SHORT COMMUNICATIONS

Some Cellular Characteristics of the Epididymal Adipose Tissue in Lean and Obese-Hyperglycaemic Mice

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Summary. 1. The nitrogen content, total water content, *in vivo* ^{125}I -albumin space, the number and size of adipocytes per unit wet weight of epididymal fat pad and the plasma volume have been studied in lean and obese mice, and in obese mice on chronic restriction of their food intake. — 2. There were fewer, larger adipocytes per unit wet weight of tissue (in young obese mice) with a proportional decrease in the vascular space and water content, and the same nitrogen content as had epididymal adipose tissue from lean mice. — 3. There was a reduction

in the nitrogen content of epididymal adipose tissue from obese mice on a restricted diet. — 4. These findings are discussed in relation to the reduced glucose metabolism and lipolysis that are apparent when metabolic data are expressed per unit wet weight.

Key words: Adipose tissue, *ob/ob* mice, isolated fat cells, cell size, cell number, plasma volume, nitrogen content.

The enormous increase in body weight of genetically obese-hyperglycaemic mice can be entirely accounted for by an increase in the fat content of the carcass [1, 2] without a proportional increase in the fat-free dry weight [1–3] and water content of the carcass [4] or in the plasma volume [4, 5], indicating no compensatory rise in the lean body mass or body water. Further, this increase in fat content is not completely abolished by restriction of their food intake [6, 7]. This suggests that a large fraction of the excess fat, which is concentrated in adipose tissue, must be relatively metabolically inert. Other authors have commented on the abnormal histology of the adipose tissue of obese-hyperglycaemic mice [8, 9] which is also macroscopically different in appearance from lean adipose tissue, and it was clear that metabolic data on epididymal fat pads from obese mice would have to be evaluated carefully and referred to common cellular or physiological parameters and not to wet weight as is common practice. Measurements on the fat pad water and nitrogen content, the number and size of its constituent adipocytes, and the ^{125}I -albumin space *in vivo*, were made in order to aid the interpretation of *in vitro* data on these pads.

Experimental

The obese and lean mice, studied at 2–4 and 6–8 months of age, have been described in a previous publication [10]. Obese mice maintained on a restricted diet from one week after weaning, are designated *ob/ob*-RD [10].

Total Nitrogen after total acid digestion of the tissue was measured in an auto analyser by a modification of the Kjeldahl method.

Coulter Counting OsO_4 Fixed Cells

The number of adipocytes per mg wet weight of epididymal fat pads was estimated by fixing adipocytes in OsO_4 and counting them using a modification [11] of method III as described by Hirsch and Gallian 1968 [12]. 8 randomly selected 30 mg fragments from pads from lean mice and 12 from *ob/ob* mice were used to obtain the data for cell size in intact pads. 3 suspensions each from the entire fat pads from lean (45 mice) and *ob/ob* mice (9) were used for the data on adipocyte suspensions. 340 determinations were made on cells from pads from lean mice, 480 from pads from *ob/ob* mice, 1420 from cell suspensions from lean mice and 1820 from cell suspensions from *ob/ob* mice.

Measurement of Cell Size

The sizing of the cells was done with a Zeiss Particle Size Analyzer TGZ3 from magnified photomicrographs of the cell suspensions.

Measurement of Plasma Volume and the *in Vivo* ^{125}I -Albumin Space of Lean and Obese Epididymal Fat Pads¹

The volume of distribution of ^{125}I -human serum albumin was used as an index of plasma volume (assuming no significant loss in the urine in 20 min). Blood was collected after 20 min by cardiac puncture

¹ In collaboration with Jennifer Elliott.

of the anaesthetized mice after which both fat pads were totally excised and chilled in clamps frozen in liquid nitrogen. Each weighed pad was homogenised separately in concentrated Teepol (a commercial liquid detergent) in the presence of a silicone antifoam agent and an aliquot counted directly in a gamma counter. The efficiency of counting ^{125}I -Albumin in 5 ml of Teepol was found to be 68% of the counts without Teepol, and the counts of ^{125}I -Albumin in the Teepol homogenate were corrected for this error.

Total Water Content

The fat pads were placed in tared vessels, weighed, and then heated in an oven at 35–40°C until no further reduction in weight occurred.

Results

In Table 1 are shown some of the cellular characteristics of the epididymal fat pads from lean and obese mice. No significant difference was observed in the nitrogen content per unit wet weight between pads from lean and obese mice either at 2–4 months or at 6–8 months of age irrespective of the weight of the sample analysed in agreement with some previous studies [3, 8, 13], although Marshall *et al.* [14] have reported it to be 30% of the lean value. The nitrogen values in Table 1 also show a significant rise with age in the nitrogen content of the obese fat pad. The nitrogen content of pads from obese mice on a restricted diet was found to be significantly reduced and was less than that found in lean or obese mice at 6–8 months of age.

Fig. 1. Histogram of percentage distribution of diameter of fixed adipocytes from ♂ lean and *ob/ob* mice 2–4 months old. *Pad*: Fixed cells prepared by incubation with 2% OsO_4 from intact pads by method of Hirsch [12]. *Cells*: Prepared by digestion with collagenase, followed by fixation with 2% OsO_4 for between 3–24 h. The median size of the 4 populations is given in the Fig. *Ordinate*: The number of particles sized as a percentage of the total number sized after multiplying by a correction factor. *Abscissa*: Particle diameter in μ on an exponential scale

Table 1. Cellular characteristics of lean and obese epididymal fat pads

	2–4 months		6–8 months	
	Lean		Lean	
Nitrogen content $\gamma\text{N}/\text{mg}$				
< 300 mg pad	—	2.09 ± 0.09 (6)	2.78 ± 0.17 (29)	2.42 ± 0.24 (12)
> 300 mg pad	1.77 ± 0.14 (4)	1.52 ± 0.06 (15) ^c	2.03 ± 0.16 (8) ^f	2.30 ± 0.22 (12) ^b
No. of cells per mg	2270 ± 285 (8)	605 ± 50 (12) ^a	—	—
Water content $\mu\text{l}/\text{mg}$	—	—	0.081 ± 0.008 (6)	0.037 ± 0.004 (7) ^a
^{125}I -Albumin space $\mu\text{l}/\text{mg}$	—	—	0.029 ± 0.002 (7)	0.007 (2)
				ob/ob RD
				1.87 ± 0.20 (4)
				1.36 ± 0.11 (15) ^{d, e, f}

^a Significantly different from lean $p \leq 0.001$. ^b Significantly different from obese 2–4 months $p \leq 0.001$. ^c Significantly different from pads of < 300 mg $p \leq 0.001$. ^d Significantly different from lean $p \leq 0.01$. ^e Significantly different from *ob/ob* 6–8 months $p \leq 0.01$. ^f Significantly different from pads of < 300 mg wet wt. $p \leq 0.05$.

Experimental details are given in the text. Values are given per mg fresh weight as the mean ± the S.E.M. with the number of determinations in parenthesis. The nitrogen content was determined on pads of differing wet weight and the results presented in two groups based on wet weight of the pads.

In young mice, the number of adipocytes per unit wet weight of fat pads from lean mice was nearly fourfold greater than in obese mice and these same fixed cells can be seen in Fig. 1 to be very much smaller in size. The median size of 480 fixed adipocytes from 6 *ob/ob* mice was 138 μ (range 113–217 μ) and 77 μ (range 52–106 μ) for 340 fixed adipocytes from 4 lean mice. Particle sizing of suspensions of adipocytes prepared by collagenase digestion [10, 15] showed a reduction in the median size of the population of particles counted from both lean and obese mice. This was particularly marked in suspensions from *ob/ob* mice showing that adipocytes from these animals were more vulnerable to lysis caused by this preparative procedure.

The plasma volume of lean mice was found to be 1.49 ± 0.05 ml (12) and this agrees well with published data on the plasma volume of mice by other methods [4, 5, 16]. The plasma volume of obese mice (1.99 ± 0.16 ml (5)) was found to be slightly but significantly higher ($p < 0.01$) than that found in lean mice, and the increase, similar to that found by Yen [5], was consistent with the small 10–20% increase in the total body water content of these mice.

Discussion

There has been considerable controversy in the literature over whether adipocytes increase in number and/or size in obesity. Investigators have also come to varying conclusions as to what changes occur in lipid, protein and water composition of adipose tissue in human obesity [17, 18], in obese mice [3, 4] and obese rats [19].

The present study provides strong evidence that the increased size of the epididymal fat pad in the young obese mouse occurs largely through an increase in size of adipocytes [20] as the number of fat cells per unit wet weight of fat pad from obese mice was considerably less. A similar conclusion has been made in mice made obese by hypothalamic lesioning [21] but different results were obtained using subcutaneous adipose tissue from older *ob/ob* mice [22]. Furthermore the results presented show that the decreased water content and plasma volume (as a percentage of carcass weight) encountered in the obese mouse, is associated with a reduction in the water content and *in vivo* ¹²⁵I-albumin space per unit wet weight of fat pad. Assuming that an increase in adipocyte size occurs largely by increase in the lipid content of the cell [23], then the fact that the amount of nitrogen per unit wet weight in pads from obese mice was not less than those from lean mice, must in all probability be due to increases in structural stromal proteins and/or cell types other than adipocytes [8, 9].

The decreased metabolism of glucose per unit wet weight, which has been demonstrated in the adipose tissue of the obese mouse both *in vitro* [8, 10] and *in*

vivo [2, 24], could be explained by the reduced number of adipocytes in the tissue; it has been shown that glucose metabolism in human adipose tissue fragments is dependent on the number of fat cells and not on their size [25]. Furthermore the decreased insulin sensitivity in the isolated fat pad of the obese mouse [10] could be explained by the increased size of the cells according to the theory [25] that insulin sensitivity is inversely related to cell size.

One of the problems which arises in interpreting data on metabolic processes and hormonal control in isolated fat cell preparations, is emphasized in the present work by the finding that during the fat cell preparation the large cells of the adipose tissue appear to undergo lysis to a much greater extent than the small cells, and this finding was particularly marked in the case of adipose tissue from obese animals.

There is much evidence showing that there is no loss of cell number in adipose tissue during acute starvation in the human [26], lean rat [27–29], obese rat [19], or during chronic restriction of diet for 3–5 months in rats [21] or obese mice [9]. However, the unelevated weights of the epididymal fat pads from acutely starved (11 days) obese mice were not associated with a significant reduction of the mean adipocyte size in epididymal adipose tissue (although great reduction occurred in subcutaneous adipose tissue) [30]. A probable explanation for this paradox is that weight reduction of the epididymal fat pads may have occurred by mobilisation of fat from small fat cells, this further reduction in their size probably resulting in their not being recognized as fat cells. The significant reduction in the nitrogen content per unit wet weight in the epididymal fat pads from obese mice on a restricted diet could thus alternatively be explained by a reduction either in the numbers of non-adipocyte cell types or stromal proteins. The ready release of fat from this tissue, which was covered with oily droplets soon after excision from the animal, also suggested that the lower nitrogen content of this tissue may be related to a release of intact protein known to occur in adipocytes and fat pads in whom lipolysis has been stimulated [31].

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