Influence of Continuous and Discontinuous Training Protocols on Subcutaneous Adipose Tissue and Plasma Substrates

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It has been shown that bouts of high-intensity exercise may reduce subcutaneous adipose tissue more than low-intensity exercise. The aim of the present study was to examine if a discontinuous training protocol is more successful in reducing adipose tissue than a continuous endurance training protocol. Fourteen untrained male volunteers were divided into two groups and trained for 10 weeks performing 3 discontinuous or 3 continuous workouts weekly (discontinuous exercise: 25 times 80 s 35% VO₂max and 40 s 80% VO₂max; continuous exercise: 50 min 50% VO₂max). The discontinuous and the continuous training resulted in a similar subcutaneous adipose tissue loss, determined by skinfold measurement, in the leg above the patella (-2.4 ± 2.4 and -2.4 ± 1.4 mm, respectively). The normalised plasma concentrations of free fatty acid, glycerol, β-hydroxybutyrate, and lactate were similar throughout the final exercise test at the end of the training period. Our data suggested that the discontinuous protocol, selected so that the average intensity was similar to that of the continuous protocol, was not better than the latter in reducing subcutaneous adipose tissue.

Key words: Subcutaneous adipose tissue, free fatty acid, glycerol, fat reducing exercise, food intake.

Introduction

The reduction of excessive body fat decreases the risk for a number of diseases including coronary heart disease and diabetes [21]. Exercise with [19,23] or even without food intake restriction [4,15,20,25] reduces fat mass. In one study [8], a reduction of adipose tissue was absent after low intensive exercise without food restriction. In order to reduce body fat most effectively, moderate endurance exercise is considered to be the best exercise mode as the absolute amount of fat oxidised after 30 min of continuous endurance exercise at 65% of maximal oxygen consumption (VO₂max) exceeds the one with 25 or 85% VO₂max [18]. Tremblay et al. [20] also compared the effects of ‘high-intensity training’ with moderate endurance training on adipose tissue of untrained volunteers. The high-intensity training consisted of continuous exercise at the beginning and continued with interval sessions during the remaining training period. Surprisingly, high-intensity training reduced subcutaneous adipose tissue more than continuous endurance training despite the fact that the high-intensity trainees spent less energy during the entire training period compared with the endurance trainees. This result was explained by an increased activity of a β-oxidation enzyme (β-hydroxyacyl coenzyme A dehydrogenase) after high-intensity workouts resulting in a better lipid utilisation in the post-exercise state. In addition, activities of two glycolytic enzymes (hexokinase and phosphofructokinase) were also increased [20]. However, Gorostiaga et al. [6] showed that continuous training is more effective in increasing muscle oxidative capacity than interval training.

As high-intensity exercise is associated with high epinephrine concentrations [1,18], lipolysis might be upregulated. Despite this upregulated lipolysis, FFA plasma concentrations are lower during high-intensity compared to moderate-intensity exercise due to reduced adipose tissue blood flow. With discontinuous exercise, the trapped FFA [9] could be released during the phase of low-intensity exercise. In contrast to epinephrine, lactic acid and insulin inhibit the lipolytic activity in the muscle and the fatty tissue [12]. Therefore, an optimal exercise protocol for a maximal fat oxidation rate should give high FFA and glycerol but low lactate plasma concentrations while spending as much energy as possible in a given time. Discontinuous exercise could be an exercising mode which fulfills these requirements.

The aim of the present study was to compare the effects of a discontinuous against a continuous training protocol during a 10 weeks period on subcutaneous adipose tissue in two groups of untrained volunteers with a sedentary job. The protocols were selected in such a way that the relative exercise intensity during the 50 min training period was identical in both groups.
Material and Methods

Subjects

Fourteen male volunteers with slight overweight and without any medication or known diseases were randomly assigned to two groups. The subjects were informed in detail and provided written consent before they participated in the study which was in line with the Helsinki declaration for human experimentation. The discontinuous exercise group consisted of 7 men, 28 to 46 years old, with a body mass index (BMI) of 25.9 ± 3.2 (SD) while the continuous exercise group consisted of 7 men, 28 to 46 years old, with a BMI of 26.9 ± 3.3. Pre-training VO2max of the discontinuous and the continuous exercise group amounted to 42.4 ± 7.5 and 42.8 ± 9.3 ml/min/kg, respectively. No comparison of the two groups reached statistical significance.

Training protocol

During the training period of 10 weeks, the subjects performed 3 exercise bouts per week on a cycle ergometer (Ergoline 900, Ergoline, Bitz, Germany). The intensity of the discontinuous exercise consisted of 80 s at 35% VO2max followed by 40 s at 80% VO2max and this 2 min lasting cycle was repeated 25 times (Fig. 1) while the continuous exercise was held constant at 50% VO2max for 50 min. In other words, averaged intensity amounted to 50% VO2max in both groups. Periodical VO2max-tests (see below) allowed to adjust the exercise intensity according to training improvements.

\[VO2\text{max-test}\]

Before the training period (W0), after three, six, and ten weeks (W3, W6, W10) the volunteers performed a VO2max-test on a cycle ergometer. The initial workload was 70 W and it increased 30 W every 2 min. The test was stopped when the subjects were unable to continue. The investigators did not encourage the subjects at any time. VO2 was measured using an open-circuit system (Oxycon β, Jaeger, Wuerzburg, Germany). VO2max was used to determine the subjects training workload assuming an efficiency of 24%. If VO2max was higher or lower by 180 ml (corresponding to 15 W) than in the previous incremental test, the training workload was raised or reduced by 15 W.

Measurement of subcutaneous adiposity

Adipose tissue was measured with a skinfold caliper at three different locations on the right side of the body (triceps skinfold on the arm, suprailiac skinfold at the waist, and the knee skinfold above the patella) in W0, W3, W6, and W10. All caliper measurements were performed by the same person and the average over 5 measurements was taken as valid result.

Blood samples

Blood samples were collected before and during the last discontinuous or continuous exercise test in W10 to measure plasma concentrations of FFA, glycerol, β-hydroxybutyrate (β-Hb), and lactate. A teflon catheter (Intracan-W, B. Braun Melsungen, Melsungen, Germany) was placed in an antecubital vein of the left arm. Nine ml blood were filled in a EDTA tube and 4.5 ml in a tube containing NH4-heparin (Sarstedt, Nümbrecht, Germany) before, as well as 15, 30, and 50 min after the start of exercise. The plasma was separated from the erythrocytes by centrifugation at 4 °C for 12 min with 4000 U/min and stored in a freezer at −80 °C until analysis. FFA concentration was analysed by a Wako NEFA-C test kit (Wako Chemicals, Neuss, Germany), glycerol and β-Hb were measured by a Sigma test kit (Sigma Diagnostics, St. Louis MO, US). The measurements were performed using a photometric-enzymatic method with the Cobas-Mira (Hoffman-La Roche, Basel, Switzerland). 20 μl venous blood were collected from the catheter at the same time to measure lactate concentration with an enzymatic method (ESAT 6661 lactate analyser, Eppendorf, Hamburg, Germany).

Control of food intake

The volunteers recorded their diet in portions over 4 days (2 week and 2 weekend days) in W0, W6, and W10. The subjects were instructed how to fill out the form. They were not informed in W0 that they had to repeat this control, to prevent them from memorising the first notations. The nutrition diary was analysed with a computer based nutrient analysis program (EBIS 2.0, E+D Partner, Stuttgart, Germany) for carbohydrate, fat, and protein contents as well as for the total amount of energy.

Statistical analysis

The results are presented as means ± SD. In addition, the blood concentrations were divided through the value of the resting blood sample to normalise the values. All results were compared with analysis of variance for repeated measures (ANOVA). When significant differences were found, a t-test with Bonferroni correction was used to locate the difference between the groups and a paired t-test was applied to locate the difference within a group. A value of p < 0.05 was regarded as significant.
Results

\( \dot{V}O_2 \text{max-test} \)

Both exercise protocols resulted in a significantly increased \( \dot{V}O_2 \text{max} \) within 6 weeks (+8.8 ± 3.7 and +4.9 ± 5.3 ml/min/kg for discontinuous and continuous exercise, respectively, \( p < 0.05 \)), whereas only the continuous exercise showed a significant increase of \( \dot{V}O_2 \text{max} \) (+3.1 ± 2.9 ml/min/kg, \( p < 0.05 \)) in W10 compared to W0 (Fig. 2).

![Fig. 2](image)

Maximal oxygen consumption (\( \dot{V}O_2 \text{max} \)) of discontinuous exercise (DE) and continuous exercise (CE) over 10 weeks. The differences between the groups were never significantly different. *: \( p < 0.05 \), compared to W0. N = 7.

Work of exercise bouts

The training workload was adjusted after each \( \dot{V}O_2 \text{max} \) determination. Averaged work of each exercise bout of 50 min duration amounted for discontinuous exercise to 130 ± 26, 144 ± 20, and 153 ± 21W and for continuous exercise to 133 ± 21, 133 ± 24, and 140 ± 18 W during W0-W3, W4-W6, and W7-W10, respectively. Work significantly increased for discontinuous exercise (+23 W, \( p < 0.05 \)) whereas it remained constant for continuous exercise throughout the training period.

Subcutaneous adiposity

Body weight of both groups did not change during the training period (Table 1). Both groups reduced the subcutaneous adipose tissue at the leg (discontinuous: −2.4 ± 2.4 mm; continuous: −2.4 ± 1.4 mm; \( p < 0.05 \)) over the training period. The skinfolds remained stable at the arm and the waist. There were no significant differences between the two groups (Table 1).

Blood substrate concentrations

During the last exercise test in W10, normalised FFA, glycerol, and lactate plasma concentrations increased significantly (\( p < 0.05 \)) within both groups (Fig. 3). \( \beta \)-Hb concentration remained constant during discontinuous and continuous exercise. The normalised plasma concentrations were never significantly different between the two groups (Fig. 3). Absolute plasma concentrations were also not significantly different with the exception of \( \beta \)-Hb after 15 and 30 min (Table 2).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Effects of a 10-week (W) exercise program on body weight (bw) and on subcutaneous adipose tissue thickness of the arm (a), waist (w), and leg (l) measured by skinfold caliper for discontinuous exercise (DE) and continuous exercise (CE). Values are means ± SD</th>
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<tr>
<td></td>
<td>DE</td>
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<td>bw (kg)</td>
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<td>a (mm)</td>
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<td>w (mm)</td>
<td></td>
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<td>l (mm)</td>
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</table>

*: \( p < 0.05 \), compared to W0, N = 7 for DE and CE

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Absolute plasma concentration of free fatty acid (FFA), glycerol (gly), ( \beta )-hydroxybutyrate (( \beta )-Hb), and lactate (lac) before (0) and during (15, 30 and 50) the exercise test at week 10 for discontinuous exercise (DE) and continuous exercise (CE). Values are means ± SD</th>
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<tbody>
<tr>
<td></td>
<td>DE</td>
</tr>
<tr>
<td>FFA (( \mu )mol/l)</td>
<td>461 ± 248</td>
</tr>
<tr>
<td>gly (( \mu )mol/l)</td>
<td>125 ± 73</td>
</tr>
<tr>
<td>( \beta )-Hb (( \mu )mol/l)</td>
<td>89 ± 56</td>
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<tr>
<td>lac (mmol/l)</td>
<td>1.05 ± 0.44</td>
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</table>

*: \( p < 0.05 \), compared between groups, N = 7 for DE and CE
**Discussion**

The main aim of the study was to examine the effects of discontinuous and continuous training, on the basis of equivalent intensity, on subcutaneous adipose tissue of the arm, waist, and leg. Both training groups significantly reduced the subcutaneous adipose tissue of the leg above the patella but no reduction was found at the arm and the waist. It is known that the abdominal subcutaneous adipose tissue is preferentially mobilised in response to energy deficits [17] which did not occur in our study. The significant reduction of fatty tissue in the leg might represent a local phenomenon as the training consisted of cycling. However, as the volunteers did not lose any weight during the training period, the small fat loss was most likely compensated by a gain in muscular tissue. The fat loss was rather small because the workload and therefore the exercise energy expenditure was lower in our study than in other studies [4,20,24]. The exercise intensities had to be selected so that the training was feasible for untrained employees with sedentary jobs. Limiting in this respect were the high-intensity bouts both with respect to intensity and duration.

Previous studies suggested that higher FFA plasma concentrations increase FFA oxidation rate during exercise [5,7]. We expected concentrations of FFA and glycerol to increase faster during discontinuous than continuous exercise after a corresponding training. Contrary to our expectation, the high-intensity exercise bouts did not result in a higher FFA plasma concentration during discontinuous compared to continuous exercise. This could implicate that discontinuous exercise was not able to increase the lipolysis activity above the one of continuous exercise given the fact that the relative intensity over time was identical in both training modes. The lactate concentration was never significantly higher during discontinuous exercise compared to continuous exercise. Therefore, the inhibiting effect of lactate on the lipolysis was similar for both exercise protocols. The fact that the concentration of the ketone body β-Hb did not change during both exercise bouts indicated that the amount of FFA utilised in the liver remained constant. As all metabolites showed an equal time course in the test of W10, it seems that the discontinuous exercise protocol was too similar to the continuous protocol and training with the discontinuous protocol could not cause a higher FFA plasma concentration.

It is known that muscular training leads to structural changes, e.g. the number of mitochondria increases [11], and also to biochemical adaptations [10]. Both effects are associated with an improved VO₂max and an increased oxidative capacity of

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**Table 3**  Energy intake measured and weighted over 4 days in week (W), 0, 6, and 10. Results are shown as total energy (TE), carbohydrates (CHO), fat (F), and proteins (P) for discontinuous exercise (DE) and continuous exercise (CE). Values are means ± SD. As consumed dietary fibres and alcohol are missing in the table, which contribute in a small amount to energy intake, there is a difference between the amount of TE and the sum of CHO, F, and P.

<table>
<thead>
<tr>
<th></th>
<th>DE</th>
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<tr>
<td></td>
<td>W0</td>
<td>W6</td>
<td>W10</td>
<td>W0</td>
<td>W6</td>
<td>W10</td>
<td></td>
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<tr>
<td>TE (kJ/d)</td>
<td>9339 ± 2996</td>
<td>9680 ± 3089</td>
<td>10062 ± 2763</td>
<td>9412 ± 1673</td>
<td>8645 ± 2767</td>
<td>7904 ± 2362</td>
<td></td>
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<tr>
<td>CHO (kJ/d)</td>
<td>3623 ± 1393</td>
<td>3850 ± 1674</td>
<td>3593 ± 1144</td>
<td>4009 ± 840</td>
<td>3499 ± 1049</td>
<td>3587 ± 1991</td>
<td></td>
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<tr>
<td>F (kJ/d)</td>
<td>3468 ± 1256</td>
<td>3581 ± 1088</td>
<td>4068 ± 1246</td>
<td>2837 ± 782</td>
<td>3109 ± 1416</td>
<td>2355 ± 650</td>
<td></td>
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<tr>
<td>P (kJ/d)</td>
<td>1426 ± 285</td>
<td>1376 ± 373</td>
<td>1469 ± 433</td>
<td>1422 ± 421</td>
<td>1158 ± 414</td>
<td>1032 ± 260*</td>
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</tr>
</tbody>
</table>

*: p < 0.05, compared to W0, N = 5 for DE and CE

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**Fig. 3** Normalised plasma concentrations of lactate, β-hydroxybutyrate (β-Hb), glycerol, and free fatty acid (FFA) before (0) and during the exercise test at week 10 for discontinuous exercise (DE) and continuous exercise (CE). *: p < 0.05 (significant increase within group). N = 7.

**Food intake**

As two volunteers of each group did not completely fill in the last nutrition diary, their data were excluded from the analysis. Food intake was significantly changed in the continuous training group (Table 3). They reduced their protein intake (~388 ± 252kcal/d, p < 0.05) without reducing the total amount of energy intake. The food intake of the discontinuous exercise group remained constant over the training period. There were no significant differences between the two groups (Table 3).
the muscle tissue. Interval (discontinuous) exercise results in a higher VO₂max compared to continuous exercise when the relative intensity of both exercises is the same throughout the training period [6]. Therefore, the discontinuous exercise of our study was expected to increase VO₂max faster than continuous exercise. This would have allowed the discontinuous trainees to exercise sooner at a higher level with a higher energy expenditure and a higher fat oxidation rate. Discontinuous as well as continuous exercise increased VO₂max within the first 6 weeks but there was never a significant difference between the two groups (Fig. 2). Exercise intensity was adjusted depending on the result of the VO₂max-test. Contradictory to the increases of VO₂max of both groups in W6, only the discontinuous exercise training group significantly increased the workload and therefore energy expenditure over the training period while the continuous exercise training group remained at the initial level. The discrepancy between the VO₂max increase and the unchanged workload is due to the small VO₂max increase of some continuous trainees who did not always translate into a higher training intensity. The required VO₂max increase for a workload adjustment was set at 180 ml VO₂ corresponding to an increase of 15 W. The surprisingly low VO₂max of both groups by W10 (Fig. 2) was most likely due to a lower motivation of the volunteers to succeed in the last test.

The results of the self-recorded diets of 5 subjects in each group indicated a change of the food intake during the training period of continuous exercise whereas the discontinuous exercise trainees maintained their nutritional habits (Table 3). The continuous exercise reduced the protein intake during the 10-week training period. The consumed amounts of the macro nutrients fat and carbohydrates influence the quantity of the substrates oxidised during exercise [3,13,22] and at rest [24]. They have an effect on energy balance [16] and therefore on body composition [14]. As the consumed protein contributes only in a negligible amount to energy expenditure, the significantly reduced protein intake should not have had an influence on the fat oxidation rate and therefore on adipose tissue during the 10-week training period. During this period, the amount of consumed carbohydrate did not change in either group. The analysis of the diaries consistently revealed rather low amounts of total energy intake. However, it is a known phenomenon that volunteers underestimate the amount of food intake [2] even if they are well instructed.

In summary, we hypothesized that training with a discontinuous exercise protocol is more efficient in reducing adipose tissue than with a continuous exercise protocol. In contrast to our expectations, the exercise protocols provided similar normalised plasma concentrations of FFA, glyceral, and lactate in week 10 and both groups showed a similar reduction of adipose tissue. It seems that the discontinuous exercise protocol, due to the moderate performance of the sedentary subjects and our intention to compare similar relative training intensities, was too close to the continuous exercise protocol. The unexpected reduction of protein intake throughout the training period of continuous exercise should not have influenced exercise metabolism.

It can be concluded that the discontinuous protocol, selected so that the average intensity was similar to the compared continuous protocol, was not better than the latter in reducing subcutaneous adipose tissue.

References

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