Abstract


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The lipolytic and the cardiac responses to 30 min of two different forms of stress — a standardized mental stress test and submaximal bicycle exercise — were investigated in non-obese healthy subjects. This was done by microdialysis of the extracellular space in the abdominal subcutaneous adipose tissue in order to determine lipolysis and electrocardiographic recordings of the heart rate. Glycerol concentrations (lipolysis index) in venous plasma and in adipose tissue dialysate as well as plasma catecholamines and determinations of the heart rate showed marked increases during mental stress (p < 0.001) and physical exercise (p < 0.001), but the patterns of response differed during the two forms of stress. All parameters rose gradually during exercise and decreased continuously in the post-exercise period. During mental stress, however, all parameters peaked within the first 20 min of stimulation and then remained at the same level until after the stress period, when they gradually declined. The maximal increase of glycerol in plasma and adipose tissue during mental stress correlated with the corresponding increase during exercise (r = 0.50–0.60). Such a relationship was not observed with plasma catecholamines or heart rate (r = 0.02–0.29). The peak level of plasma noradrenaline was an independent regressor for the peak levels of glycerol in plasma and adipose tissue as well as for the peak heart rate during mental stress and physical exercise (partial r from 0.35 to 0.64), while the peak level of adrenaline was a regressor for heart rate only during mental stress (partial r = 0.45), when multiple regression analysis was used. These results indicate that lipolysis in adipose tissue could be a valuable marker for the characterization of individual responses to various forms of stress. Noradrenaline contributes to lipid mobilization and cardiac activation during both physical and mental stress and the body adapts more rapidly to the later form of stress.

Key words

Fat cell, heart, glycerol, adrenaline, noradrenaline, exercise, mental stress

Introduction

It is well known that sympathoadrenal activation is important for physical adaptation in a wide variety of stressful situations. This reaction is usually reflected by elevated circulating levels of noradrenaline and adrenaline (6). Adrenaline, released from the adrenal medulla, acts as the circulatory hormone. On the other hand, noradrenaline, released from peripheral sympathetic nerve endings, primarily acts as a neurotransmitter but spills over into the interstitial fluid and circulation. Although both catecholamines induce similar metabolic and cardiovascular responses, the excretion depends on the kind of stressful situation to which the individual is exposed. As pointed out by Åkerstedt et al. (26), adrenaline excretion appears to be particularly responsive to mental stress as, e.g., in connection with exciting movies (19), flying (7) and performance tests in the laboratory (11). In contrast, noradrenaline excretion appears to occur mainly after physical stress (8, 16). It is not clear whether noradrenaline is also of importance for the response to mental stress. In most athletic events there is a combination of the two forms of stress.

Classical metabolic and cardiovascular responses to sympathoadrenal activation include the acceleration of lipolysis in the fat cells of adipose tissue and an increase in heart rate. It is not known whether the heart and adipose tissue respond in similar or in different ways to various forms of stress. Most of the knowledge about the sympathoadrenal effects on lipolysis derives from studies on circulating metabolites in the blood stream. These techniques provide only indirect information about the in vivo conditions. However, a microdialysis sampling technique has recently been introduced for direct studies of lipolysis in human subcutaneous adipose tissue in vivo (21, 1). With this technique it is possible to continuously monitor glycerol (lipolysis index) in the extracellular compartment of adipose tissue.
Previously, we studied the adrenergic regulation of lipolysis in vitro during exercise (2) and mental excitement (13), using microdialysis, and demonstrated a unique role of beta-adrenoceptors in lipolysis activation during these conditions. In the present investigation designed to further characterize the in vivo tissue response to different forms of stress, we have compared the lipolytic and cardiac responses to mental stress and physical exercise in a given subject. In order to evaluate the role of peripheral sympathetic activity in the tissue response, we have also related the lipolytic and cardiac responses to the venous plasma levels of catecholamines.

Material and Methods

Subjects

The study group comprised 30 healthy and non-obese drug-free volunteers (17 women and 13 men). The mean±SE age was 37±2 years (range 20—58 years) and the body mass index varied between 19 and 28kg/m² (mean 23±1 kg/m²). The women were investigated in the middle of the menstrual cycle; none of them was menopausal and none had taken oral contraceptives during the preceding 6 months. All subjects took regular light exercise, but none was involved in competitive sports. In this study they performed both moderate physical exercise and the mental stress test, although on two occasions and in a random order. The study was approved by the ethics committee of the Karolinska Institute.

Microdialysis probe

The microdialysis probe (Carnegie Medicine, Stockholm, Sweden) has been described in detail previously (22). A dialysis tubing (10 x 0.5 mm, 20,000 MW cut-off) is glued to the end of a double steel cannula. The perfusion solvent enters the probe through an inner cannula. It streams down to the tip of the probe and then upwards in the space between the inner cannula and the dialysis membrane. Thereafter, the solvent leaves the probe through an outer cannula, from which it is collected.

Experimental protocol

The dialysate probe was inserted percutaneously, using a guide cannula (diameter 1.4 mm), but without anesthesia, in the subcutaneous adipose layer to the right of the umbilicus. The inlet tubing of the probe was connected to a microinjection pump (CMA/Microdialysis AB, Stockholm, Sweden) and was continuously perfused (5 μl/min) with Ringer’s solution (content of Ringer’s solution in 1000 ml: sodium 147 mmol/l, potassium 4 mmol/l, calcium 2.3 mmol/l, chloride 156 mmol/l). Fractions of the outgoing dialysate were collected at 5-min intervals for the analysis of glycerol. The first three fractions were deleted, since earlier studies (4) had shown a transient rise in the concentrations of ATP in the outgoing dialysate during the first 15 min of dialysis, reflecting the initial trauma caused by insertion of the dialysis probe. During the dialysis experiments, venous blood samples were drawn simultaneously at regular intervals from an indwelling polyethylene catheter placed in a cubital vein for determinations of plasma catecholamines by high-pressure liquid chromatography (14) and glycerol. Under the present experimental conditions in vivo recovery of glycerol is about 10% (2). During the experiments, the pulse rate was also recorded continuously with electrocardiograms.

Response to Stress

Physical exercise

The exercise experiments were performed at 8 a.m. after an overnight fast, with the subjects seated on a bicycle ergometer, as described in detail (2). Briefly, the dialysis probes were inserted and dialysis was performed, as described above. When the subjects had rested for 35 min on a bicycle ergometer, they exercised for 30 min at two thirds of their maximum working capacity (which had been determined on a previous occasion). Then they rested on the bicycle ergometer for a further 30 min. Maximum working capacity was assessed, as described elsewhere (23). The maximum oxygen consumption (l/min) was 2.0±0.2 in the females and 2.8±0.1 in the male subjects.

Mental stress test

The mental stress test has been described in detail and is a constant stressor for 30 min (9). Briefly, following the insertion of the dialysis probes and the intravenous catheter, the subjects rested in bed for 30 min. During the resting period, the test procedure was explained to them. A modified film version of Stroop’s (10) colour-word conflict test was used as a test model. Colour-words are shown on a TV screen in various colours, the combination of words and colours being incongruous. The subject’s task is to ignore the words and name the colour of the print. In addition, there is a simultaneous oral presentation of conflicting colour-words, which also has to be ignored. Visually-presented colour-words were shown randomly for between 0.4 and 1.0 seconds and audiologically-presented words between 0.7 and 1.8 seconds. The mental stress test lasted 30 min. The subjects then rested for a further 30 min.

Analysis of glycerol

Twenty-five μl of plasma or tissue dialysate were used to analyze glycerol. An automated ultrasensitive kinetic bioluminescence assay (3) was employed to determine glycerol with a luminescence analyzer (LKB Valac, Helsinki, Finland).

Statistical analysis

The values presented are the mean±SE. Single or multiple regression analysis and the Student’s paired or unpaired t-test were used for statistical comparisons of the results.

Results

Glycerol, catecholamines and heart rate

The changes in glycerol levels in the adipose tissue dialysate and venous plasma are shown in Fig. 1. In adipose tissue (Fig. 1a) as well as in plasma (Fig. 1b) there was a gradual threefold increase in glycerol levels (p < 0.001) during exercise (= physical stress), the peak values being reached at the end of the physical stress period. During mental stress, the glycerol levels increased rapidly about twofold (p < 0.001) and the peak was reached within 10—20 min i.e., before the end of the mental stress period. The area under the curve (AUC) was greater during physical stress than during mental stress for adipose tissue glycerol (1863 ± 191 vs. 995 ± 69 μmol·l⁻¹·min, p < 0.001) and plasma glycerol (7229 ± 740 vs. 4794 ± 487 μmol·l⁻¹·min, p < 0.001).
The levels of glycerol in the dialysate are lower than those in plasma. This is due to incomplete recovery of glycerol in the microdialysis experiments. The true glycerol level can be determined in subcutaneous adipose tissue (2–3 times higher than in venous plasma) by performing calibration experiments (1). However, for several reasons, it was not possible to calibrate the adipose tissue in the present study. First, the technique is very time consuming (i.e., several hours). Secondly, it entails the exposure of adipose tissue to very high glycerol concentrations, which may artificially alter the adipose tissue metabolism in subsequent stress experiments.

The changes in the catecholamines in venous plasma and in the heart rate are shown in Fig. 2. These parameters increased gradually during physical exercise and declined during recovery, whereas they peaked rapidly before the end of the mental stress period. Adrenaline caused a threefold increase in both physical and mental stress (AUC 1374±194 vs. 1291±148 nM·min⁻¹, N.S.) but peaked earlier during mental stress. Noradrenaline increased more than threefold during physical exercise (p<0.001) and by about 50% (p<0.001) during mental stress (AUC 265±16 vs. 98±6 nM·min⁻¹, p<0.001), but it peaked earlier during mental stress. The heart rate almost doubled during physical exercise and was 40% increased during mental stress (AUC 5866±115 vs. 3978±110 nM·min⁻¹, p<0.001), but it peaked earlier during mental stress. Figs. 1 and 2 show that baseline levels (0 time) of catecholamines, heart rate and adipose tissue glycerol were higher during the exercise test than during the mental stress test. This probably reflects two different experimental situations. Before mental stress, the subjects lay in bed completely relaxed. Before exercise, they sat on a bicycle moving slightly.

Single regression analysis

In order to compare the effects of stress on the levels of glycerol, catecholamines and heart rate, the peak values of these parameters were evaluated by single regression analysis. There was no correlation between the effects of exercise and mental stress on the plasma levels of noradrenaline (NA) (r = 0.29), plasma adrenaline (A) (r = 0.02) or heart rate (r = 0.23). However, there was a positive correlation between the plasma glycerol (r = 0.60, p = 0.0005) and dialysate glycerol levels (r = 0.55, p = 0.019) when comparing the results of exercise and mental stress in the same subjects (Fig. 3a and b).

The glycerol levels in plasma and adipose tissue correlated significantly during exercise (r = 0.62; p = 0.0005, Fig. 4a). The same was true during mental stress (r = 0.61; p = 0.0003, Fig. 4b).

Multiple regression analysis

To further analyze the relative roles of the plasma noradrenaline and adrenaline levels on the glycerol levels or heart rate during physical exercise and mental stress, multiple regression analysis was performed. In this analysis noradrenaline and adrenaline were independent variables and the “peak plasma glycerol” or the “peak adipose tissue glycerol” level or the “peak heart rate” level was the dependent variable (Table 1). Noradrenaline was a significant regressor for all dependent variables during both mental stress and physical exercise. It reached statistical significance for all parameters, except the adipose tissue glycerol level during mental stress, which showed a borderline correlation (p = 0.058). However, adrenaline was a regressor for heart rate only during mental stress (partial r = 0.45).
Discussion

In this report, the effects of mental and physical stress on heart rate and lipolysis in adipose tissue were investigated in the same individual. These two types of stress were chosen because they represent ordinary daily life situations, are of importance for athletic performance and can be applied for 30 minutes or more while making quantitative and reproducible measurements of metabolic and cardiovascular changes. Cold pressure tests (18) or isometric exercise (17) can also be used as stress models. However, these interventions may be performed for 4 min, at most, if they are to be tolerated by the subjects. During such a short period of time, reliable quantitative cardiac or metabolic measurements cannot be made. A physiological increase in endogenous catecholamine levels was evoked by a standardized mental stress test and by exercise on a bicycle ergometer (physical stress). We also measured the levels of catecholamines in the antecubital vein draining the highly vascularized forearm, because we wished to study the role of peripheral sympathetic nervous activity on lipolysis and heart rate. Venous plasma noradrenaline levels are usually regarded as a valid index of "overall" whole-body sympathetic nerve activity (12, 24). Ideally, a vein draining the subcutaneous adipose tissue should be used (5). For many reasons, however, it was not possible to cannulate such a vein in the present experiments. First, it is very stressful and may influence the results of the Stroop test. Secondly, the success rate of cannulation is only about 50% indicating that up to 3/4 of the experiments risk being a failure because each subject has to be investigated twice. In this study the cardiotropic response was monitored by recording the heart rate and, as a marker of the lipolytic response, the glycerol level in adipose tissue was continuously monitored in the extracellular space of the subcutaneous adipose tissue, using the microdialysis technique. In addition, the level of plasma glycerol in the antecubital vein was measured in order to evaluate whether changes in the plasma glycerol level during stress reflect changes in lipolysis in the subcutaneous adipose tissue.

In keeping with previous investigation using the Stroop test (9, 15, 20) we found that during mental stress, the heart rate, plasma catecholamines and glycerol levels in the plasma and adipose tissue peaked before the end of the stress period. This phenomenon suggests a rapid adaptation to mental...
In contrast, adipose and plasma glycerol, heart rate and plasma catecholamine levels increased gradually during the entire period of physical exercise. This may indicate that a physical stress response is less adaptive than a mental stress response. Whether this is due to different degrees of catecholamine response (i.e., amounts of hormone production) or different patterns of catecholamine response (i.e., noradrenaline vs. adrenaline) to the two stress forms remains to be established. Dynamic muscle work requires substrate mobilization and an increase in blood flow to meet energy demands during physical stress. The physiological meaning of metabolic and cardiovascular activation during mental stress is less clear, since there is no apparent need for extra energy during the latter intervention. The increased lipid mobilization during mental stress may instead play a pathophysiological role for stress-related disturbances in lipid metabolism and cardiovascular disorders.

In athlete performance, i.e., short distance runners, it is well established that central nervous activity influences circulatory increase and muscular tension before the physical activity has begun (25). There are less data on metabolic reactions but it seems reasonable that the organism prepares to "fight or fly" and that this also includes substrate mobilization for energy supply. A mental stress situation — without involvement of dynamic muscular exercise — still results in central nervous activity which — possibly in a stereotype way — forces the organism to mobilize energy. The correlation of quantitative substrate mobilization — in this study reflected by lipolysis — for individuals during both physical and mental stress tests supports a uniform reaction to different forms of strain on the organism.

All subjects underwent a Stroop test and physical exercise, which together provide a unique opportunity to analyze various factors that determine metabolic and cardiovascular responses to markedly different stressors in a given population. There was no correlation between the effects of physical and mental stress either on the plasma levels of catecholamines or on the heart rate response to exercise and mental stress. This may reflect individual variability in sympathetic and cardiovascular responses to the different types of stressful situations, regardless of the need for energy mobilization. Alternatively, unknown specific factors may regulate the cardiac and sympathetic responses to various forms of stress. However, there was a significant correlation between both the plasma glycerol response and the adipose tissue glycerol response in the same subjects when comparing the results of physical and mental stress. The latter finding suggests that a common mechanism is responsible for the mobilization of energy substrate through lipolysis, whatever the initial reason for stress activation may be. The glycerol data also suggest that lipolysis is a more valuable marker of individual response to different stress forms in addition to cardiac activity.

Earlier studies have suggested that noradrenaline is the principal catecholamine for mobilizing energy during exercise, while adrenaline is more important during mental stress (17). However, in this study the results of multiple regression analysis indicate that venous noradrenaline contributed significantly to the heart rate response and the glycerol response to mental stress, in addition to its expected contribution to the responses to physical work. This supports the hypothesis that peripheral sympathetic nervous activity via noradrenaline contributes to energy mobilization as well as cardiovascular activity during mental stress and physical exercise.

The changes in the levels of glycerol in plasma and adipose tissue were similar during physical and mental stress and the peak levels of glycerol in the two compartments showed a significant level of correlation ($r = 0.61$ to 0.62). This suggests that the findings concerning the glycerol level in adipose tissue are not influenced in a major way by the adipose tissue blood flow. About one third of the plasma glycerol response could be attributed to abdominal subcutaneous adipose tissue as judged by the level of correlation (i.e., $r^2$). A stronger dependence is not expected, since, first, plasma glycerol represents lipolysis in several fat depots which differ in metabolic activity (2) and, second, plasma but not adipose glycerol levels are the net sum of production and utilization. No or insignificant amounts of glycerol are re-utilized by adipose tissue.

In summary, the present study indicates that lipolysis in adipose tissue could be a valuable marker for the characterization of individual responses to mental and physical stress. The data also support the thesis that noradrenaline contributes to lipid mobilization and cardiac activation during both physical and mental stress.

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| Table 1 Correlation between catecholamines, heart rate and lipolysis during stress. |
|---------------------------------------------|----------|--------|
| **Mental stress**                          | Partial r | p      |
| Plasma glycerol                            | 0.533    | 0.003  |
| Adipose tissue glycerol                    | 0.348    | 0.058  |
| Heart rate                                | 0.437    | 0.005  |
| **A**                                      | Partial r | p      |
| Plasma glycerol                            | 0.208    | 0.204  |
| Adipose tissue glycerol                    | 0.289    | 0.112  |
| Heart rate                                | 0.45     | 0.004  |
| **Physical exercise**                      | Partial r | p      |
| Plasma glycerol                            | 0.454    | 0.021  |
| Adipose tissue glycerol                    | 0.636    | 0.001  |
| Heart rate                                | 0.411    | 0.035  |
| **A**                                      | Partial r | p      |
| Plasma glycerol                            | -0.123   | 0.512  |
| Adipose tissue glycerol                    | -0.277   | 0.102  |
| Heart rate                                | 0.039    | 0.836  |

Multiple regression analysis was performed using noradrenaline (N) and adrenaline (A) together as regressors.
References


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