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ORIGINAL ARTICLE

Can alternating lower body negative and positive pressure during exercise alter regional body fat distribution or skin appearance?

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Abstract The purpose of this study was to determine whether physical activity, with and without lower body pressure, leads to increased regional fat loss in the lower extremities of overweight females. Eighty-six obese women with a female phenotype were randomly assigned into four groups: control group (C), diet only (D), diet plus exercise (DE) or diet, exercise and lower body pressure intervention (DEP). The three treatment groups followed the same diet, the two exercise groups (DE and DEP) additionally followed an endurance training program of 30 min of cycling at 50%VO_2max three times per week with or without lower body pressure. Body composition and fat distribution were assessed by DXA. Body size circumference measurements were recorded as well as subjective ratings of cellulite and skin appearance. As expected, all test groups (D, DE, DEP) showed a significant decrease ($p < 0.05$) in total body mass and fat mass. DXA revealed significant differences between the experimental groups and C. The DEP group also lost significantly more body mass and fat mass when compared with D, while no significant difference was observed between the other groups. A similar pattern was seen for circumference measurement data. A significant perceived improvement was made by the DEP group when compared with C, D and DE groups for skin condition and also between the DEP versus C and D groups for cellulite. The combination of diet and exercise is successful for weight reduction. The additional application of lower body pressure especially affects skin appearance.

Keywords Body fat distribution · Lower body negative pressure · Lower body positive pressure · Spot reduction · Training · Weight loss

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Introduction

Obesity cannot be regarded as a homogeneous condition within the body. Differences in the function of adipocytes from different regional fat deposits were found in several studies both in vitro and in vivo (Tchernof et al. 2006; Jensen 2002). Body weight or body fat can be reduced by a negative energy balance—either by an increased energy expenditure or decrease in energy intake. However, fat tissue is not removed evenly. Abdominal adipocytes have a higher responsiveness to catecholamines, resulting in an increase of lipid mobilization when compared with femoral or gluteal fat cells (Boucard et al. 1993). This is the case regardless of gender or degree of obesity. Increased lipoprotein lipase (LPL) activity is found in the gluteofemoral area, especially in younger women, which supports the female phenotype (Ferrara et al. 2002; Boucard et al. 1993). A change in lipid metabolism and regional fat distribution as a function of the hormonal status in women has been demonstrated in several studies (Kosková et al. 2007; Toth et al. 2000; Douchi et al. 2007; Pansini et al. 2008; Perdersen et al. 2004). Differences can also be found in the effects of diet and/or physical training because of differing metabolic situations, different body fat distribution and sex. Nindl et al. (2000) examined the effects of regional differences in strength and endurance training on fat and muscle mass. The 31 women in the test group lost an average of 2.6 kg of fat (1.3 kg from the abdomen and 1.1 kg in the arms), while the lower extremities showed no change in body fat. In particular, women show an increased resistance to weight loss in the gluteofemoral area, which often leads to physical dissatisfaction of body shape. This dissatisfaction, apart from the impairment of mental and physical health, can be seen as a motivation for change, since body shape can be felt directly compared with the increased risk of morbidity.

This raises the question of whether specific training that concentrates on particular areas of the body can accelerate fat loss in those regions, and thus, achieve a change in physical appearance. The term "spot reduction" describes the targeted local reduction of subcutaneous fat tissue as a result of specific exercises in that area of the body. The results of these studies are not clear (Kostek et al. 2007; Walts et al. 2008; Stallknecht et al. 2007). Stallknecht et al. (2007) addressed the question of whether blood flow and lipolysis of the subcutaneous fat tissue were affected by the activity of adjacent muscles. The local perfusion of the subcutaneous fat tissue was determined by the 133Xe washout technique. Lipolysis was calculated from interstitial and arterial glycerol concentration and blood flow. The authors showed that blood flow increases in femoral subcutaneous tissue of both legs during one-legged knee extensions. At both 25 and 55% of individual maximum work (Wmax), a significantly higher blood flow was seen; however, blood flow in the subcutaneous tissue adjacent to the working muscles was higher in the working leg as compared to the non-working leg. The authors concluded that specific training exercises can induce a so-called "spot lipolysis". Their data cannot, however, confirm a "spot reduction", since redistribution of triglycerides in the same area between training sessions is unknown. The increased lipolysis and blood flow in the femoral adipose tissue could be due to an increase in local temperature (Felländer et al. 1996; Astrup et al. 1980). Another possible explanation for increased lipolysis could be that norepinephrine levels increase locally with increased blood flow (Stallknecht et al. 2007).

Several cross-sectional studies suggest that blood flow in fatty tissue of overweight people is significantly lower than in people of normal weight, both at rest and during aerobic exercise. Based on research and conventional wisdom, in most cases obesity is associated with hypertrophy rather than hyperplasia of adipocytes (Lange 2004; Adams et al. 2005). There are very few longitudinal studies that deal with the influence of endurance training on regional blood flow in adipose tissue. Lange et al. (2001) were not able to demonstrate that 12 weeks of endurance training increased blood flow in subcutaneous abdominal tissue. Nevertheless, lipid metabolism and blood flow are closely connected. Postprandial, as well as fasting and physical activity can increase blood flow of adipose tissue by up to three times the normal level, but there are regional differences. Blood flow in visceral fat tissue is around two to three times higher than in subcutaneous fat tissue (Bülow 2004). According to Lange, blood flow in adipose tissue could be the limiting factor for the mobilization of fatty acids (FA), and consequently, FA oxidation. Bülow (2004) acknowledged that at high exercise intensities, FA concentration in arterial blood rises, and therefore, transport through the mitochondrial membrane appears to be a limiting factor. However, FA's are potentially toxic substances, so at high concentrations (above 2 mmol L^{-1}) they lead to vasoconstriction. In this respect, blood flow is limited (Bülow 2004).

In addition to cardiovascular and/or strength training, which may at least in the short term lead to increased blood flow in adipose tissue, there are other contributing physical factors to increased blood flow. One such factor is the well-known phenomenon of vasoconstriction and vasodilatation caused by temperature and/or pressure (Brown et al. 1966). If a lower body negative pressure (LBNP) or lower body positive pressure (LBPP) is applied to the lower extremity (usually from the iliac crest height downwards), there is a clear redistribution in blood volume. However, changes in micro- and macro-circulation that occur under conditions of LBPP or LBNP have yielded different findings. A negative pressure of 10–20 mmHg resulted in a displacement of 400–550 ml of blood to the lower body (Cooke et al. 2004). This volume displacement and the vascular response associated with these changes are not uniform. Kitano et al. (2005) noticed a reduction of blood flow in the arteria brachialis and in the arteria femoralis at both −20 and −40 mmHg of LBNP. Essandoh et al. (1986) studied the circulation of the forearm and leg using strain gauge plethysmography and concluded negative pressure up to −20 mmHg LBNP decreases blood flow to the forearm; however, in the lower leg no significant reduction of blood flow occurs. To maintain arterial blood pressure, muscle sympathetic nerve activity (MSNA) increases with increasing LBNP in response to central hypervolemia. In order to avoid a collapse, MSNA rapidly decreases. However, there is a disagreement about whether the increase in MSNA as a result of LBNP differs in the upper and lower extremities (Watenpaugh et al. 2004; Mack 1998; Vissing et al. 1997; Wellhöner et al. 2006). Wellhöner et al. found a decrease of skin and fat tissue blood flow at LBNP of −15 mmHg for 10 min measured by the Laser Doppler Flowmetry and 133Xe washout-technique in the area of tigh and belly. In obese individuals, blood flow to adipose tissue increased above the baseline following LBNP, while this phenomenon was not observed in persons of normal body weight. Similarly, only in the opposite direction, a blood volume shift from the lower extremity to the thoracic area occurs under high pressure conditions on the lower body sections (LBPP). The reaction of increasing LBPP on MSNA was studied by Fu et al. (1998). At 10 mmHg LBPP, there was a significant decrease in MSNA which remained at a plateau until 20 mmHg and then rose again as LBPP reached 30 mmHg, but stayed below the baseline. The authors attribute the fall in MSNA to a pressure on the cardiopulmonary baroreceptors that inhibit efferent sympathetic nerve activity to muscle. The shift of blood volume from distal regions towards the body center could lead to a vasodilatation in the peripheral blood vessels because of the baroreceptors, especially in the lower extremities (Nishiyasu et al. 2007). Undeniably, a local effect of pressure leads to changes in the peripheral blood circulation. Thus, the aim of this randomized controlled study was to investigate the effects of exercise and different lower body pressures on alterations of body mass and body fat distribution. Specifically, the purpose of this study was to determine whether physical activity, with and without lower body pressure, leads to increased regional fat loss in the lower extremities of overweight females.

Materials and methods

Subjects

The study included 86 female volunteers between the ages of 25 and 55 years. Only healthy women with a female phenotype (waist circumference <88 cm and waist to hip ratio of <0.85) and a BMI of 25–38 kg/m^2 were studied. In order to achieve a homogeneous group based on physical activity, daily physical activity was recorded using activity logs (Aadahl and Jorgensen 2003). Women with more than 2 h of physical activity per week, at moderate to high intensity, and/or physically hard-working women were excluded from the study. The study was conducted in accordance with the guidelines of the Declaration of Helsinki (1997). Upon request to the Ethics Commission, the study was deemed appropriate without further authorization.

Intervention

Subjects were randomly assigned into one of four groups of at least 20 people: control group (C); diet only (D); diet plus exercise (DE); or diet, exercise and lower body pressure (DEP). The C group served exclusively as a control and was not exposed to any form of intervention. The three test groups followed the same dietary program to control for diet differences, minimizing the effect of diet as an extraneous variable and attributing possible effects on body mass and fat mass to the training programs. The diet for these three groups was one of reduced calorie intake while including adequate nutrition with proper nutrient values from low energy density foods. For menu suggestions, analysis and evaluation of nutritional data, software (DGE-professional, version 3.2) from the German Society for Nutrition was utilized. The diet plan was designed to allow adjustments for individual energy requirement of each subject. The goal was to induce a daily energy deficit—between 350 and 500 kcal, depending on whether the subject was pre-menopausal (−350 kcal) or menopausal (−500 kcal). The protein content was increased relative to the average dietary recommendations (~15% of the daily energy supply), while the proportion of carbohydrates was selected in the lower range (~55%) and consisted mainly of whole-foods with high fiber content. The fat content was 30% of the daily energy demand and was dominated by unsaturated fatty acids (DGE 2008). Subjects in the three test groups were given a comprehensive introduction on healthy nutrition and kept daily food records during the 12-week intervention. In every 14 days, three of the days recorded in the logs (2 weekdays, 1 weekend day) were evaluated. The two remaining test groups (DE and DEP) also followed an endurance training program in addition to the diet. The training program involved exercising for 30 min three times per week at 50%VO_2max using the HypoxiS120®, which is comparable to a bicycle ergometer, conducted over 12 weeks. Both exercise groups trained on the HypoxiS120®; but, only the DEP group was exposed to a positive or negative pressure applied to the lower extremities (from below the iliac crest height).

The protocol used in the HypoxiS 120® for the DEP group included an initial constant high pressure of 7.5 mmHg over a period of 4 min. This was followed with alternating under- and overpressure of between −15 and +15 mmHg at intervals of 20 s. These settings were chosen because of the empirical experience of the company Hypoxi, Inc. Their theoretical view is as follows: initial permanent high pressure should decrease the peripheral vascular resistance or the MSNA with a simultaneous shift in blood volume to the center of the body. Subsequent changes between positive and negative pressure should lead to activation of local lipid metabolism and increased blood flow to subcutaneous fat tissue. The DE group followed the same exercise routine on the HypoxiS120® without being subjected to any under-and overpressure effects. Their training would therefore be consistent with endurance training on a conventional bicycle ergometer. The HypoxiS120® has been marketed for several years and volunteers had some expectations for its use. Subjects in the DE group were not exposed to pressure changes during their exercise bouts. Since the shell of the device shuts with an audible sound of pressure release subjects may have believed that they were performing exercise with LBPP and LBNP. Subjects in the DE group were only told that they were exercising using the "HypoxiS120 training". The training of the two groups was carried out in separate rooms and under supervision to avoid bias from one group to another.

During training, heart rate (measured with a Polar® monitor) and cycling cadence were recorded to confirm consistent training intensities. Thigh surface temperature was recorded once each week for a total of 10 measurements to provide a general estimate of changes in blood flow and metabolic activity of upper leg muscles. Pilot data revealed a high reliability ($r = 0.98$) of the temperature sensors. In order to exclude the influence of daily environmental temperature variations and adaptations to the onset of activity, values taken from 5 min after the start of exercise as compared to the end of exercise were used.

Pre- and posttest procedures

Body size and composition

Standardized anthropometric data were collected regarding age, height and weight. Measurements of circumferences were performed by an expert with a flexible, but inelastic, measuring tape according to standardized methods. Measurements before and after the training period were made by the same person according to the guidelines of Lohmann et al. (1991). The sizes of the upper arm, thigh (proximal, middle, distal and measured on both sides), hips, waist, abdomen and chest were measured. Body composition and regional fat distribution were assessed by dual energy X-ray absorptiometry (DXA) with a HOLOGIC QDR 4500 W (Hologic Inc., Bedford, MA, USA) at the Department of Nuclear Medicine and Endocrinology, Paracelsus Private Medical University Salzburg, Austria. Pre and post scans of all participants were conducted by the same trained study technologist. Each participant was measured in a standardized manner by dressing down to undergarments. In addition to whole-body measurements and pre-programed measurement of individual body regions of the arms, legs, torso and head, lower body measurements were entered manually. The labeling of the lower body was identified using a straight line along the iliac crest (crista iliaca). Quality assurance measurements for body composition were performed according to the manufacturer's guidelines.

Skin

Even if so-called cellulite is simply cosmetically disturbing and not a pathological lesion (Pavicic et al. 2006; Rossi and Vergnanini 2000), it can cause considerable distress for women. Therefore, it seems appropriate for a study of regional body fat distribution to also take this factor into account. Smalls et al. (2005) using a 10-point scale (0–9) studied quantitative measurements of the irregularities of the skin, and showed a high correlation with expert assessment, and thus, to the subjective perception of cellulite. Since cellulite is subjectively considered unaesthetic and disturbing, a subjective assessment gains importance. According to Rossi and Vergnanini 2000 cellulite can be categorized not only by strength of expression level but even after skin condition. For these reasons, the degree of cellulite and skin appearance of all subjects was assessed both before and after the intervention.using two grading scales. The skin appearance was estimated form loose tissue (1) to firm tissue (10), whereas the cellulite was graded from no cellulite (0) to severe cellulite (10).

Resting metabolic rate (RMR)

The resting metabolic rate was used as a basis to calculate the individual energy needs and was multiplied by a factor of 1.4 (PAL = Physical activity level 1.4), which corresponds to a sedentary life style with no exercise. The RMR was measured by indirect calorimetry with a metabolic analyzer of FitMate produced by Cosmed (Rome, Italy). The device was found to be a valid and reliable instrument for the determination of oxygen consumption and basal metabolism (Nieman et al. 2006). From each subject's calculated individual energy requirements, either 350 kcal (subjects before menopause) or 500 kcal (subjects in menopause) were deducted, resulting in a negative daily calorie intake, where weight loss of about 0.5 kg/week may be expected.

Cardiopulmonary exercise testing

To determine VO_2max, subjects completed a progressive test protocol on a cycle ergometer (Daum, ergo_bike 8) and VO_2max was measured using the K4b2 system of Cosmed. The initial test intensity was set at 25 W. At 2-min intervals, exercise intensity was increased by 25 W until exhaustion—either the cadence was reduced below the threshold from 70 to 80 rpm, or the maximum of the cardiorespiratory capacity was reached. The subjects completed the test at least 3-h post prandially. Test termination criteria were the achievement of maximum cardiorespiratory capacity or failure to maintain a cycling cadence >70 rpm. Individual target training heart rates at 50%VO_2max were calculated using separate slope equations. Once intensities were established, training began within a few days after preliminary investigations (pre-tests).

Table 1 Mean demographic values (±SD) for the four groups

	Diet exercise pressure group (DEP)	Diet exercise (DE)	Diet group (D)	Control group (C)
Age + SD (years)	40 + 8.3	41 + 7.6	37 + 6.4	40 + 9.9
BMI + SD	28.2 + 2.3	26.8 ± 2.0	27.2 ± 2.2	26.4 ± 2.1
DEXA in g + SD				
TBM	75,986 + 8,214	73,379 + 6,843	75,486 + 6,716	73,463 + 7,748
TBF	29,452 + 5,104	27,706 + 3,390	29,204 + 4,722	27,215 + 4,428
LBF	16,119 +2,869	15,725 + 2,056	16,894 + 3,206	14,861 + 2,733
BFL	11,423 + 2,026	11,306 + 1,484	12,197 + 2,529	10,347 + 2,091
BFA	3,225 + 770	2,814 + 268	2,964 + 536	2,929 + 596
TF	14,032 + 3,234	12,810 + 2,072	13,278 + 2,509	13,173 + 2,498
In % of TBM + SD				
LBM relative	58.2 + 3.5	59.1 + 1.8	58.3 + 3.5	59.7 + 3.3
Circumferences in cm + SD				
Arm	33.6 + 2.2	32.6 + 2.1	32.7 + 2.2	32.3 + 1.6
Waist	82.1 + 6.5	79.9 + 4.5	80.1 + 3.4	81.1 + 4.7
Hip	111 + 5.3	110.5 + 4.7	111.9 + 5.4	108.7 + 4.6
Upper leg	65.8 + 3.5	66 + 3.1	66.7 + 4	63.8 + 3

There were no significant differences in baseline data between groups; data were normally distributed

BMI body mass index, *TBM* total body mass, *TBF* total body fat, *LBF* lower body fat, *BFL* body fat legs, *BFA* body fat arms, *TF* trunk fat, *LBM* lean body mass

Table 2 Overview of the groups

Diet exercise pressure group (DEP)	Diet exercise group placebo (DE)	Diet group (D)	Control group (C)
n = 21	*n* = 20	*n* = 23	*n* = 22
12 weeks	12 weeks	12 weeks	12 weeks
30 min training	30 min training		
50%VO_2 max, 3×/week	50%VO_2max, 3×/week		
15 mmHg LBNP/LBPP			
Diet (350–500 kcal day^{-1})	Diet (350–500 kcal day^{-1})	Diet (350–500 kcal day^{-1})	No intervention

Statistical analysis

Statistical analysis and processing of descriptive data were performed with SPSS, Version 16.0. All data were normally distributed according to the Kolmogorov–Smirnov adaptation test, and presented as means and standard deviation (+SD). Using paired *t* tests, the mean of pre-and post-tests were compared within the groups. To determine mean differences between groups, MANOVA's were used, post hoc Bonferroni was utilized. The significance level was set at $p < 0.05$. To identify possible relationships of various parameters, partial correlations were calculated according to Pearson Product Moment Correlation or Spearmans rank correlation.

Results

Among the four groups, no difference based on age and BMI was found before the intervention. The mean values (+SD) for age and BMI and the results of the Pretests of DXA and circumference measurements are shown in Table 1 while research groups and the study design are displayed in Table 2. There was no significant difference in average HR for training between groups.

Diet intervention

Calories in all three test groups were similar (Fig. 1). On average, all three groups ingested about 300 kcal below their calculated energy requirements. The energy demand based on the RMR was about 300 kcal higher than the intake the people reported in their logs. There was no significant difference found among the groups neither in nutrition intake nor in distribution (Fig. 1).

Effects on regional body fat distribution and body composition: As expected, all three test groups (D, DE, DEP) showed a significant decrease in total body mass and body fat mass. A group comparison revealed a significant result between the test groups and C, but also the DEP group lost significantly more total and body fat mass compared to D, while no significant difference was observed between the

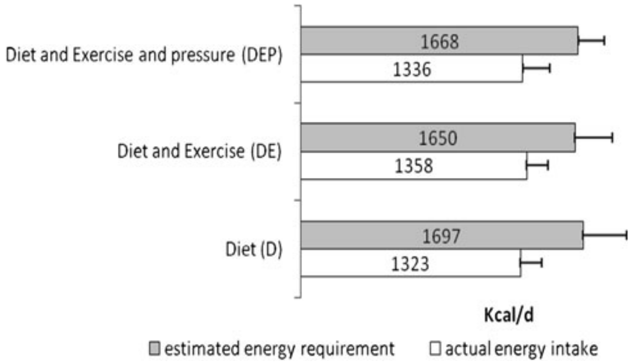

Fig. 1 Mean daily estimated energy requirement (*grey bars*) based on RMR calculation and actual energy intake (*white bars*) based on the food records

other groups. The results of the DXA measurements are shown in Table 3.

Between pre- and post-tests all three intervention groups showed significant reductions in total body fat mass. All subjects lost the most body fat, both absolutely and relatively, from the trunk. Regional fat loss in the legs was the largest for the DEP group (−1,630/+ 941 g; −3.2%); however, a significant difference was only found between the D and DEP groups ($p = 0.003$).

A similar pattern appeared in the size measurement data (Table 4). In all three test groups, significant reductions were found between the pre- and post-tests in body circumferences (upper arm, thigh, hip, chest, waist and abdomen). The multivariate analysis (MANOVA) between the groups was also significant. Not only was there a significant difference in hip circumference between the three test groups and the C group, but also between the DEP and D groups ($p < 0.006$). Thigh circumference of both exercise training groups (DE and DEP) significantly decreased as compared to the D group. Abdomen circumference also showed a significant difference between the D and DE groups ($p = 0.04$).

Subjective assessment of skin condition

Subjects of all three intervention groups rated the severity of cellulite, as well as the general condition of their skin on their thighs and buttocks in the post-test as significantly lower or better than in the pre-test (Figs. 2, 3). The

Table 3 DXA Baseline and post-tests in g + SD and differences in g and %

	Diet exercise pressure group (DEP)	Diet exercise (DE)	Diet group (D)	Control group (C)
DEXA: Baseline and Posttests in g + SD and differences (g, %)				
TBM baseline (g)	75,986 + 8,214	73,379 + 6,843	75,486 + 6,716	73,463 + 7,748
TBM posttest (g)	70,316 + 9,120	68,970 + 6,823	73,239 + 8,264	73,194 + 8,012
Difference TBM (g, %)	5,670 (7.5%)*§#	4,409 (6%)*§	2,247 (3%)*	269 (0.4%)
TBF baseline (g)	29,452 + 5,104	27,706 + 3,390	29,204 + 4,722	27,215 + 4,428
TBF posttest (g)	24,697 + 6,414	23,974 + 3,819	26,933 + 6,009	26,804 + 4,572
Difference TBF (g, %)	4,756 (16.1%)*§#	3,732 (13.5%)*§	2,271 (7.8%)*	410 (1.5%)
LBF baseline (g)	16,119 + 2,869	15,725 + 2,056	16,894 + 3,206	14,861 + 2,733
LBF posttest (g)	13,713 + 3,192	13,923 + 2,117	15,571 + 3,468	14,752 + 2,806
LBF difference (g, %)	2,406 (15%)*§	1,801 (11.5%)*§	1,323 (7.8%)*§	110 (0.7%)
BFL baseline (g)	11,423 + 2,026	11,306 + 1,484	12,197 + 2,529	10,347 + 2,091
BFL Posttest (g)	9,811 + 2,214	10,087 + 1,583	11,237 + 2,561	10,434 + 2,190
Difference BFL (g, %)	1,613 (14.1%)*§#	1,219 (10.8%)*§	960 (7.9%)*§	87 (0.8%)
BFA baseline (g)	3,225 + 770	2,814 + 268	2,964 + 536	2,929 + 596
BFA posttest (g)	2,718 + 694	2,599 + 429	2,866 + 706	2,902 + 496
Difference BFA g (%)	507 (15.7%)*§#	215 (7.6%)*	99 (3.3%)	26 (0.9%)
TF baseline g	14,032 + 3,234	12,810 + 2,072	13,278 + 2,509	13,173 + 2,498
TF Posttest g	11,411 + 4,028	10,514 + 2,185	12,070 + 3,462	12,703 + 2,737
Difference TF (g) (5)	2,621 (18.7%)*§#	2,297 (17.9%)*§	1,208 (9.1%)*	470 (3.6%)
LBM relativ basline/Posttest	58.2%/61.8%*	59.1%/62%*	58.3%/60.2%*	59.7%/60.2%

TBM Total body mass, *TBF* total body fat, *LBF* lower body fat, *BFL* body fat leg, *BFA* body fat arm, *TF* trunk fat, *LBM* lean body mass

* Significant within group ($p < 0.05$)

§ Significant between group and control group; # significant between group and diet group

Table 4 Circumference measurements- baseline, Posttests and Differences of all groups in cm + SD and differences in cm and %

	Diet exercise pressure group (DEP)	Diet exercise (DE)	Diet group (D)	Control group (C)
Upper arm baseline	33.6 + 2.2	32.6 + 2.1	32.7 + 2.2	32.3 + 1.6
Upper arm posttest	31.9 + 2.7	31.1 + 2.1	31.9 + 2.7	32.2 + 1.8
Difference	1.7 (5.1%)*§#	1.5 (4.7%)*§	0.8 (2.4%)*	0.1 (0.4%)
Chest baseline	101.2 + 7.2	99.4 + 4.7	98.9 + 4.4	98.6 + 5.9
Chest posttest	97.9 + 8	96 + 4.5	97.9 + 5.3	98.1 + 6
Difference	3.3 (3.3%)*§#	3.4 (3.4%)*§#	1 (1.1%)*	0.5 (0.5%)
Waist baseline	82.1 + 6.5	79.9 + 4.5	80.1 + 3.4	81.1 + 4.7
Waist posttest	77.5 + 7.1	75.9 + 4.6	77.9 + 5.2	80.7 + 5.3
Difference	4.6 (5.5%)*§	4 (5%)*§	2.2 (2.8%)*	0.4 (0.5%)
Abdomen baseline	91.2 + 7.9	88.8 + 6.4	90.7 + 4.8	91.6 + 6.9
Abdomen posttest	85.7 + 8.2	83.2 + 6.7	87.4 + 6	91.8 + 6.9
Difference	5.5 (6%)*§	5.6 (3.2)*§#	3.3 (3.2%)*§	0.2 (0.2%)
Hip baseline	111 + 5.3	110.5 + 4.7	111.9 + 5.4	108.7 + 4.6
Hip posttest	105.86 + 5.7	106 + 5.2	109.9 + 6.9	108.8 + 5.1
Hip Difference	5.14 (4.6%)*§#	4.5 (4.1%)*§	2 (1.9%)*§	0.1 (0.1%)
Upper leg baseline	65.8 + 3.5	66 + 3.1	66.7 + 4	63.8 + 3
Upper leg posttest	62.1 + 3.9	62.5 + 3.7	65.1 + 5.1	63.7 + 3.5
Difference	3.7 (5.7%)*§#	3.5 (4.1%)*§#	1.6 (1.9%)*	0.1 (0.1%)

* Significant within group ($p < 0.05$)

§ Significant between group and C

\# Significant between group and D

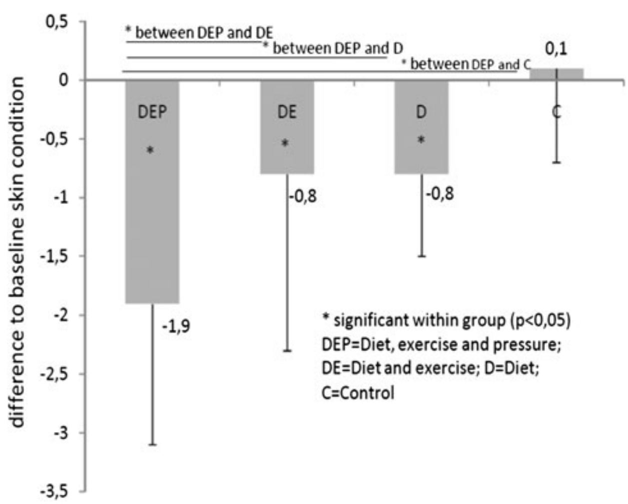

Fig. 2 Change in skin condition: difference from baseline condition (multilevel scale)

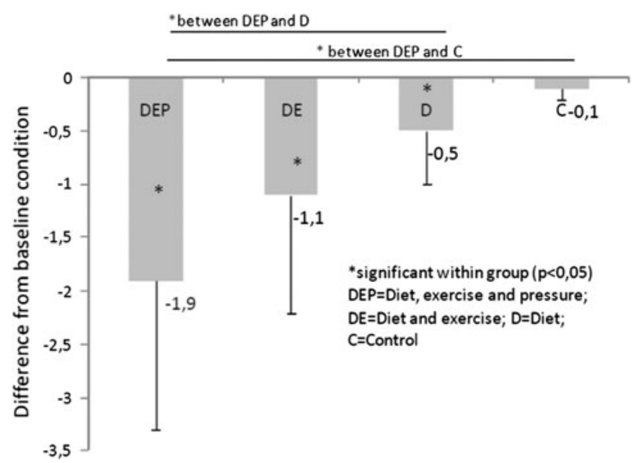

Fig. 3 Change in cellulite: difference from baseline condition (multilevel scale)

multivariate analysis showed a significant result in both parameters. In the post hoc test, a significant perceived improvement was made by the DEP group compared to the other three groups (C, D, DE) for skin condition and also between the DEP versus C and D groups for cellulite.

Discussion

The aim of the study was to investigate how diet, diet and exercise, as well as diet and exercise in combination with lower body positive and negative pressure affects changes in regional fat and overall body composition. The common perception that a reduction in fat mass can be achieved by consistent submaximal endurance training on a bicycle ergometer in combination with a modest (~350 kcal) deficit in dietary intake can be confirmed. According to this randomized controlled study, a physical activity program for previously inactive overweight women leads to greater loss of body fat than a diet only intervention. Other studies confirm these results (Hagan et al. 1986; Stiegler and Cunliffe 2006; Dunn et al. 2006), while some studies found no significant weight loss or reduction of body fat mass resulting from the addition of an exercise program (Hansen et al. 2007; Cox et al. 2003; Kraemer et al 1997; Redmann et al. 2007). Kraemer et al. (1997) divided 31 women into four groups and subjected them to either a diet only plan (an average of 1,194 kcal day^{-1}) or, a diet plus exercise plan that included either endurance or strength training. The fourth group ($n = 6$) served as control. After 12 weeks, a significant reduction in percent body fat, ranging from 4.3 to 8%, was achieved by each of the intervention groups, while fat-free mass remained unchanged. In our findings from the current study the DEP group lost 16.1% of Total body fat which is significantly more than in the diet group who lost 7.8%. Perhaps, the lack of differences among the groups in the study of Kraemer et al. was due to variance in experimental design (group size, homogeneity of the groups, control of energy intake, etc.).

There are numerous studies dealing with measures to reduce body weight and body fat as well as their impact on health. However, only a few studies show the influence of diet and exercise on local changes in body composition. Nindl et al. (2000) conducted a training study (strength and endurance training) over a period of 6 months. Subjects lost 2.6 kg of fat mass: 1.3 kg in the trunk and 1.1 kg in the arm region. A relative decrease of 31% of fat mass occurred in the arms, while the percentage change in fat tissue of the legs remained the same. Redmann et al. (2007) showed that weight loss was spread out evenly. Thus, the distribution of body fat remained essentially unchanged. These results were seen both in the group with a reduced daily caloric intake and in the diet plus exercise with no significant difference between the groups. The present study was comparable to Redman and Okura et al. (2004); fat reduction was distributed evenly over the whole body. The largest relative loss of fat mass in the test groups was in the trunk (DEP with 4.8%) and lowest in the arms (D with 1.1%). In contrast to the study of Nindl et al., a reduction in fat mass of 3.2% occurred in the legs in our DEP group. The D group only reduced fat mass of the legs by 1.9%, and the difference between these two groups was statistically significant.

Circumference measurements confirm the results of the DXA data. Waist circumference is used as a measure to assess health risk and, according to Wannamethee et al. (2005), has a stronger link to health risk than waist-to-hip ratio. The World Health Organization (WHO) recommends classification by the waist for assessing health risk, with a greater than 80 cm waist circumference for women representing an increased risk (2000, in Kiefer et al. 2006). In this study, the subjects of all three test groups had an average waist size of >80 cm, which was reduced to <80 cm after the intervention, placing them into a lower risk category according to the WHO classification.

It has long been known that a blood volume shift occurs when negative or positive pressure is exerted on the lower body. Under LBNP, initially venous return is reduced and heart rate increases to maintain cardiac output. However, in the literature there are different reports on when these responses take place. Wellhöner et al. (2006) could find no changes in blood pressure or heart rate at −15 mmHg, while Cooke et al. (2004) described a decrease in stroke volume of about 20 ml with a corresponding increase in heart rate at −10 mmHg. Results also vary when peripheral and hormonal reactions are examined. At −20 mmHg, Kitano et al. (2005) reported a reduction of blood flow in both the brachial and femoral artery. Essandoh et al. (1986) found decreased blood flow to the forearm at −20 mmHg, whereas in the leg, a measurable vasoconstriction was only seen at −40 mmHg. Responses to hormones such as plasma renin, arginine vasopressin, and aldosterone are also usually only significant at higher pressure influences (Cooke et al. 2004). Under negative pressure, a reduction in peripheral blood flow tends to occur, whereas a positive pressure typically causes a decrease in vasomotor sympathetic nerve activity. In contrast to other studies we used a combination of LBNP and LBPP with the idea to increase the blood flow of the lower Extremity and to avoid a reactive vasoconstriction. Additionally to the influence of pressure, the subjects had to perform a targeted endurance training. To our knowledge, no studies dealing with the application of LBNP and LBPP in combination with exercise and its effects on local blood flow, total body fat and regional fat loss have been performed.

A significant statistical relationship between both the fat of the legs measured in grams ($r = 0.4$; $p < 0.01$) and the relative fat content of the legs ($r = 0.6$; $p < 0.01$) with the subjective assessment of the severity of cellulite was shown. Equally significant was the correlation between the subjective rating of improvement of cellulite on the legs and loss of body fat in the legs ($r = 0.502$; $p < 0.01$), as well as the reduction of the proximal thigh circumference ($r = 0.598$; $p < 0.01$). A similar relationship was also found between the improvement of skin appearance and the extent of fat loss in the legs. The degree of cellulite and the appearance of the skin were evaluated subjectively by the subjects using a 10-point scale. This is a relatively weak measuring instrument as it is not objective. On the other hand, the subjective assessment is essential as cellulite is subjective, being cosmetically disturbing lesions which are not pathological (Pavicic et al. 2006). In this respect, the subjective assessment is the most relevant to evaluate the severity of cellulite. Smalls et al. (2005) have shown, using quantitative measures of cellulite (3D laser scanner, DXA and ultrasound), that there is a high correlation between expert assessments and the subjective perception. In the current study, the cellulite or the appearance of the so-called "orange peel" improved significantly in the DEP Group ($p = 0.001$), and significantly in the other two test groups (D, $p = 0.013$; DE, $p = 0.006$). Similarly, a subjective improvement in skin texture was observed in all three test groups ($p < 0.05$). However, the most obvious improvement was seen in the DEP group ($p < 0.000$). In a comparison among the groups, a significant difference in the change of skin appearance was seen not only compared with the C group, but also with the DE and DEP groups ($p < 0.01$). The cellulite improved most in the DEP group. There was a significant difference between the DEP group and the D or C groups ($p < 0.01$). In the literature there are no studies that examine an effect of exercise on the development of cellulite (Pavicic al 2000). Smalls et al. (2006) investigated the effect of weight loss on skin condition and showed a significant average improvement of cellulite expression by the loss of regional body fat and thigh circumference; a result that agrees to a great extent with the results of this study. The technique of manual lymph drainage (MLD) is propagated for the treatment of cellulite, even if a long-term effect has yet to be substantiated (Rossi and Vergnanini 2000; Rawling 2006). Mostly, MLD is used for the treatment of lymphedema. A reduction of the circumference and volume of limbs by MLD has been shown in several studies, although there are few randomized, controlled studies with a good research design and follow-up tests, and in some studies, no additional effect of MLD could be detected (Moseley et al. 2007; Badger et al. 2004; Lawenda et al. 2009; Damstra and Mortimer 2008). Also compression devices that produce local pressure (Pneumatic pump therapy, pneumatic compression therapy) are used for decongestion. Successes with lymph edema, wound healing disorders, circulatory disorders or "restless legs" have been reported (Kozanogly 2009; Lettieri and Eliasson 2009; Richmand et al. 1985), although the clinical relevance has been questioned in some instances (Dini et al. 1998). In these cases, however, the pressure is usually higher, up to 150 mmHg (Richmand et al. 1985). A widely held hypothesis is that cellulite is caused by a change in the microcirculation and an increased accumulation of fluid in the dermis and in the intra-or interlobular septa (Pavicic et al. 2006), thus, the use of decongesting, liquid mobilizing techniques seems logical.

It is possible that the over- and under pressure conditions applied to the lower extremities increase the mobilization of extracellular water and positively influences the skin or cellulite. This could explain the difference between the groups in the improvement of the appearance of cellulite and skin structure. In our study we detected no significant difference in regional fat loss between the DEP and DE group. One reason for this result could be due to the study design. The duration and/or combination of over- and under-pressure might have been not optimal to increase blood flow in adipose tissue. Or an increased blood flow in the area leads to a spot lipolysis but not to a spot reduction. Stallknecht (2006) indicates this fact to consider in her study. Nevertheless, the combination of exercise and pressure appears to be an interesting approach, and it may be worth further investigation. Especially, the direct measurement of the adipose tissue blood flow during exercise and pressure would be interesting. Useful and effective applications could also arise for circulatory disorders, wound healing, lymphedema or the symptoms of "restless legs". For this purpose, there is a need of additional research, investigating the effects of LBNP, LBPP or a combination of both on peripheral blood flow, as data from the few published studies only offer contrasting results.

Conclusion

The combination of diet and exercise, with or without application of a local over-and under pressure examined here has shown to be successful for weight reduction. The difference of whether this training is used with or without the application of under- or over-pressure is especially evident on skin appearance or in the improvement of cellulite. DXA is a useful instrument to show changes in weight or fat mass as well as in regional body composition. While in the current study LBNP and LBPP with exercise and diet do not appear to provide additional regional fat loss than exercise and diet alone, LBNP and LBPP may have greater implications on the cosmetic appearance of cellulite and skin conditions. As many women suffer considerably from the cosmetically disturbing changes in their skin, these results should increase the motivation to implement "Hypoxi-training" and diet program.

Ethical standard The study was conducted in accordance with the guidelines of the Declaration of Helsinki (1997). Upon request to the Ethics Commission, the study was deemed appropriate without further authorization

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