Weight loss normalizes the inhibitory effect of \( N^6 \)-phenylisopropyl)adenosine on lipolysis in fat cells of massively obese human subjects

Jorma J. OHISALO,1,2 Johanna M. KAARTINEN,2 Susanna RANTA, Pertti MUSTAJOKI,3
Stanely P. HRENIUK,4 Kathryn F. LANOUE3 and Louis F. MARTIN3,4

1Department of Medical Chemistry and 2Department of Medicine, University of Helsinki, Helsinki, Finland, and 3Department of Molecular and Cellular Physiology and 4Department of Surgery, PennState University College of Medicine, Hershey, Pennsylvania, U.S.A.

(Received 25 March/26 May 1992; accepted 11 June 1992)

1. Fat cells were isolated from massively obese patients at or before gastric bypass, from other patients after normalization of body weight after gastric bypass or gastroplasty (post-bypass patients) and from control subjects of a stable normal body weight.

2. The inhibition of isoprenaline-stimulated lipolysis by \( N^6 \)-(phenylisopropyl)adenosine in the presence of adenosine deaminase was much attenuated in cells from the massively obese patients as compared with those from normal-weight control subjects, but was normal in cells from post-bypass patients.

3. Isolated fat cells of the massively obese patients were larger (913 ± 197 pl, mean ± SEM) than those of the normal-weight group (437 ± 95 pl). The volume of cells from the post-bypass patients was only 125 ± 49 pl, although the body mass index of this group was almost exactly the same as that of the normal-weight control subjects.

4. Although epidemiological studies have suggested that genetic factors are important in the development and maintenance of obesity, these results demonstrate that the changes observed in the inhibitory regulation of lipolysis in obesity are secondary.

INTRODUCTION

Although it is evident that obesity can only develop under a positive energy balance, there is much evidence that it has a strong genetic background. In a large study of adoption, the relative body weight of the adopted person was not correlated with that of the foster parents but was strongly correlated with that of the biological mother [1,2]. It is also clear from scientific studies, everyday medical practice and common experience that although almost anybody can lose weight by dieting, this loss is practically never permanent. All this speaks for a role of metabolic factors in the development and maintenance of obesity, although the extent of their importance is not yet clear.

Lipolysis, the release of free fatty acids and glycerol from adipocytes, is stimulated by \( \beta \)-adrenoceptors and inhibited by \( \alpha \)-adrenoceptors, insulin and adenosine [3–6]. Adenosine is a local effector that is released from nerve endings or formed from ATP released in this way [6]. This nucleoside has a very short half-life and therefore can only exert a local effect [6]. The response of the adipocyte to the inhibitory action of adenosine is modulated by many conditions, such as hypothyroidism [7,8], ageing [9] and lactation [10]. The regulation of lipolysis is altered in morbid obesity. The concentration of free fatty acids in plasma is elevated, but by less than one would expect with the increased adipose tissue mass if the rates of lipolysis were unchanged. Indeed, turnover studies with labelled free fatty acids in plasma show that lipolysis is inhibited \textit{in vivo} in obese subjects [11]. However, fat cells isolated from obese donors have been shown to display an attenuated response to inhibitors of cyclic AMP accumulation and lipolysis, such as adenosine [12], clonidine and prostaglandins [13]. We have recently reported that there is more adenosine in the adipose tissue of obese than in normal-weight subjects. This may explain the inhibition of lipolysis \textit{in situ} and, by homologous and heterologous desensitization, the decreased inhibition by different agents in isolated cells [14]. This could be one of the molecular mechanisms causing and/or maintaining obesity. Comparisons between obese and normal-weight subjects cannot differentiate between primary and secondary factors. With the good results of modern obesity surgery and the concentration of a number of such operations in our hospitals, it has now become possible for us to investigate massively obese patients and such patients after bariatric surgery when they have reached a normal or almost normal body weight in order to determine whether alterations in adenosine

Key words: adenosine, adenylate cyclase, cell size, gastric bypass, hormone regulation, metabolism, obesity.

Correspondence: Dr Jorma J. Ohisalo, Department of Medical Chemistry, University of Helsinki, Siltavuorenpenkeri 10a, 00170 Helsinki, Finland.
sensitivity are secondary or are genetically pro-
grammed.

MATERIALS AND METHODS

Patients and subjects

Three different groups of tissue donors were
studied. The massively obese group consisted of
patients that underwent or were considered for a
vertical banded gastroplasty or gastric bypass. The
post-bypass group consisted of patients that had
been massively obese but had reached a normal
weight after such an operation. The post-bypass
patients had lost an average of 44% of their initial
body weight. The time between the operation and
tissue sampling was 31.8 ± 8.1 months and a stable
body weight had been achieved. The control group
consisted of subjects of a stable normal weight that
had undergone routine surgery and donated a
sample at that time or volunteered to give a surgical
adipose tissue biopsy under local anaesthesia. Fig. 1
shows the body mass indices (weight in kg divided
by the square of height in m) of the groups used in
this study. The initial body mass index of the post-
bypass group was the same as that of the obese
patients and the present body mass index was very
close to that of the normal-weight control subjects.
All subjects except for one in the obese group were
female; their ages were 36.0 ± 4.4, 38.4 ± 4.5 and
45.8 ± 5.5 years (means ± SEM) for the normal-
weight, obese and post-bypass groups, respectively.
One of the obese patients and one of the post-
bypass patients had diet-controlled type 2 diabetes;
their dose–response curves were not different from
those of the others in their respective groups and
there was one such patient in each group so that
this fact probably did not affect the comparison of
these two groups.

All samples were taken from lower abdominal
subcutaneous adipose tissue.

Preparation of fat cells and studies of lipolysis

Fat samples of three subjects in each group were
taken under general anaesthesia in the morning
after an overnight fast. Glucose was not infused. A
wedge-shaped sample of fat was removed at the
beginning of the operation and placed in warm
saline solution (150 mmol/l NaCl) for transportation
to the laboratory. Since it was not possible to
obtain all such samples in elective operations, the
samples of two subjects in each of the three groups
were taken from the lower abdominal subcutaneous
adipose tissue under local anaesthesia with 1% (w/v)
lignocaine.

Fat cells were isolated by a modification of the
method of Rodbell [15] in the presence of collagen-
ase (2 mg/ml) under constant shakag at 2 Hz at
37°C in a buffer containing 125 mmol/l NaCl,
5 mmol/l KCl, 1 mmol/l CaCl2, 2.5 mmol/l MgCl2,
1 mmol/l KH2PO4, 4 mmol/l glucose, 2% BSA and
25 mmol/l Tris at pH 7.4. After 20 min, the cells
were filtered through a nylon cloth and were
washed three times with the same buffer without
collagenase. Median cell diameter was estimated by
direct microscopy of isolated cells. For studies of
lipolysis, 100 μl of isolated fat cells was incubated
for 60 min in 1 ml of the same buffer at 37°C under
constant shaking at 1 Hz with the addition of
adenosine deaminase (1 μg/ml) and other effectors as
described below. Adenosine itself cannot be used in
metabolic studies with adipocytes because it is
rapidly taken up by the cells and phosphorylated or
deaminated. Fat cells also release adenosine into the
incubation medium, sometimes enough to cause full
inhibition of lipolysis. Therefore, we added adeno-
sine deaminase to the medium to remove endoge-
nous adenosine and then added different concen-
trations of N6-(phenylisopropyl)adenosine. This
analogue is neither a substrate nor an inhibitor of
adenosine deaminase and cannot be phosphorylated,
but it is an adenosine receptor agonist. Adenosine
deaminase was dialysed against 150 mmol/l NaCl
immediately before use. The incubations were termi-
nated by adding 100 μl of 35% perchloric acid
followed by centrifugation and subsequent neutrali-
zation or by boiling the samples for 2 min. Glycerol
was assayed spectrophotometrically or chemilumi-
metrically [16].

Reagents

Adenosine deaminase (type VIII from calf intesti-
tine), R-N6-(phenylisopropyl)adenosine, fatty acid-free
bovine fraction V albumin and isoprenaline HCl
were purchased from Sigma Chemical Co. (St Louis,
MO, U.S.A.), collagenase from Worthington Bio-
Statistical analysis

Factorial and two-way repeated measures analysis of variance were performed using the Macintosh StatView 512 program.

RESULTS

We have shown that the inhibitory response of adenylyl cyclase to N6-(phenylisopropyl)adenosine is attenuated in fat cells [12] and in fat cell plasma membranes [14] in obesity. Therefore, it was of interest to see if this change could be reversed by weight loss. Adipocytes were isolated from the three groups of tissue donors. The volumes of the cells from obese patients, normal-weight control subjects and post-bypass patients were 913±197, 437±95 and 125±49 pl, respectively (means ±SEM). All groups were different from the other groups (P<0.05 by analysis of variance). Inhibition of isoprenaline-stimulated lipolysis in the three groups of isolated cells by N6-(phenylisopropyl)adenosine in the presence of exogenous adenosine deaminase is shown in Fig. 2. Studies of lipolysis and cyclic AMP accumulation in human adipocytes are complicated by large interindividual variations in the basal and stimulated rates of lipolysis. It seems clear that larger cells can release more fatty acids and glycerol than small cells. Therefore, all comparisons of the responsiveness of adipocytes were performed by placing the values obtained on a scale from 0 to 100% of the rate of lipolysis in the presence of adenosine deaminase and 1 μmol/l isoprenaline in that fat cell batch. These rates averaged 61, 72 and 79 pmol min⁻¹ (μl of cells)⁻¹ in the normal-weight, obese and post-bypass groups, respectively, and were maximal as they were the same as those measured in the presence of 10 μmol/l 7β-Desacetyl-7β-[γ-(N-methylpiperazino)butyryl]forskolin, which stimulates adenylyl cyclase directly and maximally. It is evident from Fig. 2 that fat cells from obese donors were much less responsive to the inhibitory action of N6-(phenylisopropyl)adenosine as far as lipolysis was concerned. Cells from post-bypass patients had normal or almost normal responses. A two-way repeated measures analysis of variance gave p=0.046 for the null hypothesis that there is no difference in the dose–response curves between the three groups. This allows the statistical comparison between subgroups. The difference between obese and post-bypass patients was significant at P=0.029.

Similar comparisons of stimulation of lipolysis were complicated by the fact that the mere removal of endogenous adenosine from the incubations results in variable stimulation of lipolysis. Sometimes this stimulation was close to maximal. Also, it has been reported that basal lipolysis is more active in adipocytes from obese donors. Because of these problems, unequivocally explainable data on differences in the stimulation of lipolysis between the three groups could not be obtained.

DISCUSSION

The present results suggest that the response of adipocyte lipolysis to an adenosine analogue is attenuated in obesity but is corrected by weight loss. The comparisons were made after stimulation by isoprenaline and, therefore, a difference in the stimulatory effect of this β-adrenoceptor agonist could explain such apparent differences. However, there is plenty of evidence suggesting that this mechanism cannot be the cause of the difference in the responsiveness to adenosine. Studies with adipocytes isolated from both experimental animals [17–19] and man [20, 21] have shown a decreased re-
sponnsiveness to β-adrenergic stimulation of adenylyl cyclase, accumulation of cyclic AMP and lipolysis in obesity. The response of palmitate turnover to infusion of adrenaline is blunted in obesity [22]. A blunted response to isoprenaline in obesity would cause a higher and not a lower responsiveness to inhibitors.

Epidemiological studies suggest that obesity has a strong genetic background, although other factors are clearly involved as well. This suggests that there may be separate basic metabolic aberrations that lead(s) to the development and maintenance of obesity [1,2]. The reported higher adenosine content of adipose tissue in obese individuals [14], reflected in decreased sensitivity to adenosine in isolated cells due to a desensitization mechanism, could lead to the development and/or maintenance of obesity. The present results with adipocytes isolated from massively obese patients and such patients after reaching normal weight suggest that the difference in the sensitivity to inhibitors of lipolysis and cyclic AMP accumulation is secondary to the obesity and not due to a primary metabolic aberration that would lead to obesity. In accordance with this finding, body composition and plasma lipid concentrations of morbidly obese patients have also been reported to be normalized after weight loss [23].

It has been speculated that the changes observed in obesity are due to the fact that fat cells from obese individuals are larger and that such larger cells have altered sensitivity to hormones [24,25]. The present results are not necessarily in contradiction with this, but the very small cells of the post-bypass patients were no more sensitive to N6-(phenylisopropyl)adenosine than those of control subjects with the same present relative body weight. This could, of course, be explained by a non-linear correlation between cell size and responsiveness and also by assuming that there is still some resistance to the nucleoside even after weight loss. However, we have reported that plasma membranes of small human fat cells are no more responsive to inhibition of adenylate cyclase than those prepared from co-isolated larger fat cells of the same individual [14]. Together with this, the present results would fit better in a model where cell size per se does not regulate responsiveness.

The present results show that the attenuated inhibitory response to adenosine in fat cells isolated from obese patients is normalized by weight loss and, together with previous data, suggest that this normalization is not caused by altered cell size per se.

ACKNOWLEDGMENTS

Financial support was received from the Academy of Finland, the Sigrid Jusélius Foundation and the American Diabetes Association, J.M.K. was a recipient of the Juvenile Diabetes Foundation Summer Research Fellowship. The skilled technical assistance of Miss Iina Kajanoja is gratefully acknowledged.

REFERENCES