FAT CELL SIZE AND LIPID CONTENT
OF SUBCUTANEOUS TISSUE IN
CONGENITAL GENERALIZED LIPODYSTROPHY

By

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ABSTRACT
Fat cell size and lipid composition of subcutaneous tissue from 3 patients
with congenital generalized lipodystrophy have been measured before
and after treatment with either pimozide, fenfluramine or hypophysectomy. The fat cell volume before treatment ranged between $9.8 \times 10^4$
and $17.7 \times 10^4 \ \mu m^3$, compared to $9.0 \times 10^4$ and $85.3 \times 10^4 \ \mu m^3$ in 15
controls. The amount of lipids was only $1/10$ to $1/50$ of normal, triglycerides
being the most heavily reduced lipid component. Neither the fat cell size nor the lipid content were affected by treatment
with hypophysectomy, pimozide or fenfluramine.

Congenital generalized lipodystrophy is characterized by extreme paucity of
fat in adipose tissue, increased rate of growth, muscular hypertrophy, and
organomegaly. The lipid and carbohydrate metabolism is seriously disturbed.
The disease is inherited as an autosomal recessive trait. The complex clinical
picture and the laboratory findings have recently been described in detail
(Seip 1971; Oseid 1973a,b; Søvik & Oseid 1973; Søvik et al. 1976). In this
paper, the volume of the fat cells in the subcutaneous tissue has been compared
with chemical analysis of the tissue lipids. Additionally it was of interest to
study the effect of treatment with certain drugs known to affect the hypothala-
mic function (pimozide, fenfluramine) and reported to be of value in the
treatment of some patients with generalized lipodystrophy (Trygstad et al.
Three patients with congenital generalized lipodystrophy, two females (A.E., 21 years and I.T., 12 years) and one male (T.H., 7 years), were studied. The clinical data have been reported previously (Seip 1959; Seip & Trygstad 1963; Oseid 1973a,b). Patient T.H. was studied both before and after 12 months of pimozide (Orap®) treatment, and patient A.E. before and after hypophysectomy. Patient I.T. was studied again at 15 years of age following treatment with fenfluramine (Ponderal®) for one year. Biopsies of subcutaneous tissue from the patients were obtained from the abdomen during local anaesthesia, and in one of the patients also from the gluteal region and the thigh.

In all controls, the biopsies were obtained from the abdomen, either as an open biopsy during abdominal surgery, or by needle aspiration. The controls were 4 healthy females of normal weight, 20–21 years of age; 5 females 12 to 15 years of age undergoing abdominal surgery or general anaesthesia because of cystoscopy; and 6 males 6 to 8 years of age undergoing general anaesthesia because of rectoscopy or operation for hypospadia or orchidopexy. There was no statistically significant difference in the fat cell size between these groups, and the results were therefore combined into one control group. This is also in accordance with the results from other groups (Brook et al. 1972; Bjørntorp et al. 1972; Novak et al. 1971; Dauncey & Gairdner 1975).

**Microscopic examination**

Specimens from all biopsies were stained with Oil red O and Sudan III (Pearse 1968).

**Fat cell volume**

Fat cell size was measured according to Sjöström et al. (1971).

Slices from the subcutaneous tissue both from our patients as well as from controls, contained in addition to fat cells also fat droplets which represented damaged and broken fat cells. Only cells with a diameter above 30 μm were identified as adipose cells and used in the calculations, because of the difficulty in differentiating very small adipose cells from fat droplets. Using this procedure, the fat cell diameters in the biopsies from our patients approximated a Gaussian distribution. This indicates that the 30 μm limit differentiates fairly well between adipose cells and fat droplets produced as artifacts during the preparation of the slices. The presence of a population of very small fat cells would, however, be overlooked by this method. If so, the mean cell volume of the fat cells has been overestimated in our three cases.

**Lipid analysis**

Lipids were extracted with chloroform and methanol as described by Bligh & Dyer (1959). The chloroform extracts were washed once with methanol–H₂O (1:1), and then taken to dryness under a stream of nitrogen. The lipids were then dissolved in a small volume of isopropanol. Phospholipids were determined according to Zilversmit (1958), and the results calculated assuming a molecular weight of lecithin of 730. Total cholesterol (Levine & Zak 1964) and triglycerides (Kessler & Lederer 1966) were determined by Auto-Analyzer methods.
RESULTS

Microscopic examination

Light microscopy of fat-stained preparations showed an abundance of connective tissue with only scattered groups of fat cells in all the biopsies (Fig. 1). The biopsies from the abdomen showed the highest number of fat cells and were therefore used to measure the fat cell size. Fig. 2 shows an unstained preparation of adipose tissue from patient I.T. It is readily apparent that the fat cell diameter differs widely, as is also found in normals (Sjöström et al. 1971).

Fat cell volume

The mean volume of fat cells in the subcutaneous tissue is presented in Table 1. In patient A.E. the first biopsy was taken 2 months before transphenoidal hypophysectomy, the second 6 months after. Patient T.H. was studied before and after 12 months of pimozide treatment, and patient I.T. before and after 12 months of fenfluramine treatment.

Fig. 1.
Subcutaneous tissue from patient T.H. before treatment with pimozide.
The preparation is stained with Sudan III. Magnification × 25.
Table 1 shows that the fat cell size in our three patients varied between $9.8 \times 10^4$ and $17.7 \times 10^4 \, \mu \text{m}^3$. In comparison, a group of 15 controls had a mean volume of $40 \times 10^4 \, \mu \text{m}^3$, and the range was $9 \times 10^4$ to $85 \times 10^4 \, \mu \text{m}^3$, which agrees with the results reported from children and young adults (Björntorp et al. 1972; Brook et al. 1972) when re-calculated using 0.915 g/ml as the density of the fat cells (Sjöström et al. 1971). The fat cell size of our patients are thus comparable to the lowest range found in these control children. Further, newborns are reported to have about the same fat cell size as found in our patients (Dauncey & Gairdner 1975; Novak et al. 1971; Whitelaw 1977).

Neither hypophysectomy in patient A.E., pimozide treatment in patient T.H. nor fenfluramine treatment in patient I.T. caused any significant changes in fat cell volume. The apparent 40% change in fat cell volume in patients I.T. and T.H. only reflects an approximate 10% change in the mean fat cell diameter. The method for measuring the fat cell diameters has a coefficient of variation of 2.5% at cell diameters between 85 $\mu$m and 110 $\mu$m (Sjöström et al. 1971). The volume determination would therefore be expected to vary with up to $10 \times 10^4 \, \mu \text{m}^3$ due to the method alone, and in addition there will be differences between different biopsies.
Table 1.
Mean cell volume of fat cells in subcutaneous tissue.

<table>
<thead>
<tr>
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<th>Mean cell volume (µm³ × 10⁴)</th>
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<tr>
<td></td>
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<tr>
<td>*Patient A.E.</td>
<td></td>
</tr>
<tr>
<td>Before hypophysectomy</td>
<td>9.8</td>
</tr>
<tr>
<td>After hypophysectomy</td>
<td>11.1</td>
</tr>
<tr>
<td>Patient T.H.</td>
<td></td>
</tr>
<tr>
<td>Before treatment with pimozide</td>
<td>11.7</td>
</tr>
<tr>
<td>After treatment with pimozide</td>
<td>16.5</td>
</tr>
<tr>
<td>Patient I.T.</td>
<td></td>
</tr>
<tr>
<td>Before treatment with fenfluramine</td>
<td>17.7</td>
</tr>
<tr>
<td>After treatment with fenfluramine</td>
<td>10.2</td>
</tr>
<tr>
<td>Controls (n = 15)</td>
<td>9.0 – 85.3</td>
</tr>
<tr>
<td>(Mean 40.1)</td>
<td></td>
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</tbody>
</table>

* In patient A.E. the mean of three different slices from the same biopsy is given, while the mean of two slices is given for patients T.H. and I.T. In the controls one slice per biopsy was measured. Both the range and the arithmetic mean are given.

Lipid content and composition

In man the total lipid content in biopsies from subcutaneous fat tissue ranges from 60% to 85% of wet weight. There is nearly no difference between slim and obese persons (Pawan & Clode 1960; Thomas 1962), and triglycerides constitute more than 90% of the total lipids (Galton 1971). Table 2 shows the amount of triglycerides, phospholipids, and cholesterol in the subcutaneous tissue from our 3 patients. The lipid content is drastically reduced, from approximately 1/10 of normal in patient T.H. down to about 1/50 of normal in patient A.E. The decrease in triglycerides accounts for most of the total lipid loss. Neither the hypophysectomy, treatment with fenfluramine, nor pimozide in patients A.E., I.T. and T.H., respectively, had any effect on the amount of lipid in the adipose tissue. This is consistent with the results from caliper measurements of skinfold thickness.

The highest amount of triglycerides was found in the subcutaneous tissue from the youngest patient (T.H.). The oldest patient (A.E.) had the lowest, and patient I.T. had an intermediate amount of triglycerides. This might
Table 2.
The lipid content of subcutaneous tissue.

<table>
<thead>
<tr>
<th></th>
<th>% of wet weight</th>
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<tr>
<td></td>
<td>Triglycerides</td>
</tr>
<tr>
<td><strong>Patient A.E.</strong></td>
<td></td>
</tr>
<tr>
<td>Before hypophysectomy</td>
<td></td>
</tr>
<tr>
<td>Abdominal</td>
<td>2.2</td>
</tr>
<tr>
<td>Gluteal</td>
<td>0.6</td>
</tr>
<tr>
<td>After hypophysectomy</td>
<td></td>
</tr>
<tr>
<td>Abdominal</td>
<td>0.5</td>
</tr>
<tr>
<td>Gluteal</td>
<td>0.2</td>
</tr>
<tr>
<td>Femoral</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Patient T.H.</strong></td>
<td></td>
</tr>
<tr>
<td>Before pimozide</td>
<td>5.0</td>
</tr>
<tr>
<td>After pimozide</td>
<td>4.1</td>
</tr>
<tr>
<td><strong>Patient I.T.</strong></td>
<td></td>
</tr>
<tr>
<td>Before fenfluramine</td>
<td>2.1</td>
</tr>
<tr>
<td>After fenfluramine</td>
<td>2.3</td>
</tr>
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</table>

indicate that the triglycerides are decreased in subcutaneous tissue with increasing age in these patients.

A tentative estimate of total body fat was based on measurements of total body water and exchangeable potassium. This gave values for lean body mass higher than the actual body weight. Apparently, these patients have in addition to their pathological adipose tissue, abnormalities in the extra- and intracellular water and electrolyte content of muscle and connective tissue. It is generally accepted that such abnormalities invalidate the use of isotope dilution techniques to estimate total body fat (Lamotte et al. 1969).

**DISCUSSION**

The total lipid content of subcutaneous tissue in 3 patients with congenital generalized lipodystrophy was very low and might reflect a reduced number of adipocytes, a reduced content of lipid in each adipocyte, or both. An increased amount of collagen fibres also contributed to the result.
The mean fat cell volume was in the lower range of healthy controls, and a low content of fat in each adipocyte could therefore not explain the extensively reduced lipid content of the subcutaneous tissue. Our results rather indicated that these patients have a greatly reduced number of fat cells in their adipose tissues, or that a major fraction of their adipocytes for some reason do not accumulate lipids. Such "praeadipocytes" would not be recognized as adipocytes by the method used.

The fact that the fat cells are small may be due to impaired deposition of glucose and free fatty acids into each fat cell. This view is supported by in vivo studies on fat and carbohydrate utilization (Oseid & Pruett 1976), and by the fact that these patients have a markedly reduced number of insulin receptors on their monocytes, combined with highly increased insulin resistance, (Oseid et al. 1977). However, it conflicts with the opinion of Letarte & Fraser (1969).

Treatment with pimozide or fenfluramine did not significantly change cellularity or lipid content of the subcutaneous tissue, neither did hypophysectomy. Nevertheless, neuropeptides from the urine of these patients cause generalized lipodystrophy in animals (Foss & Trygstad 1975), possibly in part by blocking insulin receptors (Oseid et al. 1977), and an autopsy report (Berge et al. 1976) has likewise indicated that the primary defect is a hypothalamic pituitary disturbance. However, these studies do not resolve the question whether the primary disorder lies in the fat cell (Schwartz et al. 1960) or in the hypothalamus.

REFERENCES


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