The effect of non-selective (theophylline) inhibition of cyclic AMP breakdown on norepinephrine stimulated lipolysis rate was investigated in subcutaneous adipose tissue of obese subjects. In addition, changes in interstitial glucose and lactate concentration were assessed by means of the microdialysis technique. The interaction of endogenous released insulin and theophylline on adipocyte metabolism was determined. Theophylline and norepinephrine alone increased glycerol outflow significantly. When both agents were perfused in combination, interstitial glycerol concentration increased further. The enhanced glycerol level due to theophylline application was slightly decreased by insulin. In the presence of theophylline, extracellular glucose concentration increased, in contrast to the catecholamine. Norepinephrine decreased interstitial glucose level. When both drugs were added in combination, the level of interstitial glucose increased to about 1 mM, greater than with theophylline alone. With each intervention, lactate was synthesized. Local adipose tissue blood flow was increased by theophylline and theophylline plus norepinephrine. In conclusion, post-receptor mechanisms increased norepinephrine maximal stimulated lipolysis rate in subcutaneous adipose tissue. Glucose uptake was inhibited by the non-specific inhibitor of phosphodiesterase. The effect of insulin on inhibition of lipolysis was modest but sustained in the presence of high theophylline (10^{-4} M) concentration. Phosphodiesterase activity may be relatively low in obese subjects in comparison with lean subjects. In lean subjects theophylline caused a transient reversal of the antilipolytic effect of insulin.

**Key words:** microdialysis, theophylline, lipolysis, adipose tissue, obesity
INTRODUCTION

In fat cells, a large number of metabolic processes are controlled by the adrenergic system including both fat and carbohydrate metabolism. Catecholamines are primary hormones for stimulation of fat cell metabolism, and the effects are induced by stimulation of the cAMP cascade. Metabolic events are mainly connected to increments in cAMP levels, cAMP protein kinase activation and phosphorylation of various target proteins (1). After stimulation of cAMP, hormone sensitive lipase (HSL) is phosphorylated and consequently lipolysis is increased (2). For carbohydrate metabolism, membrane associated carrier proteins, called GLUT4 and GLUT1 that bind glucose and facilitate its transfer across the plasma membrane, mediate glucose uptake into the fat cell. In vitro, it has been shown that small increments of cAMP concentrations in fat cells promote translocation of GLUT4 to the plasma membrane and stimulate glucose transport (3).

Phosphodiesterase is an enzyme with a crucial function in the cAMP cascade and fat cell metabolism. The enzyme breaks down cyclic AMP to inactive 5′-AMP so that phosphorylation of many downstream proteins is inhibited. Insulin exerts its antilipolytic effect by stimulation of a particular subtype (i.e., type 3) of phosphodiesterase (PDE3) so that intracellular cAMP content is decreased leading to a reduction in hormone-sensitive lipase activity and hence in lipolysis rate (4,5).

Theophylline, a member of the methylxanthine group of chemicals, is a nonselective inhibitor of phosphodiesterase. This means that, through inhibition of phosphodiesterase, the cAMP cascade remains fully active. Reports from previous studies investigating the effect of theophylline in lean subjects on subcutaneous adipose tissue metabolism have shown that the agent increased lipolysis rate (6). In addition, in the aforementioned study, the effect of theophylline on blood flow was investigated. The authors reported a vasodilatory effect of theophylline on vasculature, indicating an increase in blood flow (6).

Insulin is the major physiological antilipolytic hormone (7). The antilipolytic action of insulin was originally thought to be due to a decrease in tissue levels of cAMP (8), as the result of either inhibition of adenylate cyclase (5,9) and/or stimulation of cyclic nucleotide phosphodiesterase (10,11). Previous studies have demonstrated that the antilipolytic effect of insulin in fat cells is abolished in the presence of phosphodiesterase inhibitors of the methylxanthine class, such as theophylline (12). In vivo studies in subcutaneous adipose tissue of lean subjects have demonstrated that the antilipolytic effect of insulin, can be abolished by inhibition with the nonselective phosphodiesterase inhibitor theophylline (13).

In this study we aimed to investigate the phosphodiesterase effect on adipose tissue metabolism in subcutaneous adipose tissue of obese subjects by means of the microdialysis technique. Changes in lipolysis rate, e.g. interstitial glycerol concentration and blood flow, and changes in extracellular glucose and lactate concentration were assessed after perfusion of adipose tissue with the phosphodiesterase inhibitor theophylline. Further, the adrenergic agent
norepinephrine was administered to adipose tissue alone and in combination with theophylline to examine the interaction between stimulation of adrenergic receptors, a consequent increase in cAMP-concentration, and the inhibition of the breakdown of increased levels of cAMP.

In a second protocol an iv-glucose load was given to obese subjects. We intended to investigate whether, in the presence of theophylline, endogenous insulin could inhibit lipolysis rate.

METHODS AND MATERIALS

Subjects

The study group comprised 18 obese female subjects aged 29 - 50 years (mean 36.8 ± 7.8 SD years). Clinical characteristics are given in Table 1, and values are represented as mean ± SD. All women were premenopausal. Four subjects were on anti-hypertension medication (ACE-inhibitors). Two days before microdialysis experiments medication was stopped. Weight was stable in the three months prior to the study. Study participants performed no specific physical exercise program. The study was approved by the Ethics committee of Ulm University. The subjects were given a detailed description of the experiments and their written informed consent was obtained.

Table 1. Clinical characteristics of study subjects.

<table>
<thead>
<tr>
<th></th>
<th>female subjects (n = 18)</th>
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<tbody>
<tr>
<td>Body mass index (kg/m²)</td>
<td>40.9 ± 4</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>129 ± 4</td>
</tr>
<tr>
<td>Percent body fat (%)</td>
<td>40 ± 5</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>74 ± 16</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>136.5 ± 22</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>87.0 ± 14</td>
</tr>
<tr>
<td>HbA1 (%)</td>
<td>6.8 ± 0.7</td>
</tr>
<tr>
<td>Blood glucose (mg/dL)</td>
<td>99.4 ± 21</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>19.4 ± 12.9</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.9 ± 0.8</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>1.4 ± 0.9</td>
</tr>
</tbody>
</table>

Values are means ± SD

Study protocol

The subjects were investigated in the supine position at 8.00 am after an overnight fast. Body composition was measured by bioelectrical impedance analysis (BIA). For the microdialysis experiments, catheters (30 x 0.3 mm Cuprophane, 3000 Da cut-off, glued to 50 and 100 mm long sections of nylon tubing) were inserted in abdominal subcutaneous adipose tissue of the patients. No anesthesia was needed. The catheters were connected to a high-precision pump (Perfusor VI, Braun, Melsungen, Germany) that delivered a flow rate of 2.5 µL/min and the catheters were perfused with saline buffer. For blood flow measurement, ethanol (100 mM) was added to perfusion
solutions. Concentrations of ethanol were measured in the dialysate, and the ratio of ethanol outflow to inflow was determined (14). A low ratio indicates an escape of ethanol from the dialysate that, in turn, mirrors a rise in local blood flow. No dialysate was collected before 45 min after catheter implantation. Dialysate was collected every 15 min. The experiments started at time 0 min.

Protocol one: To investigate the effect of theophylline on metabolic activity with and without the presence of norepinephrine, three catheters were inserted in subcutaneous adipose tissue of 9 subjects each. Each catheter was perfused with saline for 60 min for the determination of the basal value of glycerol, glucose and lactate concentration in dialysate, and to estimate changes in blood flow. Thereafter, two catheters were perfused with either theophylline (10^{-4} M) or norepinephrine (10^{-4} M), respectively for 180 min. The third catheter was perfused with theophylline (10^{-4} M) and norepinephrine (10^{-4} M) in combination. Sixty min after basal measurement both agents were perfused simultaneously.

Protocol two: To investigate the effect of insulin on theophylline stimulated activity in subcutaneous adipose tissue, an iv-glucose load was given in the course of the experiment. One catheter was inserted in subcutaneous adipose tissue of 9 subjects each. Basal values of glycerol, glucose and lactate and changes in the ethanol ratio were determined for 60 min. Thereafter, theophylline (10^{-4} M) was added to the perfusion solution for a further 60 min. At time 120 min an iv-glucose load (0.5 g/kg lean body mass) was given intravenously. The end of the microdialysis procedure was at time 240 min.

Theophylline was obtained from Altana Pharma GmbH (Konstanz, Germany), and norepinephrine came from Hoechst Marion Roussel (Bad Soden, Germany).

Glycerol concentrations in dialysate were determined by bioluminescence (15). For glucose, lactate and ethanol measurement, two consecutive samples were combined. Glucose and lactate were measured electrochemically (YSI analyzer 2300 STST, Yellow Springs Instruments Co., Ohio, USA). Ethanol concentrations were determined by gas-chromatography (16).

Statistical analysis

Data are given as the mean ± SEM unless otherwise stated. The effects of the drugs over the study period were first analyzed by one-way ANOVA for repeated measurement with time as the factor of the analysis. Then a statistical comparison of the curves was performed using two-way ANOVA for repeated measures with time and drug as factors of the analysis and a Tukey post-hoc test. The software package SPSS/Inc. (SPSS, Inc., Chicago, IL) was used for statistical analysis.

RESULTS

The adipose tissue glycerol release measured under basal conditions for 60 min remained constant (Fig 1, Panel A). When the perfusion of theophylline (10^{-4} M) was started, the concentration of glycerol in dialysate increased about threefold during the next 45 min and then remained stable until the end of the experiment at time 180 min (F=19.2, p<0.001). When norepinephrine (10^{-4} M) was added to the perfusion medium, glycerol output increased more rapidly to about five times the basal value. In contrast to the stable glycerol level observed after theophylline stimulation, in the presence of norepinephrine, after the peak level at time 120 min, glycerol concentration gradually declined (F=23.4, p<0.001). When both agents were perfused simultaneously the pattern of glycerol release was almost identical to that observed with norepinephrine. However,
glycerol outflow increased further to about six times the basal level and the decrease in glycerol concentration was delayed in comparison with norepinephrine stimulation alone (F=32.9, p<0.001). Statistical comparison of the three curves revealed a significant difference, as judged by two-way ANOVA (F=4.3, p<0.05) and post-hoc analysis.

Fig. 1. Effect of theophylline (▲) (10⁻⁴ M), norepinephrine (●) (10⁻⁴ M), and theophylline (10⁻⁴ M) plus norepinephrine (10⁻⁴ M) in combination (○) on glycerol (Panel A), glucose (Panel B), and lactate (Panel C) dialysate concentration in subcutaneous adipose tissue of 9 obese subjects after basal measurement for 60 min. Panel D shows the effect on changes in the ethanol ratio. ▲ p<0.001 vs. baseline, ● p<0.05 vs. baseline, * p<0.05 theophylline plus norepinephrine vs. norepinephrine, † p<0.05 theophylline plus norepinephrine vs. theophylline.

Values are mean ± SEM.
The levels of glucose concentration in response to theophylline and norepinephrine stimulation are shown in Fig. 1, Panel B. The basal glucose concentration remained unchanged. The addition of the nonspecific phosphodiesterase inhibitor theophylline to adipose tissue increased the level of glucose in the dialysate significantly during the first 30 min and maintained this level during the remainder of the experiment (F=8.6, p<0.001). In contrast, norepinephrine induced a small, but significant decrease in dialysate glucose level at 90-120 min after the start of agent stimulation and this lower glucose level was unchanged until the end of the experiment (F=2.5, p<0.05). Perfusion with theophylline and norepinephrine in combination caused a steady increase in glucose concentration over the entire perfusion period (F=46.6, p<0.001). The final glucose concentration in the dialysate was over three times higher than the basal level. The three curves were significantly different (F=11.9, p<0.001). The increase in microdialysate glucose concentration in the presence of theophylline plus norepinephrine was significantly higher than with theophylline alone during the last hour of the study period as calculated by post-hoc analysis (p<0.05).

Studies for the evaluation of changes in lactate concentration in adipose tissue are shown in Fig 1, Panel C. The responses elicited in this study, an elevation of lactate concentration, were similar for theophylline, norepinephrine or both agents in combination (theophylline: F=8.6, p<0.001, norepinephrine: F=11.5, p<0.001, both agents in combination: F=3.6, p<0.001). The perfusion of theophylline plus norepinephrine induced a slightly higher increase in dialysate lactate concentration, however the concentrations were not significantly different from that induced by theophylline or norepinephrine alone.

The kinetics of adipose tissue metabolites may be influenced by changes in local blood flow. Fig 1, Panel D shows that blood flow remained unaltered under basal conditions. Similar results were obtained with both theophylline and theophylline plus norepinephrine, and these were markedly different from those obtained with norepinephrine stimulation. In situ perfusion with theophylline and theophylline plus norepinephrine induced a significant increase in local blood flow over the entire study period (theophylline: F=28.0, p<0.001, theophylline plus norepinephrine F=12.4, p<0.001). In contrast, norepinephrine decreased local blood flow (F=6.3, p<0.001), but returned back to baseline value during the course of the experiment.

To investigate the effect of insulin on stimulated phosphodiesterase activity a glucose load was given intravenously (Fig 2). Theophylline was first added to the perfusion solution at 60 minutes. In the presence of theophylline, glycerol concentration in the dialysate increased significantly (F=51.2, p<0.001) (Fig 2, Panel A). At time 120 min the iv-glucose load was given. In response to the glucose load, the extracellular glycerol concentration declined significantly (F=6.3, p<0.001), but was still elevated until the end of the study procedure.

The results for glucose concentration in response to theophylline treatment followed by the iv-glucose load are depicted in Fig 2, Panel B. Theophylline
increased extracellular glucose levels during the first 60 min of application (F=17.3, p<0.001). As expected, after the iv-glucose exposure glucose concentration increased further, the peak concentration being measured 30 min after iv-glucose. Thereafter, a sharp fall in glucose level was observed until the end of the study procedure (F=20.6, p<0.001).

Fig. 2. Effect of theophylline (▲) (10⁻⁴ M) on glycerol (Panel A), glucose (Panel B), and lactate (Panel C) dialysate concentration in subcutaneous adipose tissue of 9 obese subjects after basal measurement for 60 min. After 120 min an iv-glucose load (0.5 g glucose / kg lean body mass) was given. Panel D shows the effect on changes in the ethanol ratio. *p<0.001 vs. highest glycerol release.

Values are mean ± SEM.
The corresponding results for changes in lactate concentration are shown in Fig 2, Panel C. In the presence of theophylline and after the iv-glucose load the lactate level increased significantly (theophylline: $F=6.1$, $p<0.01$, theophylline plus iv-glucose load: $F=4.8$, $p<0.01$).

An increase in adipose tissue blood flow was induced by theophylline ($F=5.1$, $p<0.05$) (Fig 2, Panel D). The observed change in blood flow was not affected after the iv-glucose load.

DISCUSSION

Theophylline applied to subcutaneous adipose tissue of obese subjects increased glycerol concentration in the microdialysate. A constant marked stimulation of lipolysis was induced by the phosphodiesterase inhibitor. This finding is in accord with previous studies showing an enhanced glycerol concentration in interstitial fluid due to theophylline in subcutaneous adipose tissue of lean subjects (6,13). However, the theophylline effect differs in obese and lean subjects, such that, in obese subjects a lower theophylline concentration was sufficient to induce a similar increment in glycerol output to that observed in lean subjects (6,13). Thus, it appears that in lean subjects a higher concentration of theophylline is necessary to induce a similar increase in glycerol concentration. The mechanism underlying this difference may be a higher phosphodiesterase activity in lean than in obese subjects. Phosphodiesterase activity is stimulated by insulin (17), but in insulin resistant states such as obesity phosphodiesterase activity may be relatively low. Indeed it has been shown that in insulin-resistant IRS-1 (-/-) mice induction of phosphodiesterase activity by insulin was reduced (18). This effect could explain the observed difference between obese and lean subjects, since in our study obese subjects showed insulin resistance characterized by elevated HbA1 and insulin levels. Changes in phosphodiesterase activity may enhance free fatty acid (FFA) levels that are known risk factors for long-term development of glucose intolerance and progression to type 2 diabetes mellitus (19).

In general, whole-body energy homeostasis depends on the precisely regulated balance of lipid storage and mobilization. In adipose tissue lipolysis is exquisitely sensitive to inhibition by insulin and lipolysis is strongly stimulated by catecholamines in the circulation or released from sympathetic nerve terminals in adipose tissue. Disturbances in the regulation of lipolysis, like insensitivity of adipocytes to the antilipolytic effect of insulin or lowered catecholamine-mediated lipolytic response are of importance in the aetiology of obesity and type 2 diabetes mellitus (20). In the adipose tissue there is a fine network of adrenergic terminals distributed predominantly around the small arteriolar blood vessels but also innervating venous blood vessels and even directly innervating fat cells themselves (21). In addition, adipocyte lipolysis is regulated by other circulating counter-regulatory hormones (22). Moreover, adipose tissue itself is also an
endocrine tissue, releasing hormone signals to the brain, regulating food intake and energy homeostasis. One such hormone, leptin, is synthesized and secreted from the adipose tissue, enters the brain and interacts with its receptor in hypothalamic nuclei and regulates ingestive behaviour and energy balance. The effects of brain-adipose tissue signaling and brain-gut signaling on food intake and energy homeostasis may be crucial for lipid storage and mobilization (23).

During times of increased energy demand, lipolysis in adipocytes is activated by hormones such as catecholamines. It is well known that catecholamines enhance membrane adenylyl cyclase activity in fat cells and hence lipolysis rate by increasing cAMP concentration (24). Therefore, when norepinephrine was applied to subcutaneous adipose tissue, glycerol concentration increased significantly, as expected. In our study a high norepinephrine concentration was used to induce maximal glycerol release in interstitial fluid. However, sustained adrenergic stimuli or chronic administration of adrenergic agents may induce desensitization of adrenergic receptors (25). This phenomenon has been shown in vivo in subcutaneous adipose tissue of lean (26) and obese subjects (27). In the present study we observed that after the peak level, glycerol concentration declined in interstitial fluid, consistent with tachyphylaxis.

The perfusion of theophylline in combination with norepinephrine produced an additional increase in glycerol concentration in the microdialysate. This finding implies that when lipolysis is maximally stimulated by norepinephrine, the desensitization of adrenergic receptors may be the limiting factor for catecholamine-stimulated lipolysis rate. However, changes in post-receptor mechanisms could increase the lipolysis rate still further, as shown by the observed enhancement in microdialysate glycerol concentration after simultaneous perfusion of adipose tissue with theophylline and norepinephrine. Thus, there appears to be additional capacity to activate hormone-sensitive lipase, which is abundantly expressed in adipocytes, through downregulation of phosphodiesterase. Further, since the activity of phosphodiesterase may be diminished in insulin-resistant states (18) a chronic release of free fatty acids into the bloodstream would be expected to occur, a condition that would contribute to further insulin resistance and type 2 diabetes.

In the present study the antilipolytic action of insulin was investigated after pretreatment of adipose tissue with theophylline that inhibits the breakdown of cyclic AMP and increases lipolysis. After the iv-glucose load, endogenous insulin was released and in response the glycerol concentration in the dialysate decreased significantly by about 20%, but still remained at an elevated level. Insulin could not fully inhibit theophylline induced lipolysis and this finding implies that theophylline restricts the ability of insulin to inhibit lipolysis. It has been shown previously that insulin could not significantly decrease the extent of lipolysis obtained with 1-methyl-3-isobutylxanthine in fat cells from Wistar rats (7). Likewise, in situ studies in subcutaneous adipose tissue of lean human subjects, using the insulin clamp technique to investigate the effect of insulin on

theophylline stimulated lipolysis rate, demonstrated a small and transient, although significant, decrease in dialysate glycerol after the start of the insulin infusion (13). The authors reported that perfusion of adipose tissue with theophylline almost completely abolished the glycerol-lowering effect of hyperinsulinemia (13). In contrast in our study in obese subjects we observed a marked and steady decrease of glycerol concentration due to insulin stimulation. This finding may be explained by the higher production of leptin in obese than in lean subjects. It has been shown that leptin and insulin share similarities in their signal transduction pathways by activation of phosphodiesterase 3B (28, 29). Although the effect of leptin is less intense than that of insulin, both hormones are involved in the reduction of cAMP (28). In our study the high concentration of leptin, which strongly correlates with obesity, plus the endogenous insulin may have caused the steady decrease of glycerol outflow seen in the presence of theophylline.

The adipocyte is highly sensitive and responsive to insulin with regard to glucose transport (30). Insulin does this by activating translocation of GLUT4 vesicles to the cell membrane and promoting glucose disposal into adipocytes. The GLUT4 transporter may be modulated by cAMP, since it has been shown in rat adipocytes that the non-selective β-adrenoceptor agonist isoproterenol stimulates both GLUT4 translocation and glucose transport at low concentrations, although glucose transport is inhibited at high concentrations, due to an inhibition of the transport activity of cell surface GLUT4 in white adipocytes (31). However, as reported recently, glucose uptake occurs in adipocytes without the action of insulin via the α1-adrenoceptor (32). These mechanisms could explain our finding of decreased microdialysate glucose concentration in the presence of norepinephrine (Fig 1B).

In contrast, in the presence of theophylline we observed an increase in microdialysate glucose concentration that mirrors an increase in extracellular glucose level. This result suggests that a smaller amount of glucose has been taken up by the adipocytes compared with basal conditions. A possible explanation for this result is the concentration of theophylline used. It has been shown previously that treatment of isolated fat cells with the β-agonist isoproterenol in high concentration stimulated cAMP-dependent protein kinase and phosphorylation of a serine residue on the C-terminal of GLUT4 and inhibited glucose transport without decreasing the number of transporters (33). Further studies have shown that in rat adipocytes dibutyryl-cAMP causes a dose-dependent acute translocation of GLUT4 with an increased 2-deoxyglucose uptake at low dibutyryl-cAMP concentration, but an inhibition of 2-deoxyglucose uptake, to below basal levels, at high dibutyryl-cAMP concentration (34).

In the present study, when theophylline and norepinephrine were perfused simultaneously into adipose tissue, glucose concentration in the dialysate was forced up compared with theophylline alone. The result suggests an additive effect on cAMP concentration induced by norepinephrine stimulation and by the inhibition of cAMP degradation by theophylline. It is possible that glucose uptake into adipocytes was thereby inhibited by high cAMP-concentration.
In adipose tissue, glucose is metabolized to glycogen, CO$_2$, glyceride-glycerol, glyceride-fatty acids, lactate and pyruvate (35). In our study, lactate concentration increased in subcutaneous adipose tissue by perfusion with norepinephrine or theophylline, respectively. Interestingly, although both agents induced different responses in regard to changes in glucose concentration in extracellular fluid, e.g. an increase of glucose level in the presence of theophylline and a decrease of glucose level in the presence of norepinephrine, lactate was synthesized to a similar extent with either treatment. When both agents were perfused in combination, lactate synthesis was higher than was observed after theophylline stimulation alone. After the iv-glucose load, lactate synthesis increased even further. Our experiments revealed different increases in lactate synthesis in response to differential stimulation of lipolysis. In general, it has been shown that various conditions, either stimulation or inhibition of lipolysis, affect the rate of glucose metabolism to a great extent (36, 37). For instance, in epididymal rat adipocytes, lactate production is stimulated by norepinephrine (38). With the addition of insulin, all products of glucose metabolism are enhanced, but most of the glucose is converted to lactate (35). Thus, our findings are consistent with the observations of others, of lactate synthesis in adipose tissue (39,40) and the observation that an increase in insulin is followed by a release of lactate (41). The production of lactate may result from the uptake and glycolytic degradation of interstitial glucose by adipocytes. Alternatively, lactate may be synthesized from the degradation of glycogen stores within the adipocytes. The latter pathway may be important in the case of administration of theophylline to adipose tissue, when glucose concentration is increased in extracellular fluid and possibly the uptake of glucose by adipocytes is decreased.

Interstitial blood flow may be of importance for changes in metabolites observed in subcutaneous adipose tissue. An increase or inhibition in interstitial flow could reduce or increase the concentration of metabolites in the interstitial fluid and could lead to an incorrect interpretation of the data. Using the microdialysis ethanol technique, a decrease in the ethanol ratio and hence an increase in local blood flow, was observed in the presence of theophylline. This finding is in accordance with results from studies performed in subcutaneous adipose tissue of lean subjects (6). In contrast, norepinephrine induced a decrease in local blood flow, an observation that has been reported in previous studies (32). In the presence of both agents in combination, local blood flow increases. This finding implies that the vasodilatative effect of theophylline overcomes the vasoconstrictive effect of norepinephrine and corroborates the findings that PDE-inhibitors act as vasoactive compounds (42). In our study, in the theophylline experiment and after the iv-glucose load, local blood flow was increased. Other studies have found no evidence of any significant change in interstitial flow in adipose tissue during systemic insulin infusion (13). Insulin alone is believed not to have a direct effect on adipose tissue blood flow (43,32). Rather, it is likely to be an important mediator, possibly acting via adrenergic stimulation (43).
Therefore, the observed increase in blood flow in the present study may be due to
the effect of PDE-inhibition.

The observed alterations in glycerol, glucose and lactate concentrations might
theoretically be due to changes in adipose tissue blood flow. However, changes in
blood flow were not consistent with changes in metabolite levels. Thus, we
suggest that vascular cells and adipocytes are regulated independently in
subcutaneous adipose tissue.

In summary, this study indicates that theophylline induced lipolysis by
inhibition of phosphodiesterase activity in subcutaneous adipose tissue of obese
subjects. In comparison with lean subjects, phosphodiesterase activity may be
reduced in obesity. The inhibition of cAMP degradation could further increase
maximal adrenoceptor-stimulated lipolysis by norepinephrine. High levels of
phosphodiesterase inhibition decreased glucose uptake by adipocytes, an effect
that is strengthened in the presence of adrenergic activity, and may lead to
glucose intolerance. Conversely, enhanced degradation of cAMP may be a
useful target mechanism for drugs directed against high levels of FFA and
against insulin resistance.

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