Relationship between the tissue level of cyclic AMP and the fat cell size of human adipose tissue

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Abstract The relationship between mean fat cell size, maximal tissue cyclic AMP concentration, and glycerol release was investigated in human subcutaneous adipose tissue incubated in vitro with or without isoprenaline or noradrenaline added at maximal effective concentrations. Basal and stimulated glycerol release and cyclic AMP concentration were each related to the fat cell size. Whether or not the phosphodiesterase inhibitor theophylline was present in the incubation system, basal and noradrenaline-induced cyclic AMP levels were significantly correlated with the fat cell size. The noradrenaline-induced cyclic AMP levels resulted in twice as rapid glycerol release as could be expected from the basal ratio between glycerol release and cyclic AMP. Furthermore, both basal and noradrenaline-induced glycerol release in relation to the cyclic AMP levels were more rapid in enlarged fat cells. It is concluded that basal and catecholamine-induced production of cyclic AMP is related to the fat cell size and that a quantitative relationship exists between rate of lipolysis and maximal tissue levels of cyclic AMP in human adipose tissue. Basal and noradrenaline-induced lipolysis are probably regulated by different mechanisms and the lipolytic sensitivity to cyclic AMP seems increased in large fat cells.

Supplementary key words lipolysis · adenylate cyclase · triglyceride lipase · phosphodiesterase

A major function of adipose tissue is to store and release fatty acids according to the requirements of the body. It is well established that the rate of lipolysis by human adipose tissue is strongly dependent on the adipocyte size so that large fat cells hydrolyze triglycerides more rapidly than do small cells (1–8). This relationship has been demonstrated both in the basal state and after stimulation with catecholamines, which are the only hormones with pronounced lipolytic activity in human adipose tissue (9).

The mechanism for increased lipolytic capacity of larger fat cells is as yet unknown. Since cyclic AMP (cAMP) mediates the action of lipolytic hormones (10), this study was undertaken to find out whether or not the relationship between the rate of lipolysis and the size of the human fat cell was dependent on the maximal tissue level of cAMP, which was observed after ten minutes of incubation (11). Experiments were performed with and without the phosphodiesterase inhibitor theophylline to allow studies on the relationship between cell size and the production of cAMP.

MATERIALS

Subcutaneous adipose tissue was obtained from 42 normal-weight patients of both sexes undergoing routine abdominal surgery and from 6 patients submitted to intestinal shunt operation because of obesity. None showed evidence of endocrine disorder. Their mean age was 40 (range 18–72) years. They were fasted overnight and only saline was given until adipose tissue was removed, which was done at the beginning of surgery. General anesthesia was induced with Narkotal (Astra, Sweden) and maintained with Leptanal (Leo, Sweden). The study was approved by the Ethical Committee of the Karolinska Institute.

METHODS

Adipose tissue, divided into portions of approximately 50 mg each, was pre-incubated for 30 min in Krebs–Henseleit–bicarbonate buffer with 40 mg/ml of dialyzed bovine serum albumin (Armour Pharmaceutical Co. Eastbourne, England. Lot No. R 970). After 2 hr of incubation in the same type of medium, aliquots were removed for glycerol determination (12). When cAMP was determined, adipose tissue was pre-incubated and incubated in albumin-

Abbreviations: cAMP, cyclic AMP; NA, noradrenaline; ISNA, isoprenaline.

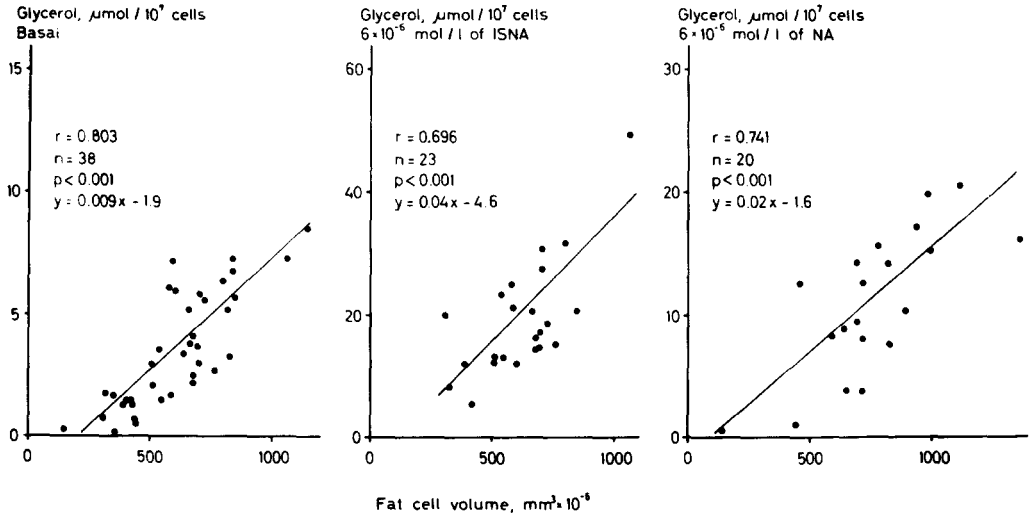

Fig. 1. Relationship between mean fat cell volume and glycerol release. Human subcutaneous adipose tissue was incubated for 2 hr in the presence or absence of 6×10^{-6} mol/l of isoprenaline (ISNA) or noradrenaline (NA). $n = 3-5$ in the individual experiments.

free buffer, since albumin interferes with the cAMP assay (13). Unless otherwise stated, 10 mmol/l of theophylline (ACO, Sweden) was added to the buffer solutions in the cAMP experiments to inhibit phosphodiesterase. After 10 min of incubation, when the hormone-induced peak tissue level of cAMP is known to occur (11), adipose tissue was removed for determination of cAMP by a modification (13) of the protein-binding method of Gilman (14). Noradrenaline bitartrate (Astra, Sweden) and isopropylnoradrenaline-HCl (Winthrop, England) were added in vitro. Both agents were dissolved in the medium to give a final concentration of 6×10^{-6} mol/l, known to induce maximal stimulation of the rate of lipolysis and cAMP production (11, 15). Further details have been described elsewhere (16).

The fat cell diameter was determined by the method of Sjöström, Björntorp, and Vrána (17). One hundred cells were measured and the mean cellular content was calculated according to the method of Hirsch and Gallian (18). The fat cell number was calculated from the mean cellular triglyceride weight and the total triglyceride content of the fat portions. Statistical analyses were performed according to those described by Snedecor and Cochran (19).

RESULTS

The rate of glycerol release was a linear function of the mean fat cell size (**Fig. 1**) when adipose tissue was incubated under basal conditions or in the presence of noradrenaline (NA) or isoprenaline (ISNA). No influence of age and sex was observed.

It is seen in **Fig. 2** that whether adipose tissue was incubated in basal medium ($r = +0.67$, $P < 0.001$) or in the presence of NA ($r = +0.58$, $P < 0.01$), the maximal tissue level of cAMP was significantly correlated with the fat cell size. Also the ISNA-induced cAMP level was significantly correlated with the cell size ($r = +0.60$, $P < 0.001$).

Fig. 3 shows the effect of theophylline on cAMP in adipose tissue incubated under basal conditions. The cAMP level was cell-size dependent whether adipose tissue was incubated in the presence of theophylline ($r = +0.82$, $P < 0.001$) or not ($r = +0.64$, $P < 0.01$). The net increase of cAMP due to theophylline was also significantly related to the fat cell size ($r = +0.77$, $P < 0.001$). **Fig. 4** shows that similar results were obtained for the effect of theophylline on the NA-induced cAMP levels ($r = +0.89$, $P < 0.01$ with theophylline; $r = +0.92$, $P < 0.01$ without theophylline; and $r = +0.84$, $P < 0.01$ for net increasing effect of theophylline), but not on the ISNA-induced cAMP concentration (uncharted experiments). It is seen in Figs. 3 and 4, that the slopes of the regression lines for cAMP vs. fat cell size in the NA experiments are almost identical with those obtained under basal conditions, whether theophylline was present or not. On the other hand, the intercepts were more positive in the NA experiments.

In order to elucidate the lipolytic response to the cyclic AMP level of a given size of fat cell, we have in **Fig. 5** used the data of Figs. 1, 3, and 4, and then calculated the ratio between the glycerol

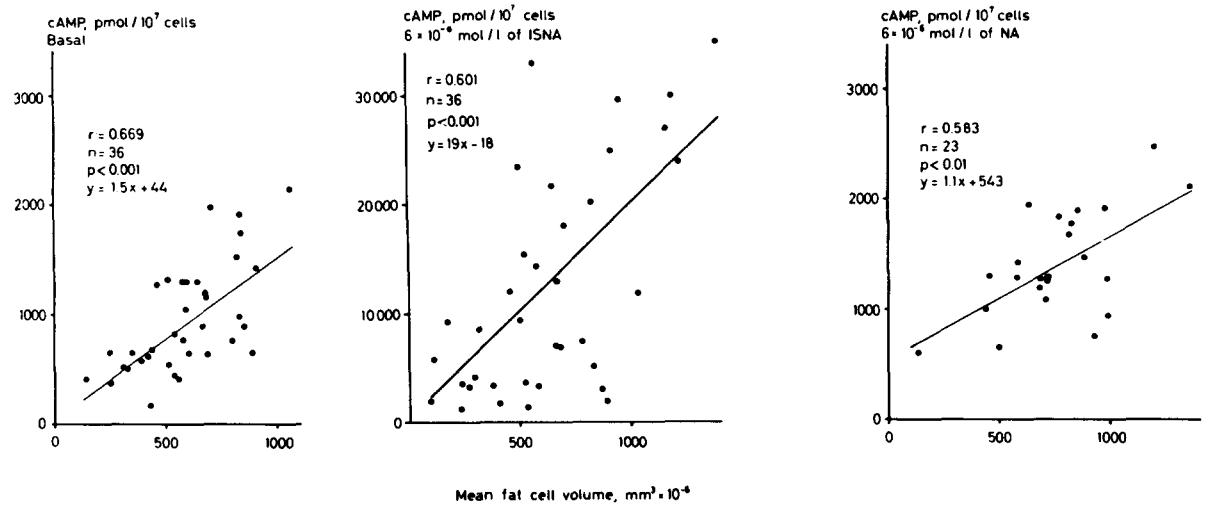

Fig. 2. Relationship between mean fat cell volume and maximal tissue level of cyclic AMP (cAMP). Human subcutaneous adipose tissue was pre-incubated in buffer containing 10 mmol/l of theophylline for 30 min and then incubated for 10 min in the same type of medium in the presence or absence of 6×10^{-6} mol/l of isoprenaline (ISNA) or noradrenaline (NA). $n = 3-4$ in the individual experiments.

release and the cyclic AMP level in experiments without theophylline. The rate of the glycerol release per unit cyclic AMP level was more pronounced for large fat cells, whether NA was present or not. Independently of the size of the fat cells, the rate of glycerol release was about twice as rapid from adipocytes exposed to noradrenaline as from those incubated under basal conditions.

DISCUSSION

The present investigation shows that the maximal cellular level of cAMP is significantly and positively related to the fat cell size. This correlation existed, first, whether or not theophylline was used in the buffer system and, second, whether or not adipose tissue was incubated under basal conditions or in the

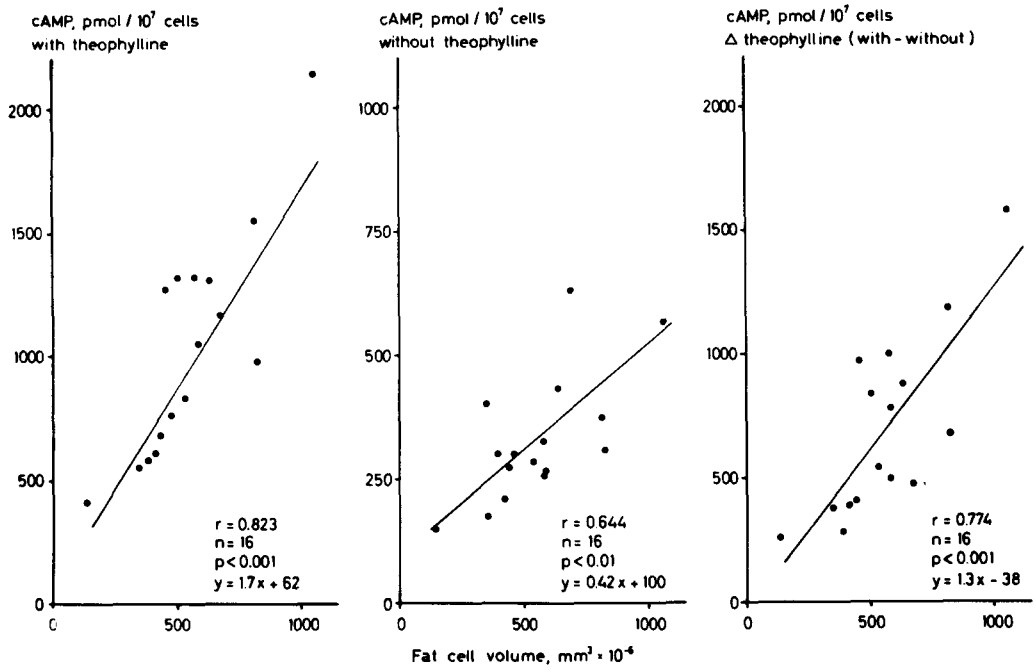

Fig. 3. Influence of theophylline on the relationship between mean fat cell size and basal tissue level of cyclic AMP (cAMP). Human subcutaneous adipose tissue was pre-incubated for 30 min and incubated for 10 min in Krebs–Henseleit–bicarbonate buffer with or without the addition of 10 mmol/l of theophylline. $n = 3-4$ in the individual experiments. Left panel records experiments with theophylline and center panel shows experiments without theophylline.

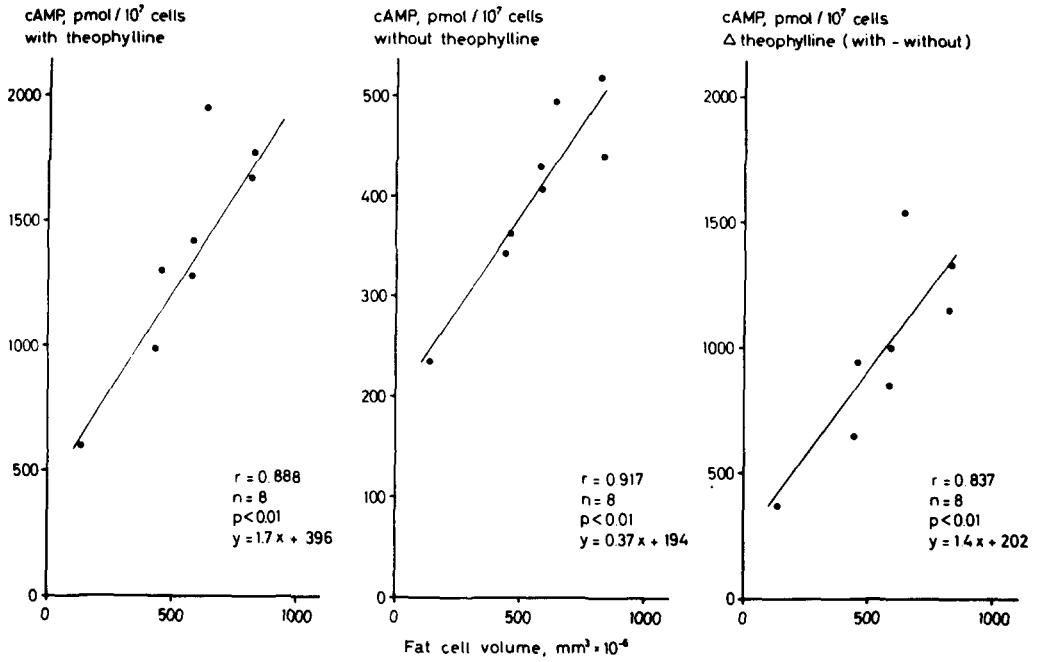

Fig. 4. Influence of theophylline on the relationship betwen mean fat cell size and noradrenaline-induced maximal tissue level of cyclic AMP (cAMP). Human subcutaneous adipose tissue was pre-incubated for 30 min in Krebs–Henseleit–bicarbonate buffer and then incubated in the same type of medium together with 6×10^{-6} mol/l of noradrenaline. To some pre-incubation and incubation buffers 10 mmol/l of theophylline was added. Theophylline experiments are in the left panel. The center panel records experiments without theophylline.

presence of ISNA or NA. Basal stimulated rates of lipolysis were also correlated with the fat cell size, which confirms several earlier studies in man (1–8). Thus, the increased capacity of large human fat cells to release fatty acids in the basal state and after catecholamine stimulation is well explained by the increased ability of the adipocyte to form cAMP. The close relationship between cAMP and fat cell size of human adipose tissue indicates strongly that, under basal conditions and in the presence of NA, cAMP originates predominantly from adipocytes and that stromal cAMP contributes very little to the tissue levels of cAMP. However, it might be suggested from Fig. 2 that some cAMP measured might come from the stroma, since a small positive intercept is observed in the regression line for the basal cAMP experiments.

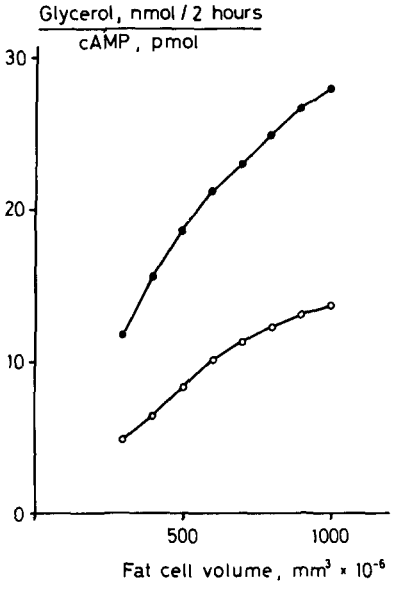

Fig. 5. Ratio between basal (○) or noradrenaline-induced (●) glycerol production and tissue level of cyclic AMP (cAMP) in human adipose tissue at different fat cell volumes. Glycerol and cAMP values for cell volumes in the range of 300–1000 $mm^3 \times 10^{-6}$ were obtained using the regression equations in the left and right panels of Fig. 1 (glycerol experiments) and the center panels of Figs. 3 and 4 (cAMP experiments without the use of theophylline).

It has been shown that the lipolytic sensitivity to NA is of the same magnitude in large and in small human fat cells (20). Therefore, the increased capacity of large fat cells to produce cAMP is not explained by an increased number of adrenergic receptors in the large cells. Nor can the results be due to reduced capacity to break down cAMP because of decreased phosphodiesterase activity in large adipocytes, since the effect of theophylline on the cAMP level was a linear function of the cell size. A more plausible explanation would be that the relationship between cAMP and fat cell size is due to increased adenylate cyclase activity in large fat cells.

The cyclic AMP concentration and the rate of lipolysis were also related to the fat cell size when adipose

tissue was incubated in the presence of a maximally effective concentration of ISNA (Fig. 1). This indicates that the maximal capacity of both triglyceride lipase and adenylate cyclase is increased in large fat cells in agreement with our previous findings (21). The interrelationship between lipolytic effect, cAMP concentration, and fat cell size supports a previous investigation where indirect evidence was given for a positive correlation between ISNA-induced cAMP level and fat cell size (11).

When Figs. 1 and 2 are compared, it is observed that ISNA is about twice as potent a stimulator of the rate of lipolysis as NA, whereas the effect of ISNA on cAMP production is approximately ten times as great as that of NA. This is in agreement with our previous observation (11) showing a semilogarithmic relationship between lipolysis (linear) and cAMP levels (log) in human adipose tissue incubated with ISNA.

It is reported that no correlation exists between the rate of hormone-induced lipolysis and the level of cAMP of the rat adipocyte (22), whereas the present investigation speaks for a quantitative relationship between rate of lipolysis and maximal cAMP concentrations in human fat cells. The difference between our results and previous findings (22) could be explained either by species differences or by the fact that we used intact adipose tissue, unlike most of the previous investigators who used isolated adipocytes. Although the experimental conditions for the cAMP and the glycerol experiments differed, some general information could be obtained from the results recorded in Fig. 5. First, about twice as much glycerol was produced per unit of cAMP when adipose tissue was incubated with NA in comparison with the basal state. This was true for small and for large fat cells and indicates that different mechanisms exist for basal and hormonal activation of lipolysis. Second, the exchange of glycerol produced per unit of tissue cAMP increased as the fat-cell volume increased. This was observed for both basal and NA-induced lipolysis and suggests that triglyceride lipase is more readily activated by cAMP when fat cells are enlarged. This conclusion is further supported by the comparison of the regression lines for basal and the NA experiments in Figs. 1, 3, and 4. In the absence of theophylline (center panels in Figs. 3 and 4) or in the presence of the phosphodiesterase inhibitor (left panels in Figs. 3 and 4) NA affects only the intercepts of the regression lines, showing that the net effect of NA on cAMP production is of the same order of magnitude in small and large fat cells. In the lipolysis experiments, however, shown in the left and right panels of Fig. 1, NA increased both the slope and the intercept of the regression curve. Thus, the net lipolytic effect of NA was more pronounced in larger fat cells.

Several reports have suggested that the hormone-induced cAMP level would represent an unphysiological overproduction of cAMP in adipose tissue (23–29) and that cAMP is sequestered into different compartments with only a small pool being responsible for the transmission of the hormonal effect (25, 26, 30–33). Our results are in contrast to those ideas, since the correlations between cAMP and lipolysis on the one hand and fat cell size on the other hand were observed both when the phosphodiesterase inhibitor theophylline was added and when it was omitted in the experiments with cAMP. Thus, cAMP seems to be produced and degraded in physiological amounts corresponding to the requirement for activating lipolysis by human adipocytes. Furthermore, the catecholamine-induced cAMP level of human adipose tissue very probably represents a single compartment of the nucleotide, as we have suggested earlier (11).

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