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CHANGES IN MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION CHARACTERISTICS IN DIETARY OBESE RATS

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ABSTRACT:

Obesity was induced by feeding a high calorie, high protein diet to weaning rats. Liver mitochondria were isolated from obese and normal rats after the dietary treatment. Although the mitochondrial pellet protein per gram-wet-weight liver was almost double that of the controls, the oxidative phosphorylation characteristics revealed that the respiratory control ratio of mitochondria from obese rats was less than 50% that of the controls, whereas the ADP/O ratios were similar. The basal and CCCP-induced ATPase activities in mitochondria from obese rats was about 60% that of the controls. 65 - 80% of the ATPase activity in mitochondria from obese rats was oligomycin-sensitive compared to 97 - 99% in control rats. These results suggest that obesity affects energy metabolism in animals.

INTRODUCTION

The ability or otherwise of obese subjects to mobilize energy stores during ordinary stresses or ambient temperature changes has been identified as a major problem in the pathogenesis and bioenergetics of obesity syndrome. The need for better techniques of assessment, devoid of ambiguous interpretation during rehabilitation and management of obesity has been advocated (1). The energy expenditure and thermogenesis of obese subjects, using direct and indirect calorimetry revealed that diet-induced thermogenesis increases during over-feeding (2,3). However, Jequier and Schutz (4) asserted that there is a thermogenic defect which plays a significant role in the pathogenesis of obesity.

Various workers on the bioenergetics of obese subjects have adopted different methods including respiration chambers (4), basal metabolic rate, diet-induced thermogenesis and the assessment of energy expenditure due to physical activity (5). In view of the thermogenic defects observed in obesity, the difficulties in respiration and the low energy expenditure in relation to size coupled with the variable existing methods of assessment of energy expenditure in obese subjects, we have decided to investigate

energy generation through a ATP hydrolysis, a situation that may explain the reduced thermogenesis observed in obesity as previously reported by Jequier and Schutz (4) and Sjostrom, (13).

It was noted that ATPase activity in mitochndria from obese rats was less oligomycin-sensitive (oligomycin sensitivity 60-80%) than the control whose oligomycin sensitivity was between 97-99% (Table 4). This observation points to yet another abnormality in the mitochondria from obese subjects.

These defects in oxidative phosphorylation characteristics associated with obesity provide interesting data on the molecular basis of abberant energy metabolism in obese subjects which should provoke further investigations.

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Table 2: Respiratory Control Ratio (RCR) and the ADP:0 Ratio of Liver mitochondrial isolated from obese and control rats

Mitochondrial SOURCE	Respiratory Control Ratio (RCR)		ADP: 0 RATIO	
	Glutamate + Malate	Succinate + Rotenone	Glutamate + Malate	Succinate + Rotenone
Obese rats	2.03	1.68	2.56	1.78
Control rats	4.33	4.25	2.81	1.97

Values given are means of ten separate determinations. Individual values were within the range mean +15%.The reactions medium in a total volume of 1.7 mL at 25oC contained 20 mM Tris-HCl pH 7.4, 210 mM mannitol, 70 mM sucrose, 0.5 mg/ml BSA, 3 mM Pi, 1 mg/ml mitochondrial protein and either 5 mM glutamate plus 5 mM malate or 5 µM succinate plus 4 mM rotenone. When added, the concentration of ADP was 500 nmoles (294 µM).

Table 3: Protein content in the mitochondria isolated of obese and normal rats

Mitochondrial source	Liver weight	Total mitochondrial pellet protein	mg protein/g wet weight liver
Obese rats	10.90	87.36	8.01
Control rats	7.10	30.02	4.23

Values given are means of ten separate determinations. Individual values were within the range mean +10%.

Table 4: ATPase activity in mitochondria isolated from obese and control rats

Mitochondrial	ATPase activity (nmoles/min/mg. protein) BASAL		CCCP-INDUCED	
	-Oligomycin	+ Oligomycin	-Oligomycin	+ Oligomycin
Obese rats	215.00	78.00	480.00	96.00
Control rats	370.00	10.00	720.00	8.00

Values given are smeans of ten separate determinations. Individual values were within the range mean +15%. ATPase activity was determined in a medium containing 50 mM Tris-acetate buffer pH 7.4, 2 mM ATP, 1 mM MgCl, 2 mM PEP, 13 µg/ml LDH, 32 µg pyruvate kinase, 0.2 mM NADH and 0.1 mg/ml mitochondrial protein, 3 µM CCCP and 4 µM oligomycin.

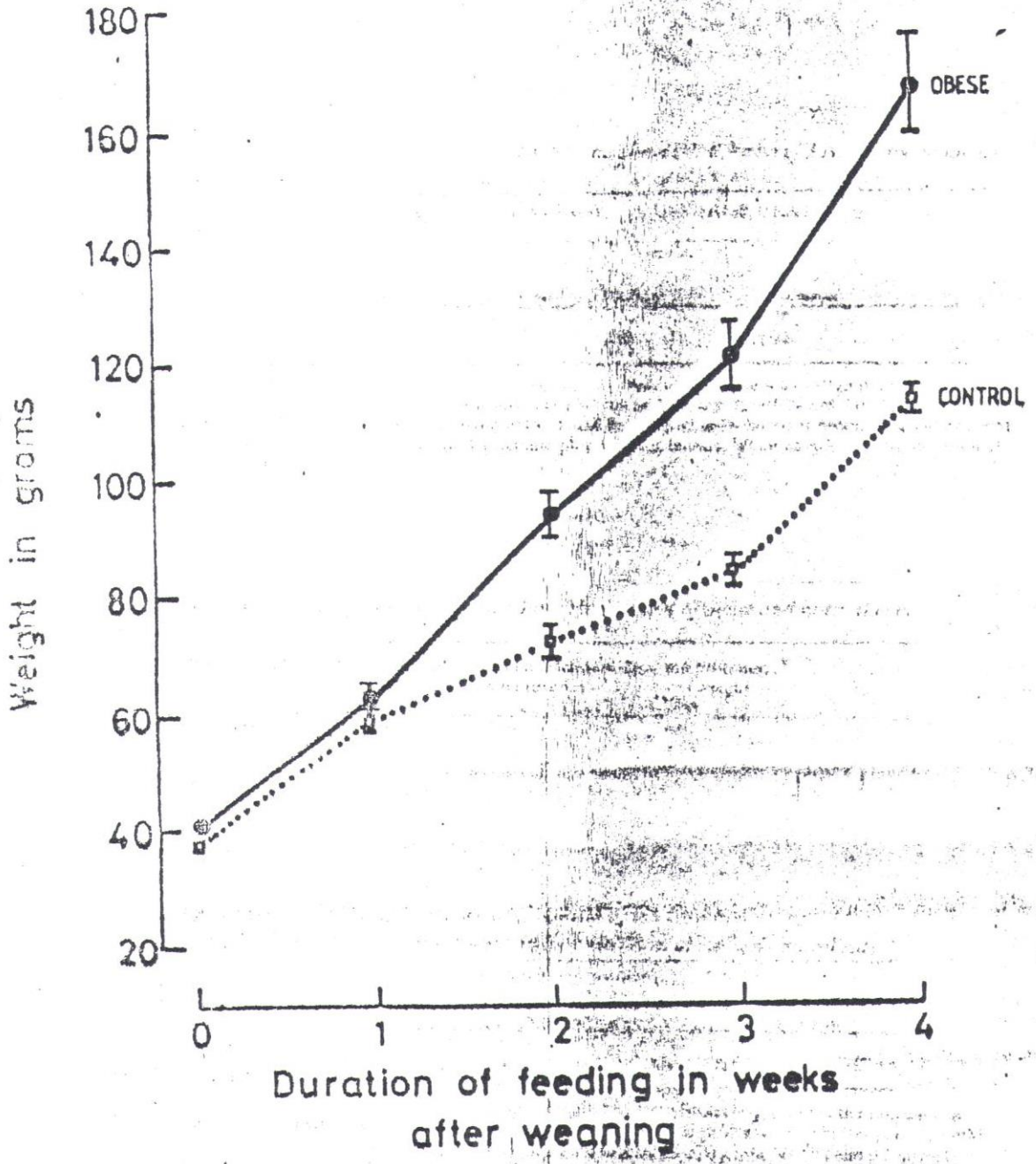

Fig. 1: Body weight of experimental (obese) and control rats during feeding treatment.

Table 1: Composition of diet for the induction of obesity in weaning rats.

Major component	Percentage composition	
	OBD	CPD
Protein	18.00	18.00
Corn oil	6.00	4.00
Carbohydrate	70.70	67.00
Mineral salts	4.00	4.00
Vitamin (premix)	1.00	3.00
DL-Methionine	0.20	–
Dietary fibre	–	4.00
	100.00	100.00
Energy density, KJ/g	17.10	15.73

OBS = Obese Diet (high caloric, high protein)

CPD = Commercially Produced Diet (control diet)

Vitamins (Premix) = Multivitamins capsule - Trade name

"Vivioptal" manufactured in West Germany by Dr. Gerhard Mann, Chem-Pharm, Fabrik Gmbk, 1000 Berlin.

RESULTS AND DISCUSSION

The average body weight of the experimental (obese) rats after the feeding treatment was 108.0g while that of the control rats was 113.7g as shown in figure 1, indicating an excess weight of about 50% in the experimental animal. This excess weight, compared to the normal, portrays a simulation of diefary obesity in experimental rats.

The respiratory control ratio (RCR) of the mitochondria isolated from obese rats was lower than that of the control when either malate plus glutamate was used as the respiratory substrate or when succinate was used (T ble 2). In both cases the RCR of the mitochondria from the obese rats was over 50% lower than the control.

These results indicate that the mitochondria from obese rats may be defective, a situation that may well be associated with uncoupling of oxidative phosphorylation and possibly ATP synthesis. It has been shown by (10, 11, 12) that in pathological obesity, severe fatty change occurs in liver, the accumulation being greatest in periportal hepatocytes.

The reduced RCR observed in mitochondria from obese rats confirm the speculations of Jequier and Schutz (4) and Sjostrom (13) that there is a defect in energy metabolism which leads to reduced thermogenesis in obese subjects. The defective oxidative phosphorylation characteristics might be similar to that observed in kwashiorkor animals (14) which also suffer from fatty infiltration of the liver and marked impairment in the functions of electron transfer complexes as reported by Olowookere and Olowookere and Olorunsogo (15). In man, mechanical impairment of respiration due to fatty infiltration of the respiratory muscles which leads to Pickwickian syndrome has also been associated with defective oxidative phosphorylation as reported by Maclennan and Tzagoloff, (16).

The ADP/0 ratios obtained with the two respiratory substrates were about the same showing a variation of only 10% between the experimental and the control. The obese rats, however, have persistently lower ADP:0 ratios. The mitochondrial protein expressed as mg per gram wet weight liver was almost double that of the control(Table 3). This also almost parallel the overall growth pattern of obese rats compared to control (Fig. 1).

Studies on the mitochondrial ATPase measured in the direction of ATP hyrolysis (Table 4) indicate that both the basal and CCCP induced activity in obese rats was about 40% lower than in the control. This observation suggests that in obese rats the enzyme has lower capacity to hydrolyse ATP than in control rats, thus suggesting a lower rate of

the mitochondrial oxidative phosphorylation characteristics indiet-induce obese rats. Base-line data on the energy production capacity of obese animals which will be provided by oxidative phosphorylation parameters are crucial to the understanding of the over-all energy metabolism and thermogenesis in obesity syndrome.

MATERIALS AND METHODS

Dietary induction of obesity in weaning rats:

Twenty weaning rats of the Sprague Dawley strain, selected from litter-mates were equally divided into two groups. Each group contained equal numbers of male and female. The rats were separated from their mothers at 21 days of age and kept at 18-22°C under a 12th light and 12h darkness period. For a period of 30 days the first group of rats (experimental) was placed on a high calorie, high protein diet, while the second group (controls) was placed on a commercially produced purina chow (Table 1). The rats were kept individually in plastic metabolic cages. Both the controls and the experimental rats were allowed free access to water and food for the same period. The weight of each rat was taken weekly.

Preparation of rat liver mitochondria:

Rat liver mitochondria were isolated using 0.25M sucrose containing 0.5 mg/ml bovine serum albumin (fatty acid free) to bind free fatty acids which could interfere with the coupling of the mitochondria. The procedure used was essentially as described by Hogeboom et al. (6). All preparations were carried out at 4°C.

Determination of oxygen consumption:

The oxygen consumption rate was recorded polarographically by an oxygen electrode operated at 25°C in a closed and magnetically stirred glass chamber. The reaction medium contained 210mM mannitol, 70mM sucrose, 20 mM Tris-HCl buffer, pH 7.4, 3 mM phosphate and 1 mg/ml mitochondrial protein in a total volume of 1.70 ml. The substrate used was either 5 mM potassium malate plus 5 mM potassium glutamate or 5 mM succinate. In the case of the latter, 4 mM rotenone was included in the reaction mixture. Respiratory control ratio (RCR) was calculated as the ratio of the rate of oxygen consumption during state 3 respiration to that of state 4 respiration as described by Chance and Williams (7).

The ADP:0 ratio was calculated as the ratio of the amount of ADP (nmol) used to the amount of oxygen (n atoms) consumed in the presence of ADP.

Protein determination:

Mitochondrial protein was estimated using biuret method as described by Jacobs and Jacobs (8).

Determination of ATPase activity:

ATPase activity was measured by coupling the reaction to the pyruvate kinase and lactate dehydrogenase systems and measuring the oxidation of NADH spectrophotometrically at 340 nm. The composition of the reaction mixture for ATPase assay was as described by Pullman et al. (9).