasmic α -Glycerophosphate Dehydrogenase (m μ moles NADH Formed/min./mg. Protein)	
			lb.	kg.	ft. in.	cm.		Expt. A *	Expt. B *	Expt. B	Expt. A	Expt. B
L.J. ..	29	F	310	140.6	5 10	178	None Enlarged sella	19.8	15.0	5.0	17	18
J.R. ..	41	F	399	181	5 6	168		29.2	30.0	9.7	30	17
D.C. ..	15	F	370	167.8	5 6	168	None Dermoid cyst in 3rd vent.	32		0.4	21	
N.S. ..	15	F	270	122.5	4 5	135		46		3.4	20	

* Expt. A and B performed one month apart.

phosphate is an essential precursor for the synthesis of triglycerides and can be synthesized only in adipose tissue, whereas the other precursors, fatty acids, probably originate in the liver as well as in adipose tissue (Bortz *et al.*, 1962; Jacob, 1963). A slight reduction in activity of this enzyme ($P=0.05$) was found in adipose tissue of obese patients. However, the activity of the mitochondrial enzyme which oxidizes glycerol phosphate was found to be markedly reduced in adipose tissue of obese patients. This would be expected to increase the local supply of cytoplasmic glycerol phosphate if the supply from glycolysis remains unimpaired. Since the concentration of cytoplasmic glycerol phosphate is probably rate-limiting in the synthesis of triglycerides (Tzur *et al.*, 1964), the physiological effects of its diminished oxidation by mitochondria might be a prolonged stimulation of lipogenesis. It is difficult to assess from the present studies the quantitative contribution that this might make to the development of obesity over the long term.

It was not possible to show an increase in the pool of glycerol phosphate in adipose tissue of obese patients, and if the mitochondrial defect described here is in fact leading to an increased availability of cytoplasmic glycerol phosphate the pool turnover must be increased. Hood and Björntorp (1966) came to a similar conclusion, that the pool of cytoplasmic glycerol phosphate in adipose tissue of obese patients was diluting the incorporation of ^{14}C -glycerol into lipid fivefold when compared with normal.

SUMMARY

An enzymatic defect has been observed in adipose tissue of some obese patients which would be expected to increase the availability of α -glycerol phosphate for the synthesis of triglycerides. Two cases of obesity, with presumed lesions in the hypothalamus, did not display this defect to the same extent as their obese controls. The possibility is considered that extreme

obesity of early onset is associated with an enzymatic defect in the metabolism of α -glycerol phosphate.

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Medical Memoranda

Fatal Pneumococcal Septicaemia after Splenectomy

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There seems to be little doubt that splenectomy predisposes to infections in infancy and childhood, severe pneumococcal infections being particularly common (Finland, 1961). However, few cases have been reported in adults. The following report is of a case of fatal overwhelming pneumococcal infection with adrenal haemorrhage six and a half years after splenectomy for hereditary spherocytosis.

CASE REPORT

A 31-year-old housewife was admitted to Stoke Mandeville Hospital on 1 July 1964. When aged 9 years she had a three-weeks episode of jaundice with pale stools. At 26 she developed jaundice and abdominal pain six weeks after giving birth to a stillborn baby. She was admitted to another hospital and was found to have enlarged liver and spleen. The serum bilirubin was 16.4 mg./100 ml., haemoglobin 13.2 g./100 ml., reticulocytes 12%; red cells showed changes of spherocytosis; direct Coombs test was negative; red-cell

fragility test showed that haemolysis began at 0.6% and was complete at 0.35% sodium chloride. Gall stones were seen on a radiograph of the abdomen. Hereditary spherocytosis was diagnosed, and on 30 January 1958 an uneventful splenectomy and cholecystectomy was carried out.

For six years after the splenectomy she remained in good health, and her haemoglobin on several occasions was normal.

On the afternoon before admission she felt vaguely unwell and complained of headache. In the evening she began to shiver and complained of aches in the muscles. These symptoms intensified during the night, and the following morning it was noted that her skin was covered with a purpuric rash. She also had coughed up some blood. On admission the same morning she was drowsy, and widespread dark-blue petechiae covered almost the entire skin area and visible parts of the mucous membranes. The heart rate was 100/min., the carotid and femoral pulses were feeble, and the blood pressure was not recordable. A diagnosis of septicaemia with adrenal failure was made.

She was given 100 mg. of hydrocortisone and noradrenaline intravenously. Saline fluids and plasma transfusions, penicillin intravenously and intrathecally, and oxygen were also given. Despite treatment she continued to deteriorate and died five hours after admission. Blood and cerebrospinal fluid taken on admission grew *Streptococcus pneumoniae*.

At necropsy the main findings were petechial haemorrhages on most internal organs and massive bilateral adrenal haemorrhages.