The effects of combined thermal and electrical muscle stimulation (cTEMS) on fatness and fitness

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Scientific environment

The present thesis is based on studies carried out at the Department of Heart Disease, Haukeland University Hospital and the Department of Clinical Science, University of Bergen, between 2009 and 2012.

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CONTENT

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SUMMARY

Electrical muscle stimulation (EMS) may have potential as an alternative to regular physical exercise and as an adjunct to dietary caloric restriction. However, the effects of regular EMS in obese, sedentary individuals are not known. Further, previous studies using EMS have methodological deficiencies. Regular physical exercise and dietary caloric restriction remains the cornerstone of lifestyle intervention and has positive effects on all modifiable risk factors related to obesity-related metabolic disease. The potential of EMS as an alternative to ordinary exercise in individuals unable or unwilling to perform physical training needs to be explored.

The aims of our studies, were to

- investigate the physiological effects of adding superficial heat to electrical muscle stimulation (combined thermal- and electrical muscle stimulation: cTEMS).
- explore the effects of different stimulation protocols, varying in electrical pulse types and intensity of heat stimulation.
- analyse the effects of prolonged cTEMS in obese sedentary persons on cardiorespiratory fitness, adipose tissue and lipid metabolism
- investigate the potential benefit of cTEMS as an adjunct to a dietary weight-reducing intervention

cTEMS showed similar physiological response as exercise training corresponding to a moderate intensity. While electrical stimulation induced muscular work with a dose-response effect on oxygen uptake, increased heat intensity conferred additional increase in growth hormone and hemodynamic response due to vasodilation. When given three times weekly over 8 weeks, we found that cTEMS improved aerobic fitness in obese sedentary individuals with an unchanged lifestyle. We found a significant improvement in the functional capacity towards higher fatigue resistance with improved oxygen uptake and workload, both at the anaerobic threshold and at peak intensity. While there were no changes on body weight or body composition during this relatively short intervention period, we observed increased lipolytic activity and increased mitochondrial fatty acid oxidation activity in adipose tissue.

Used as an adjuvant to a weight–reducing dietary intervention, the regular use of cTEMS conferred a significantly larger reduction of visceral fat than the diet alone. Although there was no change in peak oxygen uptake, the lowering of both the respiratory exchange ratio and the energy expenditure during exercise, indicated cTEMS related training effects.

In conclusion, cTEMS display physiological response similar to regular exercise and may prove useful, both as an alternative to training in obese sedentary individuals and as a supplement to a dietary intervention.

SELECTED ABBREVIATIONS

EMS	Electrical muscle stimulation
cTEMS	combined thermal and electrical muscle stimulation
$VO_{2\ max}$	Maximum oxygen uptake
BMI	Body mass index
CVD	Cardiovascular disease
VAT	Visceral adipose tissue
RER	Respiratory exchange ratio
LDL	Low-density-lipoprotein
HDL	High-density-lipoprotein
LCD	Low carbohydrate diet
$VO_{2\ peak}$	Peak oxygen uptake
HR	Heart rate
CPT	Carnitine palmitoyl transferase
PPAR	Peroxisome proliferator-activated receptor
PGC-1α	PPAR γ co-activator 1α
GH	Growth hormone
TC	Total cholesterol
apoB	Apolipoprotein B100
apoA1	Apolipoprotein A1
VLDL	Very large density lipoprotein
SCAAT	Subcutaneous abdominal adipose tissue
LPL	Lipoprotein lipase

LIST OF PAPERS

Paper I

Espen Rostrup MD, Monika H.E. Christensen, MD, Reinhard Seifert, BSc,

Jan Erik Nordrehaug MD, PhD

Physiological effects of combined thermal and electrical muscle stimulation (cTEMS) in healthy individuals: A pilot study

Scand J Clin Lab Invest; 2012; 72 (3); 237-245

Regional Committee for Medical and Health Research Ethics
(Western Norway Ref: 263.08)

Paper II

Espen Rostrup MD, Grete Slettom MD, Reinhard Seifert BSc, Bodil Bjørndal PhD,

Rolf K. Berge PhD, Jan Erik Nordrehaug MD PhD

Effect of combined thermal and electrical muscle stimulation on cardiorespiratory fitness and adipose tissue in obese individuals

Eur J Prev Cardiol 2013 Mar 20, EPub Ahead of Print

Regional Committee for Medical and Health Research Ethics
(Western Norway Ref: 2009/1273)

Paper III

Espen Rostrup MD, Reinhard Seifert BSc, Rolf K. Berge PhD, Jan Erik Nordrehaug MD PhD

Electrical muscle stimulation in obese subjects during weight loss: a randomized trial

Manuscript

Regional Committee for Medical and Health Research Ethics (Western Norway Ref: 2010/2360)

INTRODUCTION

Electrical muscle stimulation

Already around 2500 B.C. the ancient Egyptians used electricity for the treatment of medical conditions. The electrical catfish, *malapterurus electricus,* is able to generate up to 400 volts due to electrical organs of muscular origin [1] This characteristic was, among other things, used for the treatment of pain, gout and migraine. Due to its healing powers, the electrical catfish was adapted as the emblem of the early pharaoh Narma [2].

Fig 1.
Electrical catfish - malapterurus electricus

Fig 2.
Electrical catfish depicted in the Tomb of Kagemni at Saqqara
(Courtesy of Rosalind Park)

Today, electrical muscle stimulation (EMS) is mainly used in order to limit the loss of muscular function and/or restore muscle function after injury. Attention towards the use of EMS as an alternative to exercise training, as a mean to promote exercise training and a more active lifestyle however, has been increasing. Primarily this attention has been concentrated on EMS-evoked exercise, such as EMS-induced leg cycling exercise in patients with spinal cord injury. A systematic review of relevant literature found that regular EMS-evoked exercise in spinal cord injured patients had shown transition in muscle fibre towards greater fatigue resistance and significant increase in oxygen uptake (l/min) and exercise metabolism (J/kg/s) [3].

EMS without involving performance of external work has however, also in healthy individuals, been proven to produce a physiological response consistent with cardiovascular exercise at mild to moderate intensities [4] . EMS, producing rhythmical contractions in lower extremity muscles at a frequency of 4 Hz, when given regularly

over six weeks, significantly increases maximum oxygen consumption (VO2 max), 6-min walking distance and quadriceps strength. There was no observed changes in body mass index (BMI) [5].

Further, in healthy male subjects, chronic low frequency EMS leads to significant transition in patterns of myosin heavy chain isoforms in the fast-to-slow direction in skeletal muscle and induces changes in enzyme activity indicating an increase in aerobic-oxidative capacity [6].

Also patients with chronic heart failure can benefit from the use of EMS. Degenerative changes in their skeletal muscles, involving mitochondrial content and ATP synthesis, capillary density and reduced enzyme activities of terminal substrate oxidation, contribute to reduce the physical performance in these patients. Regular use of EMS counteracts these changes, leading to increased exercise capacity measured by VO2max, increased maximal workload and improved 6-minutes walk test [7].

A major limitation, when stimulating muscle electrically, has been the associated subjective discomfort [8-10]. Heat, applied superficially, is frequently used in relation to various muscular conditions and is known to reduce muscular pain [11,12]. Further, heat exposure leads to an increase in muscle blood flow and tissue circulation [9,13] and could in theory serve as a supplement to EMS. However, whether adding heat to EMS increases stimulation tolerance or leads to a more effective recruitment of muscle fibres, has so far not been explored.

A variety of electrical current characteristics has been used in EMS research, but are usually unsatisfactory described [8,14]. Systematic comparisons of electrical current characteristics, keeping other stimulation parameters fixed are lacking and thus it still remains unclear what are the optimal electrical characteristics on exercise parameters and standard effect evaluation with peak oxygen uptake.

Potential of EMS in populations at risk

A number of components have been found to increase the risk for cardiovascular disease. In the INTERHEART study, a large, international, standardised, case-control study, the effect of risk factors for coronary heart disease in 15152 cases and 14820 controls were analysed. The two most important risk factors are smoking and

abnormal lipids, accounting for about two-thirds of the population attributable risk. Abdominal obesity (OR 2,24) and exercise (OR 0,72) however, also play an important role in the risk of an acute myocardial infarction [15]. In selected populations, EMS could potentially play a role in counteracting both, either as an alternative to regular physical exercise or as a supplement to a weight-reducing intervention.

The importance of intervention to reduce the load of risk factors is made visible by the high lifetime risk. In the Framingham study the lifetime risks of coronary heart disease for individuals at age 40 were 49 per cent in men and 32 per cent in women. Even those who were free from disease at age 70 had a lifetime risk of 35 per cent and 24 per cent in men and women, respectively [16]. Thus, the potential risk reduction by increasing physical activity, or perhaps only muscular activity in individuals unable to exercise for various reasons, could be significant.

Obesity and fitness

Obesity is not a homogenous condition and in which way the body fat is distributed seems to be a determinant for the risk of cardiovascular disease (CVD) [17]. While subcutaneous obesity mostly is related to a normal metabolic profile, visceral obesity is the most prevalent manifestation of the metabolic syndrome, a constellation of metabolic abnormalities including insulin resistance, atherogenic dyslipidaemia, hypertension and inflammation [17,18]. The accumulation of visceral adipose tissue (VAT), which also correlates with smaller ectopic fat depots such as pericardial fat, is associated with increased risk of cardiovascular disease beyond BMI or waist circumference [19]. When analysing the effects of diet and exercise, the reduction of VAT induce greater beneficial effects on parameters of the metabolic syndrome than the reduction of subcutaneous fat [20]. Thus it seems important that VAT is given special focus when evaluating lifestyle interventions.

Obese individuals may have special metabolic features that could influence standard endpoint evaluation. Exercise capacity is often assessed in a standardised way using oxygen uptake and respiratory exchange ratio (RER; ratio between exhaled CO_2 and inhaled O_2). RER and consequently the lipid oxidation, measured in kcal/min, are lower in individuals with atherogenic dyslipidaemia than in those without, both at rest and

during exercise. Further, RER during exercise correlates well with levels of triglycerides, and both at rest and during exercise, RER is negatively correlated with low-density-lipoprotein (LDL) particle diameter and high-density-lipoprotein (HDL) cholesterol [21]. Thus a reduced RER after a period of parallel intervention, even without a corresponding change in peak oxygen uptake, could reflect positive metabolic effects from the intervention.

In obese individuals and type 2 diabetics, skeletal muscle display reduced oxidative enzyme capacity, increased glycolytic activity and increased lipid content, independent of fiber type, resulting in a reduced capacity to oxidize fat [22]. Subsequently this can lead to further increase in the storage of fat in both adipose tissue and inside muscle cells. Both fat oxidation and fat mobilisation however, may be improved through regular exercise [23].

Low cardiorespiratory fitness is an independent and strong predictor of CVD-death and all-cause-mortality with relative risk comparable traditional risk markers [24]. Another parameter of physical fitness, muscular strength, as assessed by one-repetition-maximum, is also independently associated with risk of death from all causes, even after adjusting for cardiorespiratory fitness [25]. The use of prolonged EMS has been shown to improve both aerobic capacity and strength in healthy sedentary individuals [5], but the potential exercise effect and effect on cardiovascular risk factors of EMS in obese and sedentary individuals have not yet been examined.

Lifestyle intervention

Diet

Dietary induced weight-loss has shown a reduction in a wide range of cardiovascular risk factors [26-30]. Although some studies indicate that different macronutritional compositions of the diets may generate different metabolic effects [26,28], the positive effect on atherosclerosis itself, as measured by regression of carotid vessel wall volume, appears to be mediated by the weight-loss induced decline in blood pressure [27].

Whether some macronutrient compositions may be more effective in inducing weight loss than others remain debated. While an often referred study by Sacks et al. did not find any significant differences in weight loss induced by different diets [30], a recently published meta-analysis by Bueno and colleagues indicate that individuals assigned to a very-low-carbohydrate diet may achieve a greater weight loss than those assigned to a low-fat diet in the long term [31]. One reason for the diverging results may be poor adherence to diets, a concern mentioned specifically in an accompanying editorial to the study by Sacks and colleagues [32].

Although newer studies have not found any evidence to conclude that dietary saturated fat per se lead to an increased risk of CVD [33-35], some concerns have been raised about a potential unfavourable change in LDL cholesterol from low carbohydrate diets [36,37]. Two large randomized trials however, found favourable changes in cholesterol and lipids with a low carbohydrate diet compared to other diets [26,28] and studies that included in-depth analysis of lipoprotein subclasses found changes in LDL leading to larger, less atherogenic LDL particles[38-41]. A potential exercise effect of EMS could modify the effects of low-carbohydrate-diets (LCD) even further.

Exercise

Physical activity is recognized as a first-line component in the management of obesity and cardiovascular risk [42] because of its pleiotropic effects on modifiable risk factors related to the cardiometabolic risk [43]. Increased physical activity may reverse the obesity-associated atherogenic dyslipidaemia, consisting of reduced HDL cholesterol, high levels of triglyceride and increased number of small, LDL particles [18,44]. When studying sedentary overweight men and women, Kraus et al. found a clear beneficial effect from a eight months exercise-program on lipoproteins and lipoprotein subclasses, even in the absence of significant weight loss [45]. A high amount of exercise resulted in greater improvements, although exercise also with lower amount and at lower intensity also had better responses than the control groups.

Despite the undisputed positive health effects set off by increased physical activity [46-49], the long-term weight reducing effects from exercise alone is small [50,51] ranging from 1-3 kg over a time period of 4 months to 16 months [51].

Physical exercise confers metabolic, hormonal, biochemical and hemodynamic changes affecting parameters such as catecholamines [52], lactate [53], growth hormone [54] and lipids [44,55]. Whether EMS can mimic these particular effects of exercise, if used regularly, is either poorly described or not evaluated.

Diet + Exercise

The primary goal of a dietary hypocaloric weight-loss intervention is the reduction of excess body fat. However, a concomitant loss of lean mass is often observed, accounting for as much as a third of the total weight loss. This observation seems to be independent of whether the diets emphasized fat, proteins or carbohydrates and more related to the total reduction in energy intake rather than the macronutrient composition of the diet [56].

Although still debated, there are several indications, that adding exercise to a calorie-restricted diet, may reduce the loss of fat free mass during weight loss by as much as 50% with aerobic exercise, providing a better lean mass-preservation than strength training [57]. However, when Redman et al. precisely matched and controlled the caloric restriction and thereby corrected for the caloric impact of adding exercise to diet, combining both did not result in further favourable changes in body composition [58].
In this aspect it is important to notice that while a weight-reducing intervention with diet or diet + exercise induce a larger total weight-loss than the reduction of fat, indicating a concomitant reduction of lean mass, an intervention with exercise alone cause a larger reduction in fat mass than the total weight reduction itself because of the simultaneously increase in lean mass [50].

In critically ill patients, small-scale studies has shown that EMS may preserve muscle mass and thus appears to be an useful adjunct to prevent muscle wasting and catabolic protein degradation [59,60]. Further, in a recently published study by Kemmler and Stengel, 12 months of so-called whole-body electromyostimulation exercise showed positive effects on parameters of sarcopenia in sedentary older female adults [61].

The potential of EMS as an adjunct to weight reducing diets however, remains unexplored.

Fat metabolism in adipose tissue

The major energy reserve of the human body is stored in the adipocytes, but due to anatomy, an integrated study of the function of the adipose tissue is not easy accessible. Microdialysis, a special in-vivo technique that allows continuous sampling of macromolecules from the interstitial space, was first described by Lönnroth et al. in 1987 [62]. The microdialysis system consists of a double-cannula probe connected to a perfusion pump that accurately provides a continuous flow, normally in the range of 1-10 µl. The perfused fluid passes a dialysis membrane and via the interstitial space it returns back over the membrane as microdialysate and ends up in a collector. The use of microdialysis has led to major advances in the understanding of adipose tissue metabolism [63] such as the role of growth hormone [64], atrial natriuretic peptide [65] and catecholamines [66] in fatty acid mobilisation and the prolonged effect from physical exercise on lipolysis [67].

Adipose tissue metabolism is highly complex and often the analysis of blood lipids does not reflect actual changes in adipose tissue [63]. A major advantage of the microdialysis technique is to monitor the tissue directly. To our knowledge, this technique has so far not been used to evaluate the effects of EMS.

Knowledge gaps

The previous overview indicates that the use of electrical muscle stimulation has shortcomings due to lack of methodological studies on electrical pulse type parameters, using a typical exercise level indicator, such as maximal oxygen uptake, as an endpoint. An accepted limitation of EMS is the subjective discomfort during stimulation. Whether adding superficial heat to EMS can enhance the physiological training effects or improve tolerance is not known.

There are indications that regular use of EMS may mimic the effects of ordinary physical exercise on oxygen uptake. The effects on other physiological variables however, such as catecholamines, growth hormone, lactate and hemodynamic parameters, are not systematically studied. The effects of EMS on adipose tissue are unknown and finally, prolonged EMS has not been tested in overweight sedentary subjects, or as a possible adjuvant to dietary intervention in obese or overweight subjects.

AIMS OF THE THESIS

The general aim of this study programme was to evaluate the potential effects of cTEMS as an intervention to counteract the increased disease risk from inactivity and obesity.

Paper 1

1. To test the hypothesis that combined thermal and electrical muscle stimulation was better than EMS alone to elicit physiological responses similar to exercise training.
2. To explore the effect on oxygen consumption of different electrical muscle stimulation protocols, using different electrical pulse types, variable intensity of heat and electric current.

Paper 2

1. To investigate the effects of prolonged combined cTEMS on cardiorespiratory fitness in sedentary obese subjects as measured by peak oxygen uptake (VO_2 peak).
2. To investigate the effects of cTEMS on body composition, adipose tissue and lipid metabolism.

Paper 3

1. To evaluate the potential effects of prolonged cTEMS on the effect on body composition, reduction of visceral fat and influence on blood lipids, when used as a supplement to a dietary intervention.
2. Explore the effects of a hypocaloric very-low-carbohydrate-high-fat diet on weight, body composition, lipids and lipoproteins.

METHODS

Study designs and populations

Paper I

In this pilot study we recruited healthy individuals through an invitation sent per electronic mail to all employees at the Haukeland University Hospital. The subjects in the study were between 30 and 70 years old and able to perform physical testing as planned in the study protocol. Exclusion criteria were extensive dermatological disease, pacemaker or implanted defibrillator, regular medication, known pulmonary or heart disease, other major disease or pregnancy. All subjects underwent adaptation to both the treadmill testing and cTEMS before the baseline examination and underwent two hours of fasting before each study visit. They further refrained from exercise training in the 24 hours before the visits. 10 electrical pulse types, varying in polarity, frequency, pulse-width and output mode were tested. At both low and high heat intensity; we registered the study parameters at 20% of max stimulation capacity and at each individual's threshold.

Paper II

For this longitudinal intervention study, 12 obese (BMI ≥ 30 kg/m^2) and sedentary subjects (< 20 minutes of exercise < 3 days per week) were recruited through an invitation sent to employees at the Haukeland University Hospital in Bergen, Norway. Other inclusion criteria were age between 30 and 70 years and the ability to undergo exercise testing. Individuals with a pacemaker, regular medication, cardiovascular disease, pulmonary disease, extensive dermatological disease or other primary diseases; pregnant women; and individuals who abused alcohol or drugs were excluded from the study. For completion of the study, participation in more than 70 % of the planned cTEMS sessions was mandatory. Before the baseline visit, all participants underwent one session of adaptation and tolerance testing. The inclusion was followed by an eight weeks intervention period with three cTEMS stimulation

sessions per week. During the intervention period, the participants were instructed to maintain their habitual lifestyle, including dietary habits and physical activity levels. Adherence was controlled for with a nutritional questionnaire and accelerometer.

Paper III

A total of 24 overweight (BMI ≥ 28 kg/m^2; fat percentage > 20% for men and 28% for women) and sedentary (≤ 20 minutes of exercise on ≤ 3 days per week), but otherwise healthy individuals aged between 30 and 70 was, after giving their written consent, included in the study. Before inclusion all subjects underwent a thorough medical examination. For inclusion and exclusion criteria, see table 1.

The subject was recruited through advertisement in the local press

Table 1. Inclusion and exclusion criteria

Inclusion criteria:	Exclusion criteria:
• Age 30-70 • BMI > 28 • Fat percentage > 20 % for men and > 28% for women	• Regular exercise • Pacemaker or ICD • Regular medication • Known pulmonary or cardiac disease • Other major diseases • Extensive dermatological disease • Alcohol and drug abuse • Pregnancy or planned pregnancy

After inclusion, the subjects were randomised to a ten weeks intervention with either a dietary intervention with a low-carbohydrate-high-fat diet alone or the diet supplemented with three 45-minute cTEMS sessions per week. At the baseline- and follow-up visit, all blood samples were collected in the fasting state.

Combined thermal and electrical muscle stimulation

In all three studies, we used the combination of applied heat (generated by electrical heating elements) and EMS. Ten silicone electrodes were connected to a stationary stimulator (TEI System, RÖS'S Estética S. L., Barcelona Spain).

The electrodes are incorporated into strips that generate heat and stimulation currents. Each electrode band includes an electrical heating element for thermal stimulation and two electrodes for the application of electrical current for EMS. Two different sets of electrodes were applied: three large (24 cm x 26 cm; max heating power 25 W) and seven small ones (24 cm x 17 cm; max heating power 15 W). The electrodes were attached to the pre-specified muscle groups (quadriceps, hamstrings, glutei muscles, oblique's, rectus abdominis and lower lumbar muscles) using elastic bands with Velcro, and optimal skin adhesion was obtained using a standard ultrasound gel.

Fig 3.

Illustrative photo of the application of cTEMS (Photo: Maritime Colours)

Exercise testing

Peak exercise capacity was used as baseline characteristic in study 1 and evaluated before and after the intervention periods with cTEMS in study 2 and 3. VO2 peak was assessed by ergospirometry during a standard treadmill test using a modified Bruce Protocol (Study 1) or a modified Balke protocol (Study 2 and 3). Ventilatory gas exchange was measured using an Schiller ergospirometer by breath-to-breath technique (Schiller Cardiovit CS-200 Ergo-Spiro/ Ganzhorn Power Cube). Before the baseline examination, there was one session of adaptation to the exercise test.

$VO_{2\ peak}$ was considered reached when all of the following accepted criteria were met: maximal heart rate measured at exhaustion was superior to 90% of the age-predicted maximal heart rate, RER measured at exhaustion was superior to 1.1, and the subject were not able to sustain sufficient running speed on treadmill.

Body composition analysis

A dual-energy X-ray absorptiometry (DEXA; GE Medical Systems, Lunar Prodigy DF) was used for the analysis of body composition while body weight and visceral fat area was measured using bioelectrical impedance analysis (InBody 720, BioSpace, Seoul, Korea)[68].

Microdialysis

After light epidermal anaesthesia (Emla patch 5%), one microdialysis probe (CMA 63, 20-kDA molecular-weight cut-off, CMA Microdialysis, Sweden) was inserted percutaneously into the subcutaneous abdominal adipose tissue (SCAT) 8-10 cm lateral of umbilicus. The probe was then connected to a microdialysis pump (CMA 107, CMA Microdialysis, Sweden) and continuously perfused with a sterile Ringer solution (154 mM Na^+, 6mM K^+, 2,5 mM Ca^{2+}, 160 mM Cl^-). The perfusion was set at a flow rate of 2µl/min and fractions were collected following a 30-minute equilibration period. An exchange of metabolites occurs over the microdialysis membrane, and the composition of the outflow solution reflects the extracellular fluid.

The microdialysate was analysed in a microdialysis analyser (Iscus flex , CMA Microdialysis, Stockholm, Sweden) for Lactate and Glycerol. The lipolytic activity in SCAT was expressed by dialysate-glycerol level.

Hemodynamic measurements

The hemodynamic measurements were derived from continuous non-invasive finger arterial pressure measurement, using a device designed for this purpose [69] (Nexfin HD, BMEYE - Cardiovascular Monitoring, Dallas, Texas). We did not apply cTEMS heating elements on the arms or near the finger cuffs used for the pressure monitoring. The cardiac output, stroke volume and system vascular resistance were assessed and indexed in relation to the body surface area.

The heart rate (HR) was observed continuously with electrocardiogram monitoring (Schiller CardiovitCS-200 Ergo-Spiro) and registered at rest and at maximum exercise capacity during exercise testing and after 5 minutes of steady state during each cTEMS pulse type. Blood pressure was monitored according to the standard protocol during exercise testing. During cTEMS, blood pressure was registered after 5 minutes of a steady state in the HR at each stimulation type, using a Schiller BP-200 plus blood pressure monitor.

Blood and tissue analysis

Hormonal analysis

For hormonal analysis, blood samples were drawn under standardized conditions and kept frozen at -80 °C until analysed at the Hormone Laboratory, Haukeland University Hospital. Insulin and GH were analysed using a two-site chemiluminescent immunometric assay using an Immulite 2000 (Siemens, Gwynedd, UK). The detection limit for GH was 0.01 ng/mL, and the inter-assay variations were 6.5%, 5.5% and 6.6% for concentrations of 2.6 ng/mL, 5.3 ng/mL and 17 ng/mL, respectively. Catecholamines were analysed with a radioimmunoassay, Cat Combi RIA (DRG Instruments GmbH, Marburg, Germany). The assay had an inter-assay variation for

norepinephrine of 10% at 22 ng/mL and 6.1% at 11 ng/mL. For epinephrine, the inter-assay variation was 5.6% at 4.4 ng/mL and 6.1% at 21 ng/mL.

Gene expression

In order to purify and quantify total cellular RNA spectrophotometrically, adipose tissue samples were homogenized. We evaluated RNA quality by capillary electrophoresis. Further, one microgram of total RNA was reverse transcribed using TaqMan reverse transcription agents with RNase inhibitor (Applied Biosystems, Foster City, CA, USA). We used 384-well Multiply-PCR Plates (Sarstedt, Newton, NC, USA) to perform real-time PCR on the following genes using probes and primers from Applied Biosystems: carnitine palmitoyltransferase (CPT) 1a (CPT- 1a, Hs00157079), CPT-2 (Hs00264677), hormonesensitive lipase (LIPE, Sh00943410), peroxisome proliferator-activated receptor gamma (PPARγ, Hs00174128), PPARγ coactivator 1 alpha (PGC-1α, Hs00173304), resistin (RETN, Hs00982492), uncoupling protein 1 (UCP-1, Hs00222453), UCP-2 (Hs00163349), and UCP-3 (Hs00243297). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Hs99999905) and ribosomal protein large P0 (RPLP0, 4333761T) from Applied Biosystems and 18S MP (Kit-FAM-TAMRA, RT-CKFT-18s) from Eurogentec (Seraing, Belgium) were uses as reference genes. To assess the stability of the measured reference, Normfinder algorithm15 was used. With a stability value of 0.039, RPLP0 showed the best results and was subsequently used to normalize the target gene values. Expression values were calculated as the ratio of target gene expression to RPLP0.

Clinical chemistry

Lipids were then measured enzymatically on a Hitachi 917 system (Roche Diagnostics GmbH, Mannheim, Germany) using the triacylglycerol (GPO-PAP), cholesterol (CHOD-PAP), HDL-cholesterol, and LDL-cholesterol kits from Roche (Roche Diagnostics GmbH, Mannheim, Germany). Non-esterified fatty acids and free cholesterol FS kits were from DiaSys (Diagnostic Systems GmbH, Holzheim, Germany).

Lipoprotein particle size

Lipoprotein particle size analysis was performed by Liposcience Inc, Raleigh NC, USA using proton nuclear magnetic resonance (NMR) spectroscopy. Particle concentrations of lipoproteins of different sizes were calculated from the measured amplitudes of their spectroscopically lipid methyl group NMR signals. Lipoprotein particle size was derived from the sum of diameter of each subclass multiplied by its relative mass percentage based on the amplitude of its methyl NMR signal.

Dietary intervention (Paper 3)

Each of the groups received teaching and instructions in nutrition in general and the low-carbohydrate-high-fat-diet (hereafter LCD) more specifically, both in groups and on an individual basis. Further the subjects were given a commercially available book on LCD diet (Frisk med lavkarbo by Hexeberg, S; MD PhD; ISBN 9788202322731). Preparation of the food was self-administered.

The carbohydrates were restricted until the presence of ketones was detected semi-quantitatively by urine reagent strips (Ketostix ®). Participants were told to keep their carbohydrate intake at a level where a colour change was maintained at the urine reagent strip, but optimally lower than 50 g per day. There was no restriction on energy content, but the subjects were instructed to replace the carbohydrates by not-processed fat from natural sources such at meat, fish, nuts or dairy products. They were further encouraged to eat vegetables in abundance.

A diet analysis was conducted at one point during the study. The subjects weighed and recorded all intakes of food and drinks over four days using a regular diary. The nutritional data of these registrations were analysed by study personnel using an online software tool (www.diett.no).

Lifestyle monitoring

Activity level measurement

In order to rule out possible bias affecting the results of the study, the subjects were encouraged to maintain their sedentary behaviour. The activity level over one week was measured before and at the end of the intervention period using an accelerometer in both study 2 and 3.

Participants wore an accelerometer (GT3X, The Actigraph, Fort Walton Beach, FL, USA) for seven consecutive days placed on their hip while being awake. The activity monitor is a compact (3,8cm x 3,7cm x 1,8 cm, 27g) triaxial accelerometer recording accelerations from 0,05 to 2,5 G. Data were processed by ActiLife software (The Actigraph) and total physical activity time spent in sedentary, light, moderate and vigorous physical activity was calculated.

Dietary habits (Study 2)

To detect possible changes in dietary habits, we used a 15-item self-administered questionnaire, Smart Diet [70].

Statistical methods

Paper 1

All outcome variables were converted to percentage change from rest. The association of different cTEMS stimulation protocols with mean percentage change of outcome variables from baseline were assessed with random intercept mixed effects models. Current intensity was modelled as a continuous covariate (0-100%) and heat intensity (low/high) and pulse types (B•7Hz•400μs, etc.) as cofactor. Study subjects were included as random effect and the variance structure was adjusted for heteroscedastic

residual spreads between pulse types [4,71]. Analyses were performed with R version 2.14.0 (The R Foundation for Statistical Computing, Vienna, Austria, 2011) and nlme package version 3.1-102.

Paper 2

Baselines vs. follow-up measurements were compared with non-parametric Wilcoxon signed-rank test and are presented as mean ± standard deviation.

Mean glycerol dialysate levels were estimated over time as well as the means of the relative gene expression values at baseline vs. follow-up were estimated with a random-intercept linear mixed-effects model in 2-way repeated measure configuration.

All statistical analyses were conducted with R version 2.15.2 (R Foundation for Statistical Computing, Vienna, Austria); linear mixed-effects models with package nlme-3.1-107.

Paper 3

Baseline characteristics are reported as mean (standard deviation). Differences between intervention groups at baseline were assessed with two-sided independent sample t-tests with Welch's correction for unequal variances. Endpoint variables at baseline, at follow-up and the absolute change from baseline to follow-up are reported as mean (standard deviation). Within group changes estimated by two-sided paired t-tests, between group differences in absolute change from baseline were compared with two-sided independent sample t-test with Welch's correction for unequal variances. P-values greater then 0.05 were considered statistically significant. All analyses were performed with R version 2.15.3 (R Foundation for Statistical Computing, Vienna, Austria).

SUMMARY OF RESULTS

Study I: Acute effects in healthy individuals

Multivariate analyses showed that electrical stimulation significantly increased peak oxygen uptake and the levels of lactate, catecholamine and growth hormone (GH). All outcome variables except the stroke volume index, increased significantly with every 10% increase in EMS intensity. Increasing the heat from low (38.2˚C) to high (40.7˚C) during electrical stimulation gave additional hemodynamic response and rise in growth hormone. The highest VO_2 was not affected by the increase in added heat. Further, we observed a dose-response relationship in peak oxygen uptake for increase in stimulation intensity. The highest oxygen uptake at individual maximum stimulation intensity was observed with biphasic continuous stimulation at 7 Hz ($p< 0.001$), which resulted in a VO_2 of 9.8±2.7 ml/kg/min. This represented a 3.2-fold increase in resting oxygen uptake, corresponding to 2.8 metabolic equivalents.

Study II: Prolonged effects in obese individuals with unchanged lifestyle

Eight weeks of cTEMS significantly increased VO_{2peak} from 28.9 ± 5.7 ml/kg/min to 31.7 ± 6.2 ml/kg/min ($p < 0.05$), corresponding to an average increase of 1.2 % per week. Further, we observed a 10.8% increase in peak work capacity. Oxygen uptake and work capacity also increased at the anaerobic threshold. Both at peak workload and at the anaerobic threshold, the observed increase in VO_2 and work capacity was accompanied by significant decrease in RER. Mean microdialytic glycerol concentration over 24 hours, an index of sedentary lipolytic activity, increased from 238 ± 60 μM to 306 ± 55 μM ($p<0,0001$), but no significant changes in body composition were observed. The increased lipolysis was not accompanied by any increase in free fatty acids. In addition, we found that both PGC-1α and carnitine-palmitoyltransferase-2 mRNAs were significantly upregulated in subcutaneous abdominal adipose tissue.

Study III: Prolonged effects during weight-loss

After 10 weeks both the LCD group and LCD+cTEMS group had similar significant weight loss (6.5±3.0, $p<0.001$; 8.5±3.4 kg, $p<0.001$), fat loss (4.8±3.0 kg, $p<0.001$; 5.9±2.8 kg, $p<0.001$) and loss of lean mass (1.3±0.8 kg, $p<0.001$; 1.8±2.0 kg $p<0.05$). The reduction in visceral fat area was 26.1±19.0 cm^2 ($p<0.01$) for LCD and 49.2±25.6 cm^2 ($p<0.001$) for LCD+cTEMS with a significant between-groups difference ($p<0.05$). Further, respiratory exchange ratio was significantly reduced at maximal exercise compared to LCD controls while max VO2 was maintained. There were no significant changes in serum lipids in the LCD-group. In the LCD+cTEMS-group however, we observed an increase in HDL cholesterol (0.1±0.2 mmol/l, $p<0.05$) and a decrease in triglycerides (0.36±0.22, $p<0.001$).

Since there were no group-differences in any of the measured lipids or lipoproteins, including lipoprotein subclasses, we performed a pooled analysis to investigate the dietary effects. After 10 weeks on the diet we observed an increase in total cholesterol from 4.98±0.86 to 5.55±1.76 mmol/l ($p=0.025$), LDL cholesterol from 3.33±0.69 to 3.92±1.61 mmol/l ($p=0.012$) and HDL cholesterol from 1.23±0.36 to 1.32±0.33 mmol/l. The ratio between the apolipoprotein B100 to A1 (apoB/apoA1) and between total cholesterol (TC) to HDL cholesterol however, did not change significantly. Triglycerides was reduced by 28.9% from 1.15±0.65 to 0.77±0.30 mmol/l ($p<0.001$). The NMR analysis showed no significant changes in the number of LDL particles, while there were significant changes in LDL subclasses. Large LDL particles increased by 85.4% ($p=0.003$) while we observed a 79.1% reduction in small LDLs. Further we found a 23.8% reduction in total very large density lipoprotein (VLDL) and chylomicron particles and the VLDL size was reduced by 11.2% ($p<0.001$).

DISCUSSION

Acute effects of cTEMS

In study 1, an observational clinical pilot study, we stimulated skeletal muscle with different levels of heat and modes of electrical currents. The study, which provided novel data on the integration of thermal stimulation and EMS, had two major findings:

1) Electrical muscle stimulation induced an increase in VO_2 and levels of venous lactate, catecholamine's and GH with a clear dose-response relationship.
2) Increased heat however, conferred additional hemodynamic changes and an additional increase in GH, but had no effect on the highest VO_2 or the levels of venous lactate and catecholamines.

The highest VO_2 measured using cTEMS, represented a 3.2-fold increase in resting oxygen uptake, which corresponds to 2.8 MET's.

Effect of different electrical currents

Our results indicated that a continuous low-frequency biphasic electrical pulse type at 7 Hz resulted in a significantly higher oxygen uptake compared to the other pulse types we tested, corrected for EMS intensity and heat intensity. A monophasic pulse type with a frequency of 100 Hz resulted in the significantly lowest VO_2 among the pulse types tested in the study and was therefore used as reference.

Although the cTEMS stimulator used in our studies did not allow us to freely set current characteristics such as frequency, pulse shape and duration, on-off time (duty cycle) and settings for bursting and ramping, we were able to test a broad range of EMS types. Ten different electrical pulse types were tested, varying in polarity (biphasic/monophasic), frequency (2.5 - 100 Hz), pulse width (150 - 500 µs) and output (continuous/burst). For detailed pulse type characteristic's, see table 2.

Table 2. Electrical pulse type characteristics and terminology

Pulse type term	Waveform	Frequency Hz	Pulse width µs	Burst Mode
M•18Hz•400µs	Monophasic	18	400	n/a
M•20-60Hz•450µs	Monophasic	Modulated 20-60	450	n/a
M•100Hz•500µs	Monophasic	100	500	n/a
B•2,5Hz•400µs	Biphasic Symmetrical	2,5	400	n/a
B•7Hz•400µs	Biphasic Symmetrical	7	400	n/a
B•14Hz•400µs	Biphasic Symmetrical	14	400	n/a
B•18Hz•400µs	Biphasic Symmetrical	18	400	n/a
B•50-165Hz•150µs•b	Biphasic Symmetrical	Modulated 50-165	150	6,5 sec on 2,5 sec off
B•60Hz•450µs•b	Biphasic Symmetrical	60	450	2,0 sec on 2,0 sec off
B•100Hz•150µs•b	Biphasic Symmetrical	100	150	8,0 sec on 2,0 sec off

Pulse type term: Waveform•Frequency•Pulse width•Burst Mode
Waveform: M=Monophasic; B=Biphasic
Burst Mode: b = Burst mode activated

There is a great heterogeneity between different EMS studies and the characteristics of the currents that are used are often poorly described [8]. Further, studies systematically analysing the effect of different EMS pulse types, varying one current characteristic while using otherwise identical stimulation parameters, are lacking. Therefore it is difficult to compare the outcome in our study with other EMS studies.

However, our finding that a biphasic pulse is more effective in eliciting training effect is in accordance with previous recommendations for non-obese subjects [72]; and considering the fact that in neuro-physiological studies, biphasic current requires a lower stimulation intensity than monophasic current to reach sensory perception threshold [73] this seems to be the preferred polarity in order to optimize training intensity.

When analysing biphasic square wave pulses at increasing frequencies, Minogue et al. found that oxygen uptake reached a plateau around 10 Hz [74]. This concurs with our findings where the biphasic continuous pulse type at 7 Hz resulted in a higher increase

in the oxygen uptake from reference than lower (2.5 Hz) or higher (14 Hz - 100 Hz) frequencies. The current with biphasic waveform at 7 Hz also induced a significant 80.1 % rise in lactate compared to the monophasic current used as reference, indicating anaerobic muscular activity.

Additional effects of heat

Increasing the heat from low (38.2 °C) to high (40.7 °C) did not affect the VO_2 measured during stimulation. Blood pressure, lactate and catecholamines were not affected. However the increase in heat stimulation gave a significant decrease in systemic vascular resistance index with a corresponding increase in both heart rate and cardiac index. This vasodilatation-induced hemodynamic response, did not allow the subjects to tolerate higher electrical stimulation intensity.

Other studies have suggested that an increased blood flow induced by local heat stimulation, as was found also in our study, can cause less electrical current to reach the muscle and thus increase the current required to stimulate the muscle to threshold [9]. This did not occur when we increased the heat stimulation intensity. We observed no difference in VO_2 between difference heat intensities and the attainable maximal electrical output during stimulation remained unchanged.

The discomfort associated with the peripheral electrical muscle stimulation is by many mentioned as a major restriction to the method [8-10]. We did not test for tolerability specifically in study 1, but all stimulation sessions were apparently well tolerated and none of the participants withdrew from the study because of the stimulation sessions. In our second study, where cTEMS was given regularly over 8 weeks, we asked the participants to fill out an anonymous questionnaire after the intervention period. From the nine responders, only two associated the stimulation sessions with light or moderate discomfort. Further, eight out of the nine responders reported that they would consider using cTEMS as a part of their daily routines, if available. Clearly, it seems like the combination of relatively large electrodes and superficial applied heat are alterations that make it possible to overcome the limitation of discomfort.

Ambient heat has several physiological effects and it is well known that increased temperature causes a decrease in maximal achievable oxygen uptake during physical

activity and that this effect occurs progressively with increasing heat exposure [75-77]. Although we did not find an increase in VO_2 when the heat stimulation was raised, it may still be the case that it represented a higher relative exercise intensity level due to a different physiological condition.

From previous studies it is known that both physical exercise[78] and the exposure to heat [79] can cause an increase in the concentration of plasma GH. Our observations, that both increased electrical stimulation intensity and the increase in heat intensity resulted in increased levels of GH, are in line with these previous findings. We extended previous results by showing that a 10% increase in stimulation intensity conferred a 68% increase in GH while increasing the heat intensity from moderate to high lead to a 107% increase. Although still unexplored, the repeated stimulation of the GH-axis may have potential benefits in some individuals. When given to patients with GH deficiency, GH has beneficial effects on cardiac function, exercise and atherosclerosis [80] and in patients with chronic heart failure, the administration of GH has shown positive effects on both exercise capacity and peak oxygen uptake [81].

Prolonged intervention with cTEMS

Training-effects of prolonged cTEMS

Eight weeks of cTEMS, given three times weekly in obese sedentary individuals, resulted in a significant increase in $VO_{2\ peak}$, a parameter for peak aerobic capacity. During the intervention period, physical activity and dietary habits were kept unchanged at each subject individual level and controlled for with accelerometer and a dietary questionnaire respectively.

The finding of an improved aerobic capacity was supported by an increased maximal workload during exercise testing and the occurrence of the aerobic threshold at a significant higher workload.

The increase in $VO_{2\ peak}$ in our study was comparable with other exercise interventions aiming to increase aerobic capacity. While we observed a 9.6% increase in total, corresponding to an average improvement of 1.2% per week, Tjønna et al. found an

increase of 1.0% per week when exercising at moderate intensity (70% of the maximal heart rate) and 2.2% per week when the intensity level was at 90% [82] .

The underlying mechanism for the increased maximal oxygen uptake caused by cTEMS however, is somewhat different than the observed improvement caused by regular physical exercise. In most cases, the cardiorespiratory delivery of oxygen to the working muscles is the main limiting factor of $VO_{2\ max}$ [83] and exercise induced increase in maximal oxygen uptake is caused by increased capacity to left ventricular filling and thus increased cardiac output. Factors at muscular level however, may also impose limitations on exercise capacity. Mitochondrial activity, capillary density, and afferent signalling pathways that communicate fatigue at the skeletal muscle level, plays a key role in arterio-venous oxygen extraction and thus contribute to the oxygen uptake. [83,84]. After the eight weeks intervention period, we found no changes in resting heart rate or blood pressure compared to the baseline measurements. This indicates unchanged hemodynamic and thus stroke volume and cardiac output. Nuhr et al. have previously described how long-term EMS can alter phenotypic properties of human muscle fibers resulting in both increased aerobic and anaerobic capacity [6]. Although, skeletal muscle metabolism was not investigated in our study, it is likely that changes in these phenotypic properties, towards a higher fatigue resistance, was causal to the increase in $VO_{2\ peak}$.

The influence of prolonged cTEMS on adipose tissue

8 weeks of cTEMS in obese sedentary individuals caused an increase in adipose tissue lipolytic activity. Further we found evidence of increased level of mitochondrial activity, related to the above-mentioned training-effects.

In order to be used as energy in the body, stored fat must be transported from the adipose tissue to e.g. skeletal muscle. Followed by hydrolysis into fatty acids and glycerol, the free fatty acids are transported with albumin molecules in the plasma. The use of microdialysis technique allows a direct monitoring of metabolic changes in the adipose tissue. Small portable pumps offer a further benefit of the technique, the possibility to perform mobile monitoring of interstitial metabolites in field studies [85]. Using microdialysis in subcutaneous abdominal adipose tissue (SCAAT) we were able

to monitor the lipolytic activity during a sedentary day, expressed by the interstitial concentration of glycerol.

Mean 24-hours concentration of glycerol increased significantly from baseline to follow-up indicating a rise in the mobilisation of triglycerides due to the intervention [63] (See figure 4).

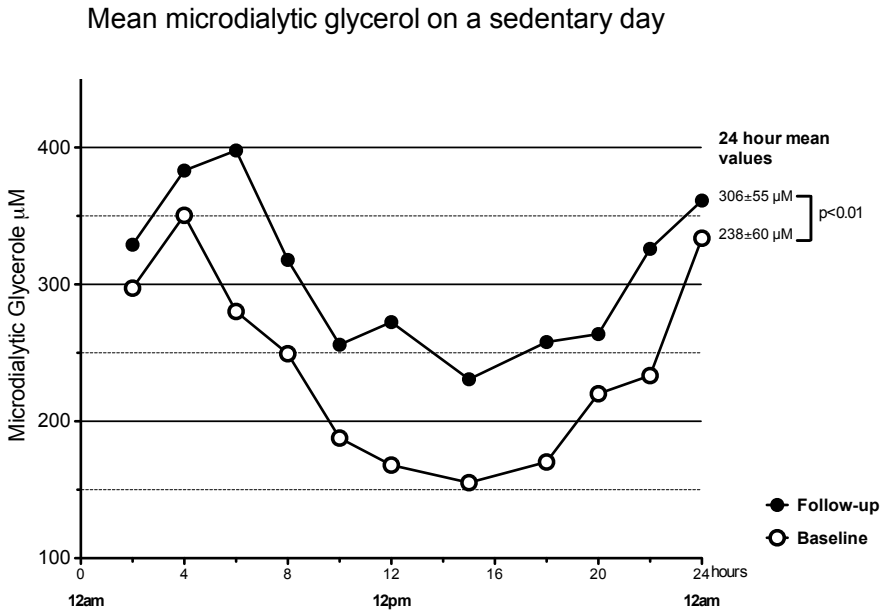

Figure 4: Mean microdialytic concentrations on a sedentary day as index of lipolytic activity in subcutaneous abdominal adipose tissue. Mean values ± standard deviation

This result is in accordance with previous findings where prolonged training intervention have been shown to improve lipid mobilisation in SCAAT [86]. Further, our observation was not accompanied by an increase in free fatty acids in the blood, indicating a concomitant increase in the ability of the skeletal muscle to oxidize the mobilized fat. Physiological pulses of GH has been shown to increase lipolysis in subcutaneous tissue [64]. Thus, the observed increase in GH, induced both by the

electrical stimulation intensity and by the heat stimulation, may have contributed to the observed increase in SCAAT lipolysis.

Although not monitored, it is unlikely that alterations in adipose tissue blood flow should have affected the concentrations of microdialytic glycerol. Microdialysis was performed on a sedentary day under controlled diet and all hemodynamic measurements remained unchanged.

Exercise-induced changes in adipocyte mitochondrial activity and gene expression in humans remain unexplored. However in rats, prolonged physical activity increases the expression of PGC-1α, which play a key role in the transcriptional regulation of genes responsible for lipid and energy metabolism, also in adipocytes [87]. After 8 weeks of cTEMS, we investigated key genes in SCAAT involved in fatty acid mobilisation and found a significant increase in PGC-1α expression in addition to a significant increase in CPT-2. Further, we found absolute, but not significant increase in the mRNA expression of other genes involved in lipid metabolism. Considering the relatively short intervention period, we interpret these findings as early adaptation to an increased fatty acid mobilisation secondary to enhanced fatty acid oxidation in skeletal muscle.

Despite previous indications that obese subjects may have both higher motor thresholds and poor tolerance to EMS [88], cTEMS was well tolerated among the participants who received prolonged cTEMS in our studies. From the total of 24 obese subjects (Study 2 and 3) that underwent stimulation session three times per week, 22 persons completed the intervention period and were included in the data analysis. Two subjects withdrew their consent, however not related to cTEMS or other methods used in the studies.

Prolonged cTEMS and metabolic efficiency

Skeletal muscle is a major player in the energy balance, contributing to more than 20 % of the total energy expenditure [89]. In healthy lean individuals, under fasting conditions, the preferred source of fuel is lipids. Decreased fat oxidation however, characterized by a higher RER, is known to be a predictor for weight gain, correlates with insulin sensitivity and often mirrors a metabolic derailment [90].

After eight weeks of cTEMS we found a significantly lower RER, both at the anaerobic threshold and at peak work capacity during tread mill testing. These findings indicate a higher ability to fat oxidation and an increased reliance on fat as fuel during exercise. Previous studies have found that the ability of the skeletal muscle to switch between fat and carbohydrate oxidation, correlates with maximal oxygen uptake and that endurance training increased the capacity for FFA mobilization and oxidation during activity at a given workload [91]. Although the increase in oxygen uptake caused by cTEMS seems to be caused by muscular changes, rather than increased cardiac stroke volume, we observed similar effects on the metabolic flexibility.

cTEMS during dietary induced hypocaloric weight loss

When cTEMS was applied in addition to a weight-reducing low-carbohydrate-diet, we found a significantly higher reduction in visceral fat accumulation compared to the diet alone. In both treatment groups, the dietary intervention caused significant, but similar weight reductions accompanied by significant reductions in body mass index, fat mass and fat percentage. In the LCD+cTEMS group however, we found a 26% reduction in visceral fat area compared to a 16% decrease in the diet-alone group. Although computed tomography remain the "gold standard" for the quantification of visceral fat, new emerging, non-radiating techniques have become available. We used multifrequency bioelectrical impedance analysis in our studies. This method offer an effective and accurate measurement of visceral fat and has in several studies been proven to correlate strongly with the findings by computed tomography [92-94].

Adding cTEMS to a low-carbohydrate-high-fat diet did not cause any significant changes in lipids or lipoproteins compared to the diet-alone group. In the group receiving both cTEMS and diet however, we found an increase in HDL-cholesterol, a reduction in triglycerides and a reduction in both size and number of triglyceride-rich lipoproteins, all changes that were not found to be significant by the dietary intervention alone. There is a close link between visceral fat accumulation on one hand and triglycerides and triglyceride-rich lipoproteins on the other hand [17,95]. Whether these changes contributed to, or were a result of the enhanced loss of visceral fat caused by cTEMS, remain unanswered.

The ratio between CO_2 production and O_2 uptake, RER, increase with exercise intensity. It is dependent on whole-body nutrient metabolism and considering that glucose has a respiratory quotient of 1.00 and fat 0.70, a higher value of RER indicates a predominance of carbohydrate oxidation whereas a lower value reflects lipid oxidation [96].

Although neither the LCD group nor the LCD+cTEMS group displayed any significant changes in the in peak oxygen uptake, as was observed when cTEMS was given to non-dieting obese individuals, we did found evidence of cTEMS related training effects. The reduction in RER was significantly larger in the group where cTEMS was used as adjuvant compared to the known reduction of RER from a low-carbohydrate-high-fat diet [97]. We interpret this as an indicator of increased aerobic capacity per workload and with a larger shift in substrate utilisation towards fatty acid oxidation than from the LCD diet alone

The exercise testing was performed under standardised conditions using the same personnel at baseline and follow-up examination and the standard criteria for peak exercise were met. A low exercise tolerance may be an explanation why the apparent increased capacity was not utilized during the test, however RER also serves as a physical fitness indicator under such conditions, correlating well with standard physical fitness parameters [98].

In healthy lean and trained individuals, fatty acids are the main fuel source. In this population determinants of RER are factors like muscle glycogen content, training volume, muscle fiber composition, lactate increase and diet [99]. In obese subject however, RER is dependent on the metabolic health and the ability to switch fuel substance [90]. A higher RER has also been shown to be a predictor for weight gain [100,101].

A further indication of cTEMS related training-effects was found when analysing the exercise parameters at a workload of 100 W during the treadmill testing. There was a significant between-group difference in the change of VO_2 at 100W with a trend towards a reduction in oxygen consumption in the LCD+cTEMS group. Further, the energy expenditure at 100W was significantly lower after the cTEMS intervention compared to baseline, corresponding to a 5% reduction. The diet-alone group

displayed no changes in the energy expenditure and the between-group difference was significant.

Adding cTEMS to the dietary intervention, caused a numeric, but not significant difference in the weight-loss compared to diet alone. There was no difference in the dietary caloric reductions between the groups and when monitoring the time spent in different activity zones during the intervention, we found similar results in both groups. When further considering that the dietary compositions in both groups were similar, ruling out differences in the thermic effect of food, only the energy expenditure during cTEMS should be able to contribute to an additional weight loss. The additional energy spent in the LCD+cTEMS group however, may have been neutralized by the earlier described observation that this group became more energy efficient during physical activity. This may in theory explain why cTEMS did not cause an additional weight loss.

Although both intervention groups displayed a significant increase in the relative lean mass (lean mass %), there was no significant difference in the absolute loss of lean mass between the groups. The loss of lean mass was 1.8±2.0 kg in the LCD+cTEMS and 1.3±0.8 kg in the LCD group, constituting 21% and 20% of the total weight loss respectively. In the POUNDS LOST trial, randomizing 424 obese individuals to different energy-reduced diets, the average loss of lean mass was 33% (-6,3 kg total weight and -2,1 kg lean mass)[56], indicating that the loss of lean mass was limited in our study.

Dietary intervention with a low-carbohydrate-high-fat diet

The dietary intervention with a low-carbohydrate-high-fat diet induced a significant loss in total weight and fat mass, while we observed a limited loss of lean mass. The diet also conferred a significant reduction in visceral fat area, an effect that was further enhanced significantly by the addition of regular cTEMS.

Although often described as a "fad diet" [102], the LCD is not a new concept. Already in 1825 Jean Brillat-Savarin published his *The Physiology of Taste,* where he described his solution to obesity, the restriction of everything that contained starch or flour [103]. As a curiosity, the Swedish name for dieting, "banta" is named after the British undertaker,

William Banting (1796-1878). In his international bestseller, *Letter on Corpulence, Addressed to the Public,* from 1863, he described how a diet low on sugar and starch had helped him loose over 20 kg and shrink his waist by 33 cm [104,105].

The dietary intervention used in our study, was per se not calorie restricted. Still the carbohydrate restriction resulted in a significant and similar reduction in caloric intake in both groups, despite ad libitum feeding. This finding is in accordance with studies where similar dietary interventions were used [26,28,29,106,107]. Reduced nutritional choices, appetite reduction due to ketones [108], a central interaction between insulin and leptin [109] and reduced food-reward [110], may all be mechanisms contributing to the caloric reductions. The exact mechanisms however, remain unanswered and were not investigated in our study.

The seemingly contradictory observation, that a diet high on fat causes a significant reduction in triglycerides and triglyceride-rich lipoproteins, is not fully understood, but several mechanisms may contribute to this consistent observation. Carbohydrate reduction appears to confer a reduction in de novo lipogenesis, indicated by a reduction in palmitoleic acid (16:1n-7) [111]. This is probably explained by reduced activity of the insulin sensitive sterol regulatory element binding protein 1c, a key regulator of de novo lipogenesis [112]. Furthermore, reduction of insulin leads to increased activity of the lipoprotein lipase (LPL) in skeletal muscle and thus to increased clearance of VLDL and its remnants [113]. Another mechanism may be increased Chylomicron clearance caused by the reduction in concomitant VLDL secretion and thus increased LPL capacity in adipose tissue [114]. Increased LPL activity may also be caused by a reduction of apolipoprotein CIII [38], which correlates with plasma triglycerides and is a known inhibitor of LPL [95].

A standard lipid measurement quantifies the cholesterol or triglyceride content of lipoproteins, but does not provide any information of lipoprotein particle number or subclasses. Therefore, in order to perform in-depth analysis of the changes in lipids and lipoproteins, we used nuclear magnetic resonance spectroscopy, an analysis which provide size-specific lipoprotein particle information [115].

In the pooled analysis of the dietary effects on lipids and lipoproteins, we found a major reduction in the concentration of triglycerides and a significant increase in both LDL-

cholesterol and HDL-cholesterol, but no changes in the apoB/apoA1 and TC/HDL-cholesterol ratios. We observed no changes in the total number of LDL particles, but there was a clear shift in LDL from the small dense particles towards the larger buoyant ones.

Summed up, all the characteristics of the atherogenic dyslipidaemia were positively altered. These findings are aligned with other studies investigating the short-term effects of carbohydrate restriction on lipids and lipoproteins [38-40,114,116,117] and are mainly thought to be downstream effects from the reduction in triglycerides [38,118].

Data from long-term effects (> 2 years) from carbohydrate restriction and on the effects in weight stable healthy individuals are still insufficient.

CONCLUSIONS

Physiological acute effects of cTEMS

cTEMS showed physiological responses similar to regular exercise training. The electrical muscle stimulation induced an increase in VO_2 with a dose-response, increasing the intensity of superficial heat conferred additional hemodynamic responses induced by the vasodilatation. The most effective stimulation, when using oxygen uptake as the effect-parameter, was found with a continuous biphasic pulse type at the frequency of 7 Hz, independent of heat and stimulation intensity.

Effect of prolonged cTEMS in obese individuals

After eight weeks of prolonged electrical muscle stimulation with added heat in obese, sedentary subjects, we found significantly increased VO_2, both at peak exercise and at the anaerobic threshold. This effect was, in all likelihood, caused by increased muscular arterio-venous O_2-extracion due to increased oxidative capacity in the skeletal muscle. Further cTEMS also conferred increased lipolytic activity and increased level of mitochondrial activity in adipose tissue. The lowering of the respiratory exchange ratio during exercise testing indicated both a higher fatigue resistance and a higher whole-body fat-oxidation. During the relatively short stimulation period used in our study, the body composition and visceral fat area remained unchanged.

Effect of cTEMS in obese subjects during weight loss

When cTEMS was used as an adjuvant to weight reducing diet, we found a significantly greater reduction in visceral fat than from the diet alone. This effect may be attributable to the observed training effects caused by cTEMS, resulting in more efficient muscle work and a metabolic switch towards higher fat oxidation in the skeletal muscle.

Summarized conclusion

Our studies provide new insight into the effects of electrical muscle stimulation. We have shown that cTEMS exerts physiological responses similar to regular exercise when analysing usual effect parameters. Further, the often-described discomfort associated with EMS was not an issue in our studies, indicating that the use of relatively large electrodes and the application of superficial heat may be alterations that can reduce this undesirable restriction.
Despite indications that EMS is not well abided in obese individuals, we found that prolonged cTEMS was very well tolerated and increased both aerobic fitness and metabolic efficiency when used as an alternative to regular physical exercise in obese sedentary subjects.

Considering that reduction of visceral fat induces greater benefit on metabolic parameters than the reduction of subcutaneous fat, our finding that cTEMS significantly enhances the reduction of VAT during weight loss is of considerable value.

In conclusion, intervention with combined thermal and electrical muscle stimulation may have a role in the lifestyle intervention in sedentary obese individuals unwilling or unable to perform regular physical exercise.

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ORIGINAL ARTICLE

Physiological effects of combined thermal and electrical muscle stimulation (cTEMS) in healthy individuals: A pilot study

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Abstract

Objective. Adding superficial heat to electrical muscle stimulation may provide added effects. In this pilot study we investigated the effects on oxygen consumption of combined thermal and electrical muscle stimulation at different levels of heat and modes of electrical stimulation. ***Design.*** An observational clinical pilot study. ***Subjects.*** A total of 14 healthy persons aged 30–70 years. ***Methods.*** Subjects were randomly assigned to stimulation with different electrical pulse types in random order. At 38.2°C and 40.7°C heat intensity we measured peak oxygen uptake, capillary lactate, catecholamines, growth hormone and hemodynamics at 20% of the maximum output (194 mA) and at each individual's maximal stimulation intensity. ***Results.*** Multivariate analyses showed that electrical stimulation significantly increased peak oxygen uptake and the levels of lactate, catecholamine and growth hormone. Increasing the heat during electrical stimulation gave additional hemodynamic response and rise in growth hormone. We observed a dose-response relationship in peak oxygen uptake for increase in stimulation intensity. The highest oxygen uptake was observed with biphasic continuous stimulation at 7 Hz ($p < 0.001$). ***Conclusions.*** Biphasic low frequency electrical muscle stimulation elicited the highest oxygen uptake; higher stimulation intensity was not obtained by adding heat.

Key Words: *Electric stimulation therapy, skeletal muscle, exercise, growth hormone, oxygen consumption, catecholamines, hot temperature*

Introduction

Increased aerobic fitness reduces cardiovascular disease risk [1]. Different methods of electrical muscle stimulation (EMS) can mimic traditional physical activity and improve aerobic fitness [2] and increasing evidence has shown also that the EMS of unloaded muscles could mimic the effects of regular exercise training and thus could serve as a substitute. In healthy individuals, EMS has been shown to increase cardiorespiratory fitness as assessed by maximum oxygen uptake ($VO_{2\ max}$) [3–5] and increase muscle strength [3,6], but it can also have clinical application [7]. The effects of EMS may be improved by various combinations of stimulation modes, however there is no general agreement as to which mode of electrical stimulation should be used [8].

Acute hormonal response to exercise training is important for skeletal muscle remodeling and long-term adaptation, and it leads to increased muscle strength and muscular hypertrophy [9]. Growth hormone (GH) is a potent anabolic hormone that stimulates protein synthesis in skeletal muscle [10], and EMS seems to stimulate GH release more than voluntary exercise [11]. Catecholamines are also known to increase during exercise training [12,13] and plasma catecholamine concentration seems to be related to the intensity of exercise; expressed as the percentage of $VO_{2\ max}$ [14].

Superficial heat application is known to reduce muscular pain [15,16] and moderate heat exposure increases muscle blood flow and tissue circulation [17,18]. Heat is widely used in various muscular conditions, but it has not been tested whether it can increase tolerance to electrical stimulation or recruit more muscle fibers as an adjuvant to EMS.

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In this pilot study, we wanted to test the hypothesis that combined thermal and electrical muscle stimulation (cTEMS) was better than EMS alone to elicit physiological responses similar to exercise training. Further, we explored the effect on oxygen consumption of different electrical muscle stimulation protocols, using different electrical pulse types, variable intensity of heat and electric current.

Methods

Subjects

Healthy individuals were recruited from the first responders to an invitation sent by e-mail to all 10,000 employees at the Haukeland University Hospital in Bergen, Norway. Subjects were eligible if they were between the ages of 30 and 70, able to perform exercise testing and their clinical examination was normal. Exclusion criteria included extensive dermatological disease, pacemaker or implanted defibrillator, regular medication, known pulmonary or heart disease, other major disease or pregnancy. A total of 16 healthy volunteers (8 males, 8 females) were recruited. Among these volunteers, 14 subjects completed all examinations and stimulation sessions and were included in the data analysis. Non of the recruited subjects were competitive athletes. Two female subjects withdrew their consent of reasons not related to the study or the methods used.

The pilot study was approved by the Regional Committee for Medical and Health Research Ethics (Western Norway Ref: 263.08) and conformed to the Declaration of Helsinki. All subjects gave written, informed consent.

Experimental setup

All examinations and experiments were performed in a designated research laboratory in the Haukeland University Hospital, Bergen, Norway.

Prior to the baseline examination, all subjects underwent two sessions of adaptation to cTEMS. Furthermore, prior to all study visits, individuals included in the study underwent 2 h of fasting but were allowed to drink water. In the 24 h before the study visits, the volunteers were asked to refrain from exercise training and the consumption of pineapples, plums, avocados and walnuts. At the baseline visit, all resting parameters were measured, and the resting blood samples collected after 30 min of supine rest.

Combined thermal and electrical muscle stimulation

In this pilot study, we used the combination of applied heat (generated by electrical heating elements) and EMS. Ten silicone electrodes were connected to a stationary stimulator (TEI System, RÖS'S Estética S. L., Barcelona Spain).

The electrodes were incorporated into strips that generate heat and stimulation currents. Each electrode band included an electrical heating element for thermal stimulation and two electrodes for the application of electrical current for EMS. Two different sets of electrodes were applied: three large electrodes (24 cm × 26 cm; max heating power 25 W) and seven small ones (24 cm × 17 cm; max heating power 15 W). The electrodes were attached to the prespecified muscle groups (quadriceps, hamstrings, glutei muscles, oblique's, rectus abdominis and lower lumbar muscles) using elastic bands with Velcro, and optimal skin adhesion was obtained using a standard ultrasound gel.

Stimulation protocol

After the baseline visit, all subjects were randomly allocated to undergo stimulation with five different cTEMS pulse types at low- (45 W) and high-intensity heat (90 W). In tests prior to the study, we measured the temperature between the electrode and the skin (mean temperature over 30 min) to be 38.2 and 40.7°C at low- and high-intensity heat, respectively. We did not measure body temperature.

A total of 10 electrical pulse types that varied in polarity (biphasic/monophasic), frequency (2.5–100 Hz), pulse width (150–500 ms) and output mode (continuous/burst) were tested (Table I).

At each stimulation visit, the participants received the designated electrical pulse type and heat intensity starting at 20% of the stimulator's maximal electrical stimulation capacity (194 mA). Blood samples were drawn, and the clinical and gas exchange variables were registered after the heart rate (HR) reached a steady state (minimum 10 min). Then, while keeping the heat intensity unchanged, the electrical stimulation intensity was increased to an individual maximum, while maintaining it below the threshold of discomfort. After the HR reached a steady state, all of the parameters were measured, and the blood samples were collected. Fever and illness was considered a contraindication to cTEMS.

Respiratory gas analysis

The ventilatory gas exchange was determined every 10 sec during the cTEMS sessions using a breath-to-breath analyzer (Schiller Cardiovit ErgoSpiro CS200/PowerCube, Ganshorn) with a facemask. The highest oxygen consumption during a steady state in the HR for each stimulation intensity was determined, and the mean during the 30 sec around the peak value was calculated, which represented the highest sustainable oxygen uptake (VO_2) for each temperature and stimulation intensity.

Table I. Electrical pulse type characteristics and terminology.

Pulse type term	Waveform	Frequency Hz	Pulse width μs	Burst mode
M•18Hz•400μs	Monophasic	18	400	n/a
M•20 − 60Hz•450μs	Monophasic	Modulated 20–60	450	n/a
M•100Hz•500μs	Monophasic	100	500	n/a
B•2,5Hz•400μs	Biphasic Symmetrical	2,5	400	n/a
B•7Hz•400μs	Biphasic Symmetrical	7	400	n/a
B•14Hz•400μs	Biphasic Symmetrical	14	400	n/a
B•18Hz•400μs	Biphasic Symmetrical	18	400	n/a
B•50−165Hz•150μs•b	Biphasic Symmetrical	Modulated 50–165	150	6.5 sec on 2.5 sec off
B•60Hz•450μs•b	Biphasic Symmetrical	60	450	2.0 sec on 2.0 sec off
B•100Hz•150μs•b	Biphasic Symmetrical	100	150	8.0 sec on 2.0 sec off

Pulse type term: Waveform• Frequency• Pulse width• Burst Mode Waveform: M, Monophasic; B, Biphasic Burst mode: b, Burst mode activated.

Hemodynamic measurements

The hemodynamic measurements were derived from continuous non-invasive finger arterial pressure measurement, using a device designed for this purpose [19] (Nexfin HD, BMEYE - Cardiovascular Monitoring, Dallas, Texas). We did not apply cTEMS heating elements on the arms or near the finger cuffs used for the pressure monitoring. The cardiac output, stroke volume and system vascular resistance were assessed and indexed in relation to the body surface area.

The HR was observed continuously with ECG monitoring (Schiller Cardiovit CS-200 Ergo-Spiro) and registered at rest and at maximum exercise capacity during exercise testing and after 5 min of steady state at each cTEMS pulse type stimulation. Blood pressure was monitored according to the standard protocol during exercise testing. During cTEMS, blood pressure was registered after 5 min of a steady state in the HR at each cTEMS pulse-type stimulation using a Schiller BP-200 plus blood pressure monitor.

Exercise testing

The maximum exercise capacity was evaluated by ergospirometry during a standard treadmill test, using a modified Bruce Protocol. The ventilatory gas exchange was measured with a Schiller ergospirometer by the breath-to-breath technique (Schiller Cardiovit CS-200 Ergo-Spiro). Before the baseline examination, all subjects underwent one session of adaptation to the exercise test.

The $VO_{2\ max}$ was considered reached when all of the following accepted criteria were met; the maximal HR measured at exhaustion was greater than 90% of the age-predicted maximal HR, the respiratory exchange rate measured at exhaustion was greater than 1.1, and the subject was not able to sustain a sufficient running speed on the treadmill.

Body composition

A dual-energy X-ray absorptiometry (GE Medical Systems, Lunar Prodigy DF) was used for the analysis of body composition.

Biochemical and hormonal analyses

Lactate was measured using a portable blood lactate analyzer (Lactate Pro, Arkray KDK, Japan). Coded reagent strips were filled by capillary blood and inserted in the device for analysis.

For hormonal analysis, blood samples were drawn under standardized conditions and kept frozen at −80°C until analyzed at the Hormone Laboratory, Haukeland University Hospital. Insulin and GH were analyzed using a two-site chemiluminescent immunometric assay using an Immulite 2000 (Siemens, Gwynedd, UK). The detection limit for GH was 0.01 ng/mL, and the inter-assay variations were 6.5%, 5.5% and 6.6% for concentrations of 2.6 ng/mL, 5.3 ng/mL and 17 ng/mL, respectively. Catecholamines were analyzed with a radioimmunoassay, Cat Combi RIA (DRG Instruments GmbH, Marburg, Germany). The assay had an inter-assay variation for norepinephrine of 10% at 22 ng/mL and 6.1% at 11 ng/mL. For epinephrine, the inter-assay variation was 5.6% at 4.4 ng/mL and 6.1% at 21 ng/mL.

Statistical analysis

All outcome variables were converted to percentage change from rest. The association of different cTEMS

Table II. Baseline characteristics; $N = 14$ (8 males, 6 females).

	All subjects $N = 14$
Age (years)	42 ± 6
Height (cm)	172 ± 10
Weight (kg)	73.6 ± 16.9
Body mass index (kg/m^2)	24.5 ± 3.6
Lean mass (kg)	50.8 ± 11.2
Fat mass (kg)	19.7 ± 7.0
Fat percentage (%)	27.7 ± 5.2
$VO_{2\ max}$ (ml/kg/min)	48.5 ± 4.7
MET_{max}	13.9 ± 1.3

Values are means ± SD; lean mass, fat mass and fat percentage measured by DEXA; $VO2_{max}$ and MET_{max} from treadmill test MET, metabolic equivalent.

stimulation protocols with mean percentage change of outcome variables from baseline were assessed with random intercept mixed effects models. Current intensity was modeled as a continous covariate (0–100%) and heat intensity (low/high) and pulse types (B•7 Hz•400 μs, etc) as cofactor. Study subjects were included as random effect and the variance structure was adjusted for heteroscedastic residual spreads between pulse types [20]. Analyses were performed with R version 2.14.0 (The R Foundation for Statistical Computing, Vienna, Austria, 2011) and nlme package version 3.1-102.

Results

The baseline characteristics of the 14 subjects are shown in Table II.

There were no complications related to the cTEMS sessions.

Stimulation intensity

The effect on outcome variables, expressed as the percentage change from resting values, is shown in Table III. EMS increased VO_2, HR and cardiac index (CI) and the levels of capillary lactate, catecholamine and GH. In addition, EMS decreased peripheral vascular resistance.

Every 10% increase in EMS intensity induced a significant increase in all outcome variables except the stroke volume index, which was not influenced by either the stimulation intensity or the increased heat intensity. A 10% increase in stimulation intensity induced a 5.9% increase in the HR ($p < 0.001$) and an 11.8% increase in the CI ($p < 0.001$). Systolic blood pressure increased by 3.7% ($p < 0.001$) and diastolic blood pressure by 1.2% ($p < 0.01$). Furthermore, the systemic vascular resistance index (SVRI) was reduced by 5.8% ($p < 0.001$). The highest VO_2 during stimulation increased by 39.2% ($p < 0.001$), which was accompanied by a highly significant increase in capillary lactate ($p < 0.001$) and plasma catecholamine levels ($p < 0.001$ for both Epinephrine and Norepinephrine). Finally, there was also a 67.7% increase in GH ($p < 0.05$).

Heat intensity

Increasing the heat from low (38.2°C) to high (40.7°C) did not increase the highest VO_2, blood pressure, lactate or catecholamines. Increased heat intensity, however, changed hemodynamic parameters. The HR and CI increased by 7.3% ($p < 0.001$) and 8.8% ($p < 0.001$), respectively, whereas the SVRI was reduced by 6.8% ($p < 0.001$). Increasing the heat intensity from the lowest to the highest level conferred an additional significant rise in GH by 106.8% ($p < 0.05$).

At 20% stimulation intensity, there was no difference in the measured VO_2 (4.8 ± 0.8ml/kg/min vs. 4.9 ± 1.0ml/kg/min) between the low heat stimulation and the high heat stimulation (see Figure 1). The same observation was made when the stimulation intensity was increased to the individual maximum.

Table III. Outcome variables as percentage change from resting values induced by every 10% increase in stimulation intensity or by increasing heat intensity from low to high intensity.

	By 10% stimulation intensity increase	By heat intensity moderate to high	Resting values
Heart rate (beats/min)	**5.9**‡	**7.3**‡	57 ± 5
Systolic blood pressure (mmHg)	**3.7**‡	1.0	118 ± 12
Diastolic blood pressure (mmHg)	**1.2**†	− 1.5	79 ± 9
Cardiac Index (l/min/m^2)	**11.8**‡	**8.8**‡	2.7 ± 0.3
Systemic Vascular Resistance Index (dyn^{X}s/m^5/m^2)	**− 5.8**‡	**− 6.8**‡	2535 ± 444
Stroke Volume Index (ml/m^2)	0.9	1.1	49 ± 7
Highest oxygen uptake (ml/kg/min)	**39.2**‡	2.0	3.1 ± 0.5
Lactate (mmol/l)	**25.0**‡	5.8	1.2 ± 0.3
Epinephrine (nmol/l)	**27.3**‡	5.4	0.20 ± 0.15
Norepinephrine (nmol/l)	**16.0**‡	6.5	2.8 ± 1.3
Growth hormone (μg/l)	**67.7***	**106.8***	1.2 ± 1.8

Differences in mean percentage change in association with different cTEMS treatment parameters were assessed using multivariate analysis (linear mixed model with random intercept).
Absolute resting values are shown as mean ± SD.
Significant changes are marked with bold figures. Significance codes: *$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$

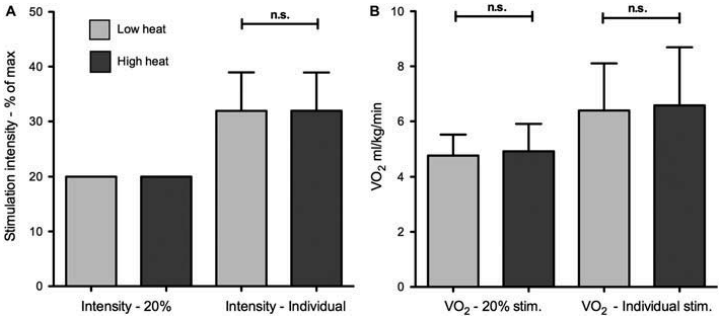

Figure 1. Stimulation intensity and oxygen uptake (VO_2) at low and high heat stimulation. (a) Mean stimulation intensity as a percentage of the maximum stimulation (194 mA) at low and high heat for fixed stimulation at 20% of the maximum and the individual maximum stimulation intensity. (b) Mean oxygen uptake (ml/kg/min) at low and high heat for fixed stimulation at 20% of the maximum and the individual maximum stimulation intensity.

The highest sustainable oxygen uptake at low heat was 6.4 ± 1.7 ml/kg/min, and at high heat, it was 6.6 ± 2.1 ml/kg/min. Individual maximum stimulation intensity did not differ between the low heat and high heat stimulations ($32 \pm 7\%$vs. $32 \pm 7\%$).

Electrical pulse types

The monophasic pulse type with a frequency of 100 Hz and a pulse width of 500 μs resulted in the lowest VO_2 increase during stimulation and was chosen as the reference for comparison in the other electrical pulse type protocols. Outcome variables are expressed as percentage change compared to the reference and are shown in Table IV.

All current intensities showed a higher VO_2 than the monophasic stimulation at 100 Hz, except for the monophasic pulse type at 18 Hz frequency (M•18 Hz•400 μs; for electrical pulse type terminology, see Table I).

The HR, CI and SVRI were all significantly altered by B•100 Hz•150 μs•b. In addition, a significant rise in HR was achieved by biphasic pulse type at 14 Hz (B•14 Hz•400 μs). In addition, a significant increase in CI was observed by biphasic pulse type at 7 Hz (B•7 Hz•400 μs). The stroke volume index and epinephrine levels were not significantly influenced by any cTEMS protocol compared to the reference pulse type. In norepinephrine however, we observed a 69.5% increase ($p < 0.001$) using M•18 Hz•400 μs and a 67.6% increase ($p < 0.001$) by B•100 Hz•150 μs•b. No cTEMS protocol showed a significant impact on blood pressure compared to stimulation with M•100 Hz•500 μs. With the exception of monophasic pulse type at 18 Hz, the significant increases of the outcome variables were consistent for the same protocols between 20% and the maximal stimulation intensity.

The rise in VO_2 achieved using the biphasic low frequency pulse type at 7 Hz (B•7 Hz•400 μs) was significantly higher than all other pulse types. At low heat, the highest oxygen uptake was 5.2 ± 0.6 ml/kg/min at 20% stimulation intensity and 8.7 ± 1.8 ml/kg/min at individual maximum stimulation ($28 \pm 3\%$). At high heat, B•7 Hz•400 μs generated an oxygen uptake of 6.0 ± 1.1 ml/kg/min at 20% stimulation intensity and 9.8 ± 2.7 ml/kg/min at individual maximum stimulation intensity ($29 \pm 5\%$). The highest VO_2, measured using B•7 Hz•400 μs, represented a 3.2-fold increase in resting oxygen uptake, corresponding to 2.8 metabolic equivalents.

Discussion

This pilot study provides novel data on the integration of thermal stimulation and EMS. The principal finding was that the electrical muscle stimulation increased VO_2 and levels of venous lactate, catecholamines and GH with a clear dose-response relationship. Increasing heat in the stimulation protocol caused additional hemodynamic changes and an increase in GH, but had no effect on the highest VO_2 or the levels of venous lactate and catecholamines. Our results also indicated that a continuous low-frequency biphasic electrical pulse type at 7 Hz resulted in a significantly higher oxygen uptake compared to the other pulse types, corrected for EMS intensity and heat intensity. A monophasic pulse type with a frequency of 100 Hz resulted in the significantly lowest VO_2 among the pulse types tested in the study.

One of the major limitations with EMS is the strong discomfort associated with the peripheral stimulation [8,17,21]. The integration of heat simulation and EMS may in theory reduce this disadvantage, but using heat as adjuvant to EMS, the increase of superficial heat from 38.2°C to 40.7°C, did not allow a higher electrical stimulation intensity. Increasing the heat from low to high intensity induced peripheral vasodilatation with a decrease in the

Table IV. Mean improvement in percentage change from rest by pulse types with M•100Hz•500μs as reference.

	VO_2 ml/kg/min	HR bpm	SBP mmHg	DBP mmHg	CI l/min/m^2	SVI ml/m^2	SVRI dyn*s/m^5/m^2	Lactate mmol/l	Epinephrine nmol/l	Norepinephrine nmol/l
Absolute values Rest	3.1 ± 0.5	57 ± 5	118 ± 12	79 ± 7	2.7 ± 0.3	49 ± 7	2535 ± 444	1.2 ± 0.3	0.20 ± 0.15	2.8 ± 1.3
Absolute values M•100Hz•500μs	4.8 ± 0.5	63 ± 5	116 ± 13	77 ± 9	3.4 ± 0.7	51 ± 12	1986 ± 495	1.1 ± 0.3	0.31 ± 0.14	3.2 ± 1.2
M•100Hz•500μs	Reference§	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Percentage change vs. reference										
M•18Hz•400μs	8.0§	4.5	3.0	−0.3	14.4	−1.6	−7.3	34.0	39.2	67.6‡
M•20-60 Hz•450μs	45.7‡,§	−0.1	6.8	1.9	10.3	−5.4	−2.2	31.8*	53.9	16.6
B•2,5Hz•400μs	62.7‡,§	−0.5	3.1	0.2	6.2	−7.1	−5.0	24.3	9.7	−3.1
B•7Hz•400μs	134.3‡	11.4	10.8	1.4	33.9†	−2.9	−12.3	80.1‡	93.4	49.3
B•18Hz•400μs	43.7‡,§	−2.9	7.0	3.5	9.8	−2.5	−4.5	46.6*	42.4	21.7
B•14Hz•400μs	32.3‡,§	7.4*	2.0	0.2	7.5	−8.1	−5.5	7.2	18.4	29.8
B•50-165Hz•150μs•b	19.5†,§	5.4	1.9	3.8	10.7	0.4	−7.2	31.4	1.6	14.9
B•60-450μs•b	50.0‡,§	3.9	7.7	6.6	13.5	−6.7	−4.1	60.1†	15.7	32.2
B•100Hz•150μs•b	34.0‡,§	7.8*	1.4	0.2	14.6*	−4.0	−7.6*	30.6†	50.5	69.5‡

Mean outcome variables at rest and during stimulation with M•100Hz•500μs as absolute values ± standard deviation.

VO_2: Highest sustainable oxygen uptake during cTEMS; HR, Heart Rate; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; CI, Cardiac Index; SVI, Stroke Volume Index; SVRI, Systemic Vascular Resistance Index.

Current terminology: Waveform•Frequency•Pulse width•Burst Mode; Waveform: M=Monophasic or B=Biphasic; Burst mode: b=Burst mode activated.

Differences in mean percentage change in association with different cTEMS currents were assessed using a multivariate linear mixed model with random intercept.

Changes induced by different cTEMS-currents are calculated with M•100Hz•500μs as reference.

Significance codes: $^*p<0.05$; $^\dagger p<0.01$; $^\ddagger p<0.001$; $^\S p<0.001$ for decreased effect on VO_2 compared to B•7Hz•400μs in post-hoc analysis.

systemic vascular resistance and thus a compensatory physiological increase in the HR and the CI. The increase in the blood flow induced by local heat application has previously been shown to cause less EMS current to penetrate into the muscles and increase the current required to stimulate the muscle [17], but we observed no significant change in the highest VO_2 by increased heat intensity. At 20% stimulation intensity, there was no difference in VO_2 at low and high heat and when we used individualized electrical stimulation intensity, there was no difference in the maximum tolerated intensity or measured VO_2 between the heat intensities.

All stimulation sessions were apparently well tolerated, and none of the participants in this study withdrew because of cTEMS. One limitation with the cTEMS device was the fixed program design. This limitation was attenuated by the application of a wide range of stimulation frequencies.

Both exercise [22] and heat exposure [23] have been shown to increase the concentration of plasma GH which is in accordance with our findings. We found an increase in GH when heat intensity was increased during cTEMS and when the electrical stimulation intensity was increased. This finding is also consistent with the work of Ftaiti et al., who showed a synergistic rise in GH due to the combination of exercise and heat exposure [24]. The long-term effect of repeated stimulation of the GH-axis through cTEMS is unclear; however, in some individuals, this increase may have beneficial effects. GH given to patients with chronic heart failure has shown positive effects on physical exercise capacity and peak oxygen uptake [25]. Furthermore, GH given to patients with GH deficiency had beneficial effects on cardiac and exercise performance, lipid profile and atherosclerosis [26].

Consistent with previous findings during isometric exercise [27], we observed a significant increase in catecholamines caused by electrical stimulation. Given that stimulation of ß-adrenoreceptors during long bed rest can attenuate deconditioning [28], our observations are of special interest. In contrast to most exercise regimes, cTEMS can be applied during bed rest.

Catecholamines increase muscle performance [14], and the increase in lactate reflected muscular work that increased beyond the anaerobic threshold. Given that the increase in HR was the same for heat and electrical stimulation, the increased oxygen consumption does not reflect the myocardial workload but must have been due to increased skeletal muscular oxygen demand. Clearly, whereas heat induces a natural physiological response, only electrical stimulation has the potential to increase muscle performance by the combination of direct electrical stimulation, the additional catecholamine effects and the possible long-term effects of GH.

Studies on nerve stimulation have shown that a monophasic current requires a higher stimulation intensity to reach the sensory perception threshold and sensory nerve action potential than a biphasic current [29]. This may partly explain our findings which indicates that monophasic currents at a given stimulation intensity are less likely to cause an increase in VO_2 than biphasic currents.

Few studies on EMS have used the same current characteristics, and, in addition, these studies have often been poorly detailed [8,30], making the comparison of the outcomes between EMS studies difficult. In this study, we used a stimulator that delivered square waveforms. Although some studies have compared the effect of different waveforms [30], we have been unable to identify studies comparing the exercise effects of different waveforms using otherwise identical stimulation parameters. Our data, however, indicate that with cTEMS, continuous pulse types at lower frequencies are better at eliciting an increase in the peak oxygen uptake as observed with other electrical stimulation modalities [31]. Further, it is well established that heat exposure causes a decrease in $VO_{2\ max}$ [32–34] and that $VO_{2\ max}$ decreases progressively with increasing ambient temperature [32]. It may therefore be the case that the VO_2 measured during cTEMS represents a higher relative exercise intensity than values measured in other studies [35–37] with conventional EMS and that increasing the heat intensity during cTEMS enhances this effect [38].

Conclusion

In conclusion, cTEMS shows physiological responses similar to exercise training. While electrical stimulation induced muscular work above the anaerobic threshold with a dose-response effect on the highest sustainable VO_2, increasing heat intensity increased GH and led to additional hemodynamic responses triggered by the vasodilatation. We found a continuous biphasic pulse type at 7 Hz frequency to be the most effective in inducing an increase in oxygen uptake, independent of stimulation and heat intensity. The clinical use and indication for the application of heat combined with EMS remains to be determined.

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Effect of combined thermal and electrical muscle stimulation on cardiorespiratory fitness and adipose tissue in obese individuals

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Abstract

Background: To better understand how prolonged electrical muscle stimulation can improve cardiorespiratory risk markers in obese subjects, we investigated the effect of prolonged combined thermal and electrical muscle stimulation (cTEMS) on peak oxygen consumption (VO_{2peak}) and body composition with subsequent lipolytic and mitochondrial activity in adipocytes.

Methods and results: Eleven obese (BMI $\geq 30\,kg/m^2$) individuals received cTEMS in three 60-minute sessions per week for 8 weeks. Activity levels and dietary habits were kept unchanged. Before and after the stimulation period, functional capacity was assessed by VO_{2peak}, and body composition was analysed. Lipolytic activity was determined in abdominal adipose tissue by 24 hours of microdialysis on a sedentary day, and adipose tissue biopsies were taken for the gene expression analysis. Eight weeks of cTEMS significantly increased VO_{2peak} from 28.9 ± 5.7 to 31.7 ± 6.2 ml/kg/min ($p < 0.05$), corresponding to an average increase of 1.2% per week. Oxygen uptake and work capacity also increased at the anaerobic threshold. Mean microdialytic glycerol concentration over 24 hours, an index of sedentary lipolytic activity, increased from 238 ± 60 to $306 \pm 55\,\mu M$ ($p < 0{,}0001$), but no significant changes in body composition were observed. In addition, PGC-1α and carnitine-palmitoyltransferase-2 mRNAs were significantly upregulated in subcutaneous abdominal adipose tissue.

Conclusions: In obese individuals with unchanged lifestyles, 8 weeks of cTEMS significantly improved functional capacity towards a higher fatigue resistance. This increase also gave rise to elevated lipolytic activity and increased mitochondrial activity in abdominal adipose tissue.

Keywords

Body composition, electrical stimulation, lipolysis, mitochondria, obesity, oxygen consumption

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Introduction

Electrical muscle stimulation (EMS) of unloaded muscles improves cardiorespiratory fitness in healthy individuals[1,2] and patients with heart failure,[3,4] but it is unclear whether EMS could serve as an adjunct approach to improve fitness and body composition in obese individuals.

Obesity is associated with an increased risk of numerous comorbidities.[5,6] While targeting obese subjects with sedentary habits and low fitness appears to be an effective strategy in improving the health of this population,[7] the possible role of prolonged EMS within this strategy remains unexplored. Muscular strength and cardiorespiratory capacity are both inversely associated with metabolic syndrome prevalence,[8] and the maintenance or improvement of physical fitness may counteract obesity-associated disease risks and

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lead to health benefits.[9] In addition to improving physical fitness, exercise training has beneficial effects on lipoprotein profiles,[10] and, even at low intensity, exercise training also tends to increase fat oxidation and lead to positive changes in the expression of genes essential for fat metabolism.[11]

Obesity may restrict both exercise training practice and performance, which makes alternative methods to promote fitness desirable. However, these same restrictions may also limit the potential benefit of EMS. For example, the electrical current threshold is reported to be higher in obese than in nonobese subjects, and their stimulation tolerance appears to diminish within one stimulation session.[12] Alterations have been proposed to reduce discomfort while maintaining the efficacy of EMS in obese populations,[13] but the potential benefits have not yet been explored.

This study aimed to investigate the effects of combined thermal and electrical muscle stimulation (cTEMS) on cardiorespiratory fitness in obese subjects as measured by VO_{2peak}. In addition, we investigated effects of cTEMS on body composition and adipose tissue.

Methods

Subjects

This study recruited 12 obese (body mass index, BMI, $\geq 30\,kg/m^2$) and sedentary subjects (< 20 minutes of exercise <3 days per week) through an invitation sent to employees at the Haukeland University Hospital in Bergen, Norway. Other inclusion criteria were age between 30 and 70 years and the ability to undergo exercise testing. Individuals with a pacemaker, regular medication, cardiovascular disease, pulmonary disease, extensive dermatological disease, or other primary diseases, pregnant women, and individuals who were abusing alcohol or drugs were excluded from the study. For completion of the study, participation in more than 70% of the cTEMS sessions was mandatory.

Among the 12 volunteers (six males and six females), one female withdrew her consent during the study. This was not related to the study or methods used. Before inclusion, all subjects underwent a clinical examination and provided written informed consent. The study was approved the Regional Committee for Medical and Health Research Ethics (Western Norway ref. 2009/1273) and conformed to the Declaration of Helsinki.

Experimental set up

The study was performed in a designated laboratory in the Haukeland University Hospital, Bergen, Norway. Prior to the baseline visit, all subjects had one session of cTEMS adaptation and tolerance testing. Prior to the baseline and follow-up examinations, the participants fasted overnight but were allowed to drink water.

Blood samples were collected in the fasting state, and the body composition was measured. Lipolytic activity in subcutaneous abdominal adipose tissue (SCAAT) was analysed for the following 24 hours using microdialysis. We also performed exercise testing and collected a SCAAT biopsy to analyse mitochondrial and lipolytic-related mRNAs. To avoid interactions, we separated the examinations (microdialysis, exercise testing, and biopsy) by at least 48 hours.

Following the baseline examinations, all subjects underwent an 8-week intervention period with three cTEMS sessions per week, followed by follow-up examinations. During the study, all participants were instructed to maintain their normal dietary habits and physical activity levels. Possible changes in lifestyle were controlled for with a nutritional questionnaire and with accelerometer.

Combined thermal and electrical muscle stimulation

In this study, we used cTEMS, a combination of applied superficial heat and electrical muscle stimulation. cTEMS was applied using a stationary stimulator (TEI System; RÖS'S Estética, Barcelona Spain).[14] The system consists of an electrical power source connected to ten silicone pads with two different sizes (24×26 cm, with maximum heating capacity 25 W, and 24×17 cm, with maximum heating capacity 15 W). Within the silicone pads, both electrode bands for the delivery of electrical current and heating elements are incorporated.

At each stimulation session, ten electrodes were attached to the designated muscle groups (two electrodes each to quadriceps, hamstrings, glutei, and obliques, and one electrode each to rectus abdominis and lower lumbar muscles) with elastic bands. Optimal skin adhesion was secured by standard ultrasound gel.

Stimulation protocol

cTEMS was delivered three times a week for 60 minutes at each individual's stimulation threshold. To secure compliance throughout the intervention period, we varied the electrical pulse types during the sessions using 2–4 different pulse types at each stimulation visit. Heat stimulation was set at 40% of the maximum heating capacity. The detailed stimulation protocols are given in Table 1.

Biopsy from abdominal adipose tissue

Abdominal adipose tissue was collected using a 14-gauge needle connected to a 10-ml syringe with a

Table 1. Electrical pulse types and cTEMS sessions per week

	Session 1	Session 2	Session 3
Time (min)	60	60	60
Pulse type 1			
Waveform	Biphasic	Biphasic	Biphasic
Frequency (Hz)	14	7	18
Pulse width (μs)	400	400	400
Modulation	n/a	n/a	n/a
Pulse type 2			
Waveform	Biphasic	Biphasic	Biphasic
Frequency (Hz)	2.5	100	2.5
Pulse width (μs)	400	150	400
Modulation	n/a	Burst	Burst
Pulse type 3			
Waveform	Biphasic		Biphasic
Frequency (Hz)	50–165		60
Pulse width (μs)	150		450
Modulation	Modulated frequency		Burst
Pulse type 4			
Waveform			Monophasic
Frequency (Hz)			100
Pulse width (μs)			500
Modulation			n/a

locking member to create vacuum (Hepafix; B Braun Melsungen, Germany). While maintaining vacuum in the syringe, 4-6 ml of fat and fluid were aspirated, cleansed in saline, frozen in liquid nitrogen, and kept at −80°C until further analysis.

Gene expression

Tissue samples were homogenized and total cellular RNA was purified and quantified spectrophotometrically. RNA quality was evaluated by capillary electrophoresis. One microgram of total RNA was reverse transcribed in 100 μl reactions using TaqMan reverse transcription reagents with RNase inhibitor (Applied Biosystems, Foster City, CA, USA). Real-time PCR was performed with 384-well Multiply-PCR Plates (Sarstedt, Newton, NC, USA) on the following genes using probes and primers from Applied Biosystems: carnitine palmitoyltransferase 1a (CPT-1a, Hs00157079), CPT-2 (Hs00264677), hormone-sensitive lipase (LIPE, Sh00943410), peroxisome proliferator-activated receptor gamma (PPARγ, Hs00174128), PPARγ coactivator 1 (PPARGC-1a (PGC-1a), Hs00173304), resistin (RETN, Hs00982492), uncoupling protein 1 (UCP-1, Hs00222453), UCP-2 (Hs00163349), and UCP-3 (Hs00243297). Three reference genes were used: glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Hs99999905) and ribosomal protein large P0 (RPLP0, 4333761T) from Applied Biosystems and 18S MP (Kit-FAM-TAMRA, RT-CKFT-18s) from Eurogentec (Seraing, Belgium). We used the Normfinder algorithm[15] to assess the stability of the measured reference genes. RPLP0 showed the best results, with a stability value of 0.039, and was subsequently used to normalize the target gene values. We calculated the relative expression values as the ratio of target gene expression to RPLP0.

Microdialysis

Lipolysis in adipose tissue was assessed with microdialysis, a technique previously described by Arner et al.[16] After light epidermal anaesthesia (Emla patch 5%), one microdialysis probe (CMA 63, 20-kDa molecular-weight cut-off; CMA Microdialysis, Sweden) was inserted percutaneously into the SCAAT, 8–10 cm lateral to the umbilicus. The probe was connected to a microdialysis pump (CMA 107, CMA Microdialysis) and continuously perfused with sterile Ringer's solution (154 mM Na^+, 6 mM K^+, 2,5 mM Ca^{2+}, 160 mM Cl^-). The perfusion was set at a standard flow rate of 2 μl/min, and fractions were collected every 2 hours following a 30-minute equilibration period. The microdialysate was kept at 4°C until it was analysed in a microdialysate analyser (Iscus Flex; CMA Microdialysis). The lipolytic activity in SCAAT was expressed as the dialysate glycerol.

The 24 hours of registration the subjects spent in their habitual surroundings. At baseline, the subjects registered their food intake and were instructed to follow exactly the same dietary intake on the follow-up registration.

Exercise testing

The cardiorespiratory fitness before and after the 8-week intervention period with cTEMS was evaluated by ergospirometry during a standard treadmill test while using a modified Balke protocol. The subjects were acclimated to the treadmill before the baseline test was performed. At a speed of 5.5 km/h, we increased the elevation by 2° every 2 minutes, during which ventilatory gas exchange was measured by an ergospirometer with a breath-to-breath analyser (Cardiovit CS-200 Ergo-Spiro/13 Ganzhorn Power Cube; Schiller).

Peak oxygen consumption (VO_{2peak}) was considered to have been reached when all of the following criteria were met: the maximal heart rate measured at exhaustion was higher than 90% of the age-predicted maximal heart rate; the respiratory exchange ratio (RER) measured at exhaustion was greater than 1.1; and the

subject was not able to sustain sufficient speed on the treadmill.

Anaerobic threshold was determined using the V-slope method.[17]

Body composition

Dual-energy X-ray absorptiometry (Lunar Prodigy DF; GE Medical Systems) was used to analyse body composition, while body weight and visceral fat area were measured using bioelectrical impedance analysis (InBody 720; BioSpace, Seoul, Korea).[18]

Lifestyle monitoring

Subjects were encouraged to maintain their sedentary behaviour, and activity levels were measured before and at the end of the 8-week treatment period. For 5 consecutive days, the participants wore a triaxial accelerometer (GT3X; The Actigraph, Fort Walton Beach, FL, USA) on their hip while awake. The data were processed by ActiLife software (The Actigraph), and the total physical activity time spent in sedentary, light, moderate, vigorous, and moderate to vigorous physical activity modes was calculated and converted into the percentage of total time spent in each activity mode. We used a 15-item SmartDiet self-administered questionnaire to monitor possible dietary habit changes.[19]

Serum lipid analysis

Serum lipids were measured enzymatically on a Hitachi 917 system (Roche Diagnostics, Mannheim, Germany) using the triacylglycerol (GPO-PAP), cholesterol (CHOD-PAP), HDL cholesterol, and LDL cholesterol kits from Roche (Roche Diagnostics). The phospholipids FS, NEFA FS (nonesterified fatty acids), and free cholesterol FS kits were from Diagnostic Systems (Holzheim, Germany).

Statistical analysis

Baseline and follow-up measurements were presented as mean ± standard deviation and compared using the nonparametric Wilcoxon signed-rank test.

Mean glycerol dialysate levels were estimated over time. Relative gene expression values at baseline and follow-up were estimated with a random-intercept linear mixed-effects model in a two-way repeated measure configuration.

Statistical analyses were conducted with R version 2.15.2 (R Foundation for Statistical Computing, Vienna, Austria) and linear mixed-effects models were analysed using package nlme-3.1–107.

Results

Eleven subjects (five female; six male) with a mean age of 44.6 ± 5.9 years completed the study and were included in the analysis. We observed no notable complications related to the cTEMS intervention.

Exercise testing

All subjects completed the exercise testing (Table 2). After 8 weeks of cTEMS intervention, we observed a 9.6% increase in VO_{2peak} ($p = 0.014$) and a 10.8% increase in peak work capacity ($p = 0.014$). The higher VO_{2peak} and workload were accompanied by a significantly lower RER. At the anaerobic threshold, both total VO_2 and work capacity increased significantly, and RER decreased ($p = 0.022$). The decrease in RER was also found at rest when analysing true resting values (from 0.87 ± 0.06 to 0,79 ± 0,06; $p = 0.028$).

Table 2. Exercise testing and body composition

	Baseline	Follow up	*p*-value
Resting systolic BP (mmHg)	129.2 ± 13.9	127.4 ± 16.9	0.575
Resting diastolic BP (mmHg)	85.0 ± 7.9	87.6 ± 8.6	0.608
Resting heart rate (bpm)	64 ± 7	67 ± 8	0.423
Peak VO_2 (l/min)	3.04 ± 0.68	3.33 ± 0.69	**0.014**
Peak VO_2 (ml/kg/min)	28.9 ± 5.7	31.7 ± 6.2	**0.014**
Peak VCO_2 (l/min)	3.93 ± 0.99	3.99 ± 0.91	0.663
Peak RER	1.28 ± 0.06	1.20 ± 0.06	**0.009**
Peak work capacity (watt)	231 ± 57	256 ± 59	**0.014**
AT VO_2 (l/min)	2.44 ± 0.59	2.65 ± 0.49	**0.002**
AT VO_2 (ml/kg/min)	23.3 ± 5.4	25.4 ± 4.7	**0.002**
AT VCO_2 (l/min)	2.64 ± 0.70	2.67 ± 0.58	0.754
AT RER	1.08 ± 0.06	1.00 ± 0.08	**0.022**
AT work capacity (watt)	174 ± 50	190 ± 40	**0.008**
Total mass (kg)	103.13 ± 14.05	106.31 ± 14.31	0.365
Body mass index (kg/m^2)	35.4 ± 3.3	35.5 ± 3.7	0.833
Fat mass (kg)[a]	43.72 ± 8.85	43.22 ± 8.46	0.959
Fat percentage (%)[a]	42.9 ± 8.3	42.5 ± 7.9	0.373
Lean mass total (kg)[a]	58.73 ± 12.92	59.00 ± 12.70	0.505
Lean mass trunk (kg)[a]	28.69 ± 6.34	29.05 ± 6.48	0.577
Lean mass arms (kg)[a]	6.24 ± 1.59	6.28 ± 1.91	0.683
Lean mass legs (kg)[a]	19.38 ± 4.86	19.19 ± 4.38	0.365
Visceral fat (cm^2)[b]	179.6 ± 31.4	177.8 ± 30.3	0.898

Values are mean ± SD for 11 patients (six male, five female). *p*-values for two-sided nonparametric Wilcoxon signed-rank test; Blood pressure and heart rate measured in supine position at rest. Respiratory values measured at peak exercise intensity and anaerobic threshold during treadmill test; [a]Measured with dual-energy X-ray absorptiometry; [b]Estimated by bioelectrical impedance analysis; AT, Anaerobic threshold; BP, blood pressure; RER, respiratory exchange ratio.

Body weight and body composition

As shown in Table 2, we observed no significant changes in body weight or body composition between baseline and follow up.

Cholesterol and lipids

During 8 weeks of cTEMS, the ratio between the total and HDL cholesterol was unchanged despite significant reductions of total cholesterol from 5.60 ± 1.37 to 5.21 ± 1.14 mmol ($p = 0.042$). Both LDL and HDL cholesterol were nonsignificantly decreased. The other lipid biomarkers remained unchanged (Table 3).

Microdialysis

Lipolytic activity in the abdominal adipose tissue was estimated by the mean glycerol concentration over 24 hours on a sedentary day. We observed a significant increase from 239 ± 60 μM at baseline to 304 ± 55 μM at follow up ($p = 0.001$) after 8 weeks of cTEMS, as shown in Figure 1. Further, we also observed an increase in mean 24-hour microdialytic lactate from 2.85 ± 0.27 mM at baseline to 3.73 ± 0.58 mM at the follow-up examination ($p = 0.002$).

Adipose tissue analysis

The gene expression in SCAAT is presented as the mean ± standard error relative expression. Resistin and UCP-1 mRNAs were not expressed in the SCAAT specimen and thus not included in the analysis.

Eight weeks of cTEMS significantly increased PGC-1α expression in the adipose tissue from 8.10 ± 1.08 to 10.00 ± 1.56 ($p < 0.05$). Further, we observed an increase in CPT-2 from 7.89 ± 0.24 at baseline to 8.82 ± 0.35 at follow up ($p < 0.001$). CPT-1a (4.43 ± 0.94 to 4.73 ± 0.67; $p = 0.445$), LIPE (3.04 ± 0.35 to 3.15 ± 0.50; $p = 0.641$), PPARγ (75.30 ± 5.53 to 77.21 ± 7.31; $p = 0.518$), UCP-2 (5.53 ± 1.09 to 5.58 ± 0.79; $p = 0.908$), and UCP-3 (41.25 ± 3.24 to 48.21 ± 3.85; $p = 0.518$) all showed slight but nonsignificant increases in mRNA expression.

Lifestyle monitoring

Dietary habits remained unchanged, as indicated by a SmartDiet score of 29.27 ± 5.16 at baseline and 29.36 ± 4.15 at follow up ($p = 1.000$). Physical habits, as measured with an accelerometer, also remained unchanged. The percentage of total time spent in the sedentary state over 5 consecutive days was $78.8 \pm 7.7\%$ at baseline and $80.0 \pm 6.7\%$ at follow up ($p = 0.232$). Additionally, $18.9 \pm 7.8\%$ of their time was spent in light physical activity at baseline vs. $18.3 \pm 6.8\%$ at follow up ($p = 0.375$), and we observed a slight decrease in time spent in moderate activity (2.3 ± 0.9 vs. $1.7 \pm 0.7\%$; $p = 0.027$). The participants barely performed heavy physical activity (0.01 ± 0.03 vs. $0.02 \pm 0.04\%$; $p = 1.000$), and no time was spent with very heavy physical activity.

Discussion

This study provides new insights into the effects of prolonged electrical muscle stimulation. In obese sedentary individuals with unchanged physical activity levels and nutritional habits, 8 weeks of cTEMS resulted in significant increases in peak aerobic capacity, as expressed by VO_{2peak}. The improved work capacity, towards a higher fatigue resistance, was both reflected in an increased maximal workload during the exercise testing

Table 3. Lipids and cholesterol at baseline and follow up

	Baseline	Follow up	*p*-value
Total/HDL cholesterol ratio	4.87 ± 1.56	4.82 ± 1.42	0.683
LDL cholesterol (mmol/l)	3.61 ± 1.02	3.41 ± 0.99	0.147
Non-HDL cholesterol (mmol/l)[a]	4.40 ± 1.41	4.09 ± 1.16	0.638
Triglycerides (mmol/l)	1.69 ± 1.06	1.45 ± 0.72	0.308
Apolipoprotein B/A1 ratio	0.80 ± 0.23	0.83 ± 0.23	0.229
Apolipoprotein B	1.09 ± 0.32	1.03 ± 0.27	0.052
Apolipoprotein A1 (g/l)	1.38 ± 0.20	1.26 ± 0.16	0.083
Phospholipids (mmol/l)	2.95 ± 0.60	2.76 ± 0.40	0.185
Free fatty acids (mmol/l)	0.50 ± 0.13	0.47 ± 0.17	0.594

Values are mean ± SD for 11 patients (six male, five female). *p*-values for two-sided nonparametric Wilcoxon signed-rank test; [a]Total cholesterol minus HDL cholesterol.

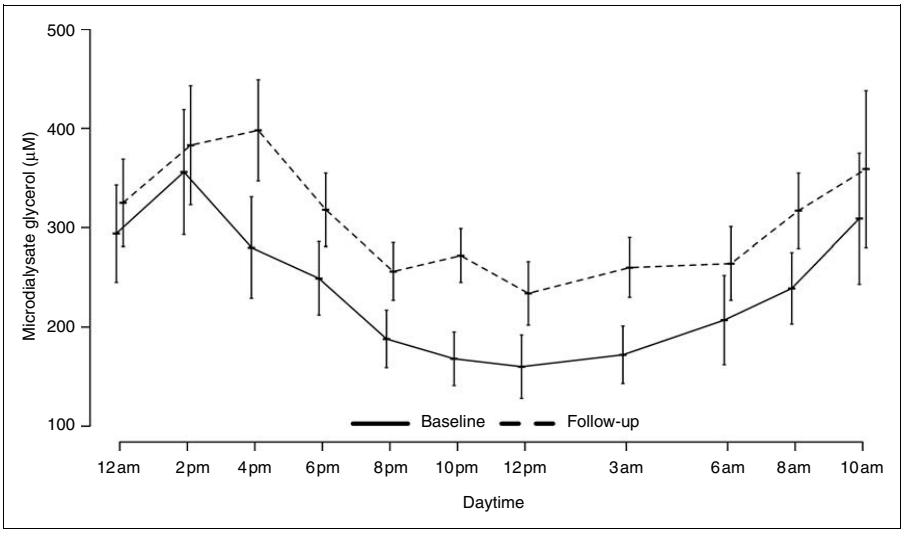

Figure 1. Lipolytic activity.
Mean microdialysate glycerol over time. Error bars denote standard error estimated by a random-intercept mixed-effects model. The group difference between baseline and follow up is 76.9 mmol/l ($p < 0.001$).

and secondary improvements in sedentary lipolytic activity and adipose tissue mitochondrial activity. To our knowledge, this is the first time such a training effect has been observed in obese individuals using EMS as an exercise training modality.

The observed changes in VO_{2peak} from baseline to follow up represented a 9.6% increase, with an average increase of 1.2% per week of cTEMS intervention. In comparison, when investigating the training effects in individuals with metabolic syndrome, Tjønna et al. observed an increase of 1.0% per week with continuous moderate exercise at 70% of the maximal heart rate and 2.2% per week with aerobic interval training at 90% of the maximal heart rate.[20] Exercise capacity is a more powerful predictor of mortality than many other established cardiovascular risk factors,[21] thus our finding may prove to have future clinical relevance for obese individuals not able or willing to participate in regular exercise training.

In healthy individuals, a rise in the maximal oxygen uptake is caused by an increase in the cardiac output, increased arteriovenous oxygen extraction, or both.[22] We have previously shown that cTEMS resulted in a 3.2-fold increase in the resting VO_2, corresponding to 2.8 metabolic equivalents,[14] an increase less likely to give a training effect on the heart. In the present study there was no change in resting blood pressure or heart rate, indicating that stroke volume and cardiac output was unaffected. The increased oxygen consumption caused by cTEMS is therefore likely to be associated with increased arteriovenous oxygen extraction by the stimulated muscle groups. This would also be in agreement with the finding of Nuhr et al.,[23] who previously showed that EMS in healthy individuals induced changes both in skeletal muscle fibre composition and energy metabolism.

Maximal oxygen uptake has been positively correlated with metabolic flexibility, i.e. the ability of the skeletal muscle to switch between fat and carbohydrate oxidation.[24] Furthermore, exercise increases the reliance on fat oxidation and the capacity for free fatty acid mobilization and oxidation during exercise at a given intensity level.[25,26] An upregulation of the anaerobic threshold due to cTEMS and the corresponding increase in metabolic flexibility is the most reasonable explanation for the observed increase in the lipolytic activity measured on sedentary days. Compared with baseline values, we found a significant increase in the 24-hour mean microdialysate concentration of glycerol at follow up, which is an indication of a rise in the mobilization of triacylglycerol and the following conversion into free fatty acids and glycerol.[27] Subsequent to the increase in interstitial glycerol, we also found a significant increase in adipose tissue lactate. This pathway remains a major source of energy for the working muscle[28] and thus the increased lipolysis provides fuel for the increased muscular utilization of fatty acids.

It is unlikely that changes in microdialytic glycerol levels resulted from blood flow alterations in adipose tissue. Central haemodynamics remained unchanged,

and microdialytic examinations were performed during a sedentary day under controlled diet. A physiological pulse of growth hormone may also activate lipolysis[29] in adipose tissue. We have previously found an acute increase in growth hormone due to cTEMS set off by both the electrical and the heat stimulation,[14] but the long-term effect of cTEMS and EMS on growth hormone is unknown and was not in focus in this study.

The effect of exercise training on gene expression in human adipocytes remains unexplored. In rats, however, 4 weeks of daily swim training resulted in a significant increase in PGC-1α, a key regulator of mitochondrial biogenesis.[30] When analysing key genes related to fatty acid oxidation in SCAAT, we found an increase in PGC-1α, essential in mitochondrial function and in regulating the genes involved in fatty acid oxidation.[31] We interpreted these findings as early adaptions to the increase in the energy-demanding lipolysis process, as shown by the increase in microdialytic glycerol in SCAAT. The significant increase in CPT-2 and the relative, but not significant, increase in the other investigated genes may support this interpretation.

Despite the observed increase in adipose tissue lipolysis, 8weeks of cTEMS did not significantly influence body composition. When investigating the effect of EMS on the body mass index of sedentary individuals, Banerjee et al.[2] found similar results. The type of exercise conferred by cTEMS may explain this, as regular steady-state exercise at moderate intensity does not seem to induce significant changes in body composition.[32]

Previously, a connection between the fat layer thickness and electrical current has been proposed,[33] and higher currents, associated with increased discomfort, are necessary to evoke muscle activation in obese subjects.[13] The stimulation modality used in this study, with relatively large electrodes, as suggested by others,[13,34] and applied heat, appear to be alterations making it possible to overcome these limitations. Superficial heat is known to reduce pain[35] and thus seem to improve tolerance to afferent electrical stimulation necessary to evoke muscular contraction.[14] In this study, cTEMS was well tolerated and administered without any significant complications or discomfort. After the end of the intervention period, an anonymous questionnaire was sent by mail to the 11 study participants, and nine responded. Only two of nine responders associated the stimulation sessions with light or moderate discomfort. Another two subjects reported being neutral, while the remaining five described light (one person), moderate (one person), or great comfort (three persons) related to cTEMS. If available, eight of the nine responders reported that they would consider cTEMS as a regular part of their daily routines.

Some limitations in our study may be considered in the evaluation of the findings. The main weaknesses are the nonrandomized design and a relatively small study population. Both diet and everyday activity may influence the results; however, when controlled, we did not find any changes in dietary habits or physical activity levels. Further, the fact that the training effect from cTEMS was observed in combination with positive metabolic and genetic findings, we consider a strength of our study.

In conclusion, we found that 8 weeks of prolonged electrical muscle stimulation with added heat in obese sedentary subjects significantly improved the functional capacity towards higher fatigue resistance. Both at peak exercise and at the anaerobic threshold, we observed increased VO_2, most likely caused by increased muscular arteriovenous O_2 extraction. Secondary to these training-effects, we found increased lipolytic activity and increased level of mitochondrial activity in adipose tissue. During this relatively short stimulation period, the body composition and visceral fat area remained unchanged. Further studies are needed to determine the possible role for cTEMS as an adjuvant in the treatment of obese individuals.

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III

Effects of electrical muscle stimulation on body composition in obese subjects during weight loss

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Abstract

Introduction: Electrical muscle stimulation shows physiological response similar to exercise training and could improve the effects of weight reducing diets. A link between exercise and reduction of visceral fat has been suggested.

Objectives: The purpose of this study was to investigate the potential effects of prolonged combined thermal and electrical muscle stimulation (cTEMS) on body composition and the reduction of visceral fat, when used as a supplement to a dietary intervention.

Methods: 22 obese (BMI ≥ 28 kg/m2; fat percentage > 20% for men and 28% for women) and sedentary, but otherwise healthy individuals were randomized to either a low carbohydrate diet (LC) or LC supplemented with cTEMS (LC+cTEMS). The intervention period was 10 weeks during which physical activity level was kept unchanged and controlled with accelerometer. cTEMS was given in three 45 min sessions per week. Body composition was assessed by Dual-Energy-X-ray-absorptiometry, visceral fat area estimated by Bioelectrical Impedance Analysis and serum lipids and lipoprotein levels were measured from fasting blood samples.

Results: After 10 weeks both LC and LC+cTEMS-group had similar significant weight loss (6.5±3.0, $p<0.001$; 8.5±3.4 kg, $p<0.001$), fat loss (4.8±3.0 kg, $p<0.001$; 5.9±2.8 kg, $p<0.001$) and loss of lean mass (1.3±0.8 kg, $p<0.001$; 1.8±2.0 kg $p<0.05$). The reduction in visceral fat area was 26.1±19.0 cm^2 ($p<0.01$) for LC and 49.2±25.6 cm^2 ($p<0.001$) for LC+cTEMS with a significant between-groups difference ($p<0.05$). Further, respiratory exchange ratio was significantly reduced at maximal exercise compared to LC controls while max VO2 was maintained. On treadmill we found a reduction in energy expenditure at a load of 100W ($p<0.05$) in the LC+cTEMS and with a significant between-groups difference ($p<0.05$).

Conclusion: When used as a supplement to a weight-reducing low carbohydrate diet, cTEMS resulted in a significantly greater reduction in visceral fat accumulation and delayed anaerobic threshold. The reduced energy expenditure at a given workload induced by regular muscle-work may in general explain the lack of weight reduction by increased physical exercise alone.

***Key words*:**

electrical muscle stimulation, obesity, weight reduction, lipoprotein, visceral fat, low carbohydratte diet, energy expenditure

Introduction

The primary aim of a weight loosing diet is the reduction of fat mass while preserving the lean body mass. Many trials however, with dietary intervention, report an unwanted loss of fat free mass [1,2], an effect influenced by the degree of caloric restriction, exercise and the rate of weight loss [1]. In a recently published work, de Souza et al. did not find any difference in the changes in body composition, abdominal fat or hepatic fat in participants assigned to different macronutrient amounts in a 6 months trial of four different diets[3]. Although some smaller studies have indicated a possible preferable effect of low-carbohydrate, the main view is that it that it still remains unclear whether a certain macronutrient composition has a preferable effect compared with others in this regard. There is no evidence from randomized controlled trials confirming the established belief that physical activity actually induce or improve weight loss [4], however, exercise training is an effective tool in the maintenance of lean body mass during hypocaloric weight loss [5,6].

Visceral adipose tissue (VAT) is more closely linked to cardiovascular disease and type 2 diabetes than other obesity indexes [7] and studies have indicated a relative greater reduction in VAT in response to exercise interventions [8]. A plausible explanation for these results has been the strong responsiveness of VAT to adrenergic activation [9].

We have previously shown that combined thermal and electrical muscle stimulation (cTEMS) shows physiological response similar to exercise training and induce muscular work above the anaerobic threshold with a corresponding increase in catecholamines [10]. Therefore, the aim of the present study was to evaluate the potential effects of prolonged combined thermal and electrical muscle stimulation (cTEMS) on the effect on body composition, the reduction of visceral fat and influence on blood lipids, when used as a supplement to a dietary intervention.

Methods

Subjects

Overweight (BMI ≥ 28 kg/m^2; fat percentage > 20% for men and 28% for women) and sedentary (≤ 20 minutes of exercise on ≤ 3 days per week) subjects, were recruited among reponders to an advertisement in the local newspaper. Additional inclusion criteria was age between 30 and 70 and years. Exclusion critieria were pacemaker or implanted defibrillator, regular medication, cardiovascular disease, pulmonary disease,

extensive dermatological disease or other primary diseases, pregnancy, alcohol- or drug-abuse. The first 24 eligible subjects from a total of 300 responders were invited for assessement and clinical examination. Tentytwo subjects completed the study and were included in the statistical analysis. Two persons (on male and one female) withdrew their consent of reasons not related to the study.

The study was approved the Regional Committee for Medical and Health Research Ethics (Western Norway Ref: 2010/2360) and conformed to the Declaration of Helsinki.

Table 1 Baseline charateristics

		LC+cTEMS n=11(6 f/5m)	**LC** n=11(5f/6m)	**Between groups**
Age	Years	44.2±6.0	50.7±7.9	n.s.
Systolic blood pressure	mmHg	121±9	128±12	n.s.
Diastolic blood pressure	mmHg	78±7	81±9	n.s.
Heart rate rest	1/min	64±8	63±7	n.s.
$VO_{2\ max}$ per kilogram	ml/kg/min	30.6±6.1	29.3±4.5	n.s.
Maximal work load	watt	237±51	231±60	n.s.
Body mass	kg	101.5±14.4	101.6±15.7	n.s.
Height	cm	175.1±9.3	174.3±9.8	n.s.
Body mass index	kg/m^2	33.1±4.6	33.4±4.1	n.s.
Lean mass[1]	kg	57.4±11.4	56.7±12.7	n.s.
Fat mass[1]	kg	41.1±10.5	42.0±7.5	n.s.
Fat %[1]	%	41.8±8.7	42.9±6.4	n.s.
Visceral fat area[2]	cm^2	190.0±47.8	174.9±42.3	n.s.
Fasting Glucose	mmol/l	5.9±0.8	5.5±0.7	n.s.
HbA_1C	%	5.8±0.7	5.5±0.3	n.s.
Insulin	mU/l	14.0±16.3	7.4±7.0	n.s.
HOMA IR		4.0±5.3	1.8±1.6	n.s.
Total cholesterol	mmol/l	4.9±0.8	5.0±0.9	n.s
LDL cholesterol	mmol/l	3.3±0.7	3.3±0.7	n.s
HDL cholesterol	mmol/l	1.2±0.3	1.3±0.4	n.s
Triglycerides	mmol/l	1.2 ±0.5	1.3±0.9	n.s.

Baseline values expressed as mean±SD;
Differences between dietary groups at baseline were assessed with two-sided independent sample t-tests with Welch's correction for unequal variances.
Blood pressure measured in supine position at rest.
Respiratory values and maximal work load was measured at peak exercise intensity during treadmill test.
[1] Measured by Dual Energy x-ray absorptiometry
[2] Estimated by Bioelectrical impedance measurement

Experimental setup

The study was carried out at the Haukeland University Hospital in Bergen, Norway. All procedures related to the study was performed in a designated research laboratory. Prior to baseline and follow-up examinations and sampling, the participants fasted overnight, but were allowed to drink water. They were also asked to abstain from the use of tobacco and chewing gum.

All blood samples were collected in the fasting state

Combined thermal and electrical muscle stimulation

As described earlier [10,11], we used a combination of applied superficial heat and electrical muscle stimulation in this study. With a stationary stimulator (TEI System; RÖS'S Estetica, Barcelona Spain) heat and EMS was applied using silicone pads attached to the designated muscle groups. The use of standard ultrasound gel aimed to secure optimal skin adhesion.

Stimulation protocol

Larger muscle groups (quadriceps, hamstrings, glutei, oblique's, rectus abdominis and lower lumbal) and overlying subcutaneous tissue was exposes to cTEMS using ten silicone electrodes. The electrodes covered a total area of 0,62 cm^2 and heat intensity was set to 40% of maximum heating capacity (72 W spread on 10 electrodes).

The LC+cTEMS group received cTEMS three times per week during the 10 week intervention period with current intensity set at individualized threshold. Each session lasted 45 minutes and a combination of square biphasic electrical pulse types were given with frequencies varying between 2,5 Hz and 60 Hz and with pulse width 400-450 μs.

Dietary intervention

Each of the groups received teaching and instructions in nutrition in general and the diet more specifically, both in the group and on an individual basis. Further the subjects were given a commercially available book on low carbohydrate diet (Frisk med lavkarbo by Hexeberg, S MD PhD; ISBN 9788202322731). Preparation of the food was self-administered.

The carbohydrates were restricted until the presence of ketones was detected semiquantitivly by urine reagent strips (Ketostix ®). Participants were told to keep their carbohydrate intake at a level where a color change was maintained at the urine reagent strip, and optimally lower than 50 g per day. There was no restrictions on the dietary energy content and the subjects were instructed to replace the carbohydrates by non-processed-fat from natural sources such at meat, fish, vegetables, nuts or dairy products. Dietary analyses were conducted at two points during the study. The subjects weighed and recorded all food intake and drinks over four days on two occastions using a regular diary. The nutritional data of these registrations was analysed by study personell using a online software tool (www.diett.no).

Exercise testing

Cardiorespiratory fitness and the energy expenditure at a workload of 100W was evaluated, before and after the eight weeks intervention period with cTEMS, by ergospirometry during a standard treadmill test while using a modified Balke Protocol. The subjects were accustomized to the treadmill before the baseline test was performed. At a speed of 5.5 km/h we increased the elevation by 2 ° every 2 minutes during wich ventilatory gas exchange was measured with a ergospirometer with breath to breath analyzer (Schiller Cardiovit CS-200 Ergo-Spiro/13 Ganzhorn Power Cube).

Peak oxygen consumption ($VO_{2\ peak}$) was considered reached when all of the following accepted criteria were met: Maximal heart rate measured at exhaustion was superior to 90% of the age-predicted maximal heart rate, respiratory-exchange-rate (RER_{peak}) measured at exhaustion was superior to 1,1, and the subject was not able to sustain sufficient speed on treadmill.

Body composition

A dual-energy X-ray absorptiometry (GE Medical Systems, Lunar Prodigy DF) was used for the analysis of body composition while body weight and visceral fat area was measured using bioelectrical impedance analysis (InBody 720, BioSpace, Seoul, Korea) [12].

Lifestyle monitoring

In order to rule out possible bias affecting the results of the study, the subjects were encouraged to maintain their sedentary behaviour and activity level was measured during the intervention period for 7 consecutive days. The participants were wearing an accelerometer (GT3X, The Actigraph, Fort Walton Beach, FL, USA) placed on their hip while being awake. The activity monitor is a compact (3,8cm x 3,7cm x 1,8 cm, 27g) triaxial accelerometer recording accelerations from 0,05 to 2,5 G. Data was processed by ActiLife software (The Actigraph) and total physical activity (PA) time spent in sedentary, light, moderate, vigorous and moderate to vigorous physical activity was calculated and converted into percentage of total time spent in different activity zones.

Serum lipid analysis

Serum lipids were then measured enzymatically on a Hitachi 917 system (Roche Diagnostics GmbH, Mannheim, Germany) using the triacylglycerol (GPO-PAP), cholesterol (CHOD-PAP), HDL-cholesterol, and LDL-cholesterol kits from Roche (Roche Diagnostics GmbH, Mannheim, Germany). Phospholipids FS, NEFA FS (non-esterified fatty acids), and free cholesterol FS kits were from DiaSys (Diagnostic Systems GmbH, Holzheim, Germany). Lipoprotein particle size analysis was performed by Liposcience Inc, Raleigh NC, USA using proton nuclear magnetic resonance (NMR) spectroscopy.

Statistical analysis

Baseline characteristics are reported as mean (SD). Differences between intervention groups at baseline were assessed with two-sided independent sample t-tests with Welch's correction for unequal variances. Endpoint variables at baseline, at follow-up and the absolute change from baseline to follow-up are reported as mean (SD). Within group changes estimated by two-sided paired t-tests, between group differences in absolute change from baseline were compared with two-sided independent sample t-test with Welch's correction for unequal variances. P-values greater then 0.05 were considered statistically significant. All analyses were performed with R version 2.15.3 (R Foundation for Statistical Computing , Vienna , Austria).

Results

The treatment groups were comparable at baseline, shown in Table I . There were no complications, side-effects or discomfort related to the cTEMS intervention or the diet.

Body composition

When analysing viceral fat area, we found a significantly larger reduction in the cTEMS+LC group compared to the LC alone group ($p = 0.027$) as shown in figure 1. In the LC-group there was a mean reduction by 26.1 cm^2 (174.9 ± 42.3 cm^2 to 148.8 ± 47.8 cm^2; $p < 0.01$) compared to a mean 49.2 ± 25.6 cm^2 reduction in the cTEMS+LC group (190.0 ± 47.8 cm^2 to 140.9 ± 38.0 cm^2; $p < 0.0001$).

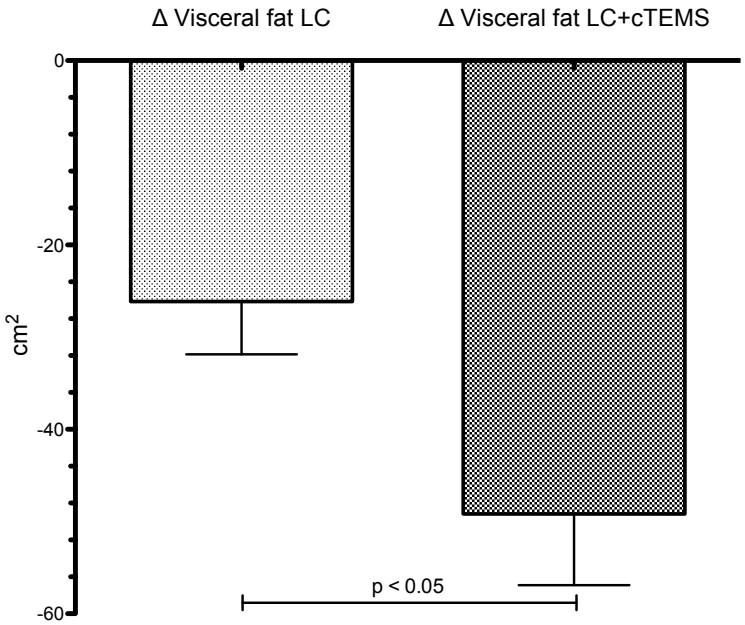

Figure 1 Changes in visceral fat area

Reported in table 2. Mean absolute change in visceral fat area from baseline to follow-up. P-values calculated with two-sided independent sample t-test with Welch's correction for unequal variances. Error bars denote standard deviation.

Both intervention groups experienced significant within group changes in body composition as shown in table 2. In the LC-group we found a weight reduction from 101.6 ± 15.7 kg to 95.1 ± 15.8 kg ($p < 0.0001$) and a reduction in body mass index from 33.4 ± 4.1 kg/m^2 to 31.3 ± 4.1 kg/m^2 ($p < 0.0001$). Further, mean fat mass was reduced by 4.8 ± 3.0 kg (42.0 ± 7.5 kg to 37.2 ± 8.5 kg; $p < 0.001$) with a corresponding reduction in mean fat percentage by 2.5 ± 2.1 % (41.5 ± 6.2 % to 39.0 ± 7.1 %; $p < 0.01$). Absolute

lean mass was reduced from 56.7 ± 12.7 kg to 55.4 ±12.7 ($p < 0.01$), but the lean percentage actually increased from 55.2 ± 6.1 % to 57.5 ± 7.1 % ($p < 0.01$).

In the the cTEMS+LC group we found similar results and with no significant between-group differences. Mean body weight was reduced from 101.4 ± 14.4 kg to 93.0 ± 12.0 kg ($p< 0.0001$) and mean body mass index from 33.1 ± 4.6 kg/m^2 to 30.4 ± 4.2 kg/m^2 ($p < 0.0001$). Fat mass was reduced from 41.1 ± 10.5 kg to 35.2 ± 10.3 kg ($p < 0.0001$) and fat percentage from 40.4 ± 8.5 % to 37.4 ± 9.5 % ($p < 0.001$). Similar to the diet-alone group, we also found a reduction in lean mass from 57.4 ± 11.4 kg to 55.6 ± 10.8 kg ($p < 0.05$) but an increase in lean percentage from 56.3 ± 8.3 % to 59.1 ± 9.2 % ($p < 0.01$).

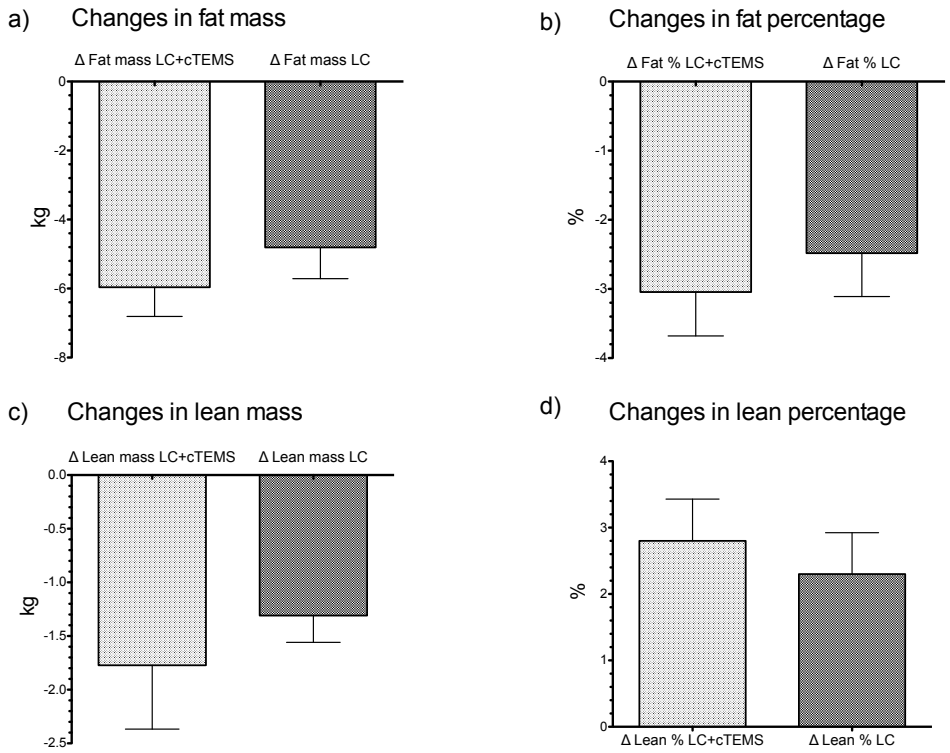

Figure 2 Changes in body composition

Reported in table 2. Mean absolute change in body composition measurements from baseline to follow-up. None of the changes were found to differ significantly between the intervention groups tested with two-sided independent sample t-test with Welch's correction for unequal variances. Error bars denote standard deviation.

Table 2 Body Composition at baseline and follow-up

		Low-carbohydrate + cTEMS group			Low carbohydrate group			
Parameter	**Time**	**Mean**	**Absolute change**	**Within group**	**Mean**	**Absolute change**	**Within group**	**Comparison of groups**
Body weight (kg)[1]	Baseline Follow-up	101.4±14.4 93.0 ±12.0	-8.5±3.4	p<0.0001	101.6±15.7 95.1±15.8	-6.5±3.0	p<0.0001	p=0.156
Body mass index (kg/m²)[1]	Baseline Follow-up	33.1±4.6 30.4±4.2	-2.7±1.0	p<0.0001	33.4±4.1 31.3±4.1	-2.1±1.0	p<0.0001	p=0.194
Fat mass (kg)[1]	Baseline Follow-up	41.1±10.5 35.2±10.3	-5.9±2.8	p<0.0001	42.0±7.5 37.2±8.5	-4.8±3.0	p<0.001	p=0.367
Fat percentage (%)[1]	Baseline Follow-up	40.4±8.5 37.4±9.5	-3.0±2.1	p<0.001	41.5±6.2 39.0±7.1	-2.5±2.1	p<0.01	p=0.535
Lean mass (kg)[1]	Baseline Follow-up	57.4±11.4 55.6±10.8	-1.8±2.0	p<0.05	56.7±12.7 55.4±12.7	-1.3±0.8	p<0.01	p=0.471
Lean percentage (%)[1]	Baseline Follow-up	56.3±8.3 59.1±9.2	+2.8±2.1	p<0.01	55.2±6.1 57.5±7.1	+2.3±2.1	p<0.01	p=0.570
Visceral fat area (cm²)[2]	Baseline Follow-up	190±48 141±38	-49±26	p<0.0001	175±42 149±48	-26.±19	p<0.01	p=0.027

Body composition parameters at baseline and follow-up expresses as mean±SD with mean absolute change±SD
p-values for within group differences by two-sided paired t-test.
p-values for comparison of groups by two-sided independent sample t-tests with Welch's correction for unequal variances.
[1] Measured by Dual Energy x-ray absorptiometry (DEXA)
[2] Estimated by Bioelectrial impedance measurement

Exercise testing

The respiratory exchange ratio attained was reduced in both groups, but significantly more after LC-cTEMS for 10 weeks. There was no difference in the mean peak oxygen uptake. In the LC group absolute oxygen uptake (3.0 ± 0.7 l/min to 3.1 ± 0.7 l/min; p=0.063) and maximal load (231 ± 60 watt to 236 ± 59 watt; p=0.222) remained unchanged with similar findings (absolute oxygen uptake 3.1 ± 0.7 l/min to 3.0 ± 0. l/min; p=0.09 and and maximal load 232 ± 58 watt to 223 ± 47 watt; p=0.391) observed in the LC+cTEMS group. The respiratory exchange ratio was 4.1 % lower in the LC group, from 1.20 ± 0.05 to 1.15 ± 0.05 (p=0.021), and 9.3 % lower in the LC+cTEMS group from 1.25 ± 0.07 to 1.14 ± 0.09 (p<0.001) resulting in a significant greater RER-reduction in the LC+cTEMS group (p=0.028) compared to controls.

At a workload of 100W we observed a significant 5% reduction in energy expenditure in the LC+cTEMS group from 464 ± 19 kcal/h to 442 ± 15 kcal/h (p<0.05). There was no change in the LC group (457 ± 24 kcal/h to 454 ± 20 kcal/h; p=0.630) and the difference between the groups was found to be significant with a p-value < 0.05. The reduction in energy expediture was accompanied by a significant (p< 0.05) between-group difference in the change in oxygen uptake at 100 W. While there was a trend towards a reduction in $VO_{2\ 100W}$ in LC+cTEMS (1.58±0.06 l/min to 1.54±0.05 l/min; p=0.070) there was no change in the LC alone group (1.57±0.08 l/min to 1.58±0.06 l/min;p=0.448).

When measuring muscle strength with 1RM, none of the groups showed any significant changes from baseline to follow-up. 1RM went from 189 ± 48 kg to 189
± 45 kg (p=0.376) in the LC group and from 176 ± 9 kg to 180 ± 6 kg (p=0.156= in the LC+cTEMS group.

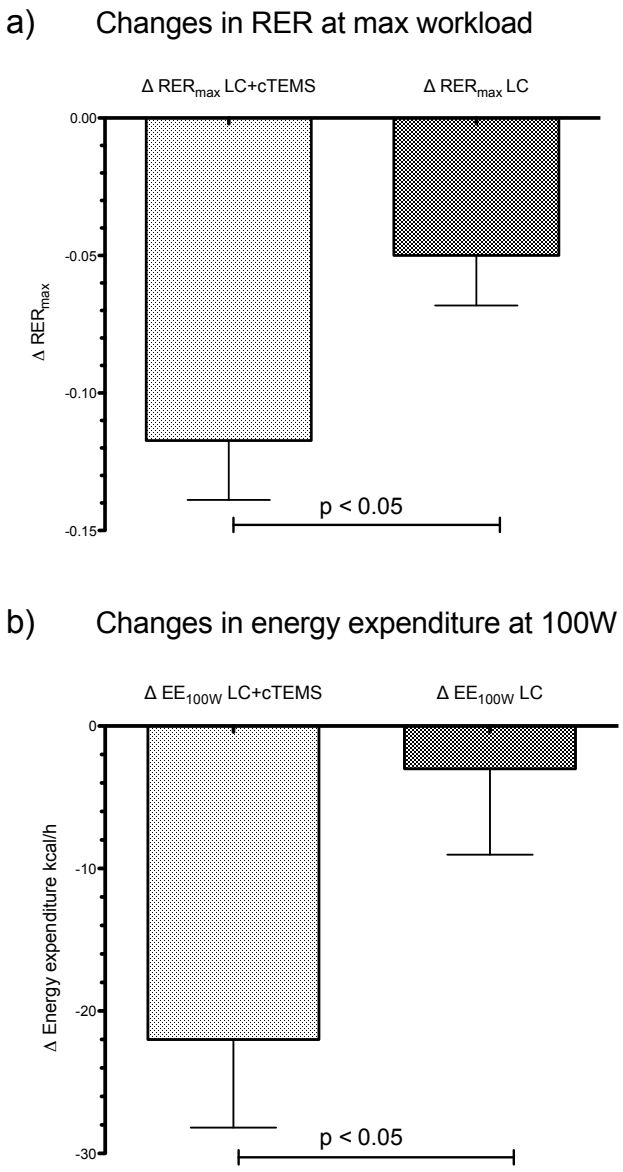

Figure 3 Changes in respiratory exchange ratio at maximum load and energy expenditure at 100 W

Mean absolute change in EE_{100W} and RER_{max} during treadmill test from baseline to follow-up between intervention groups. P-values calculated with two-sided independent sample t-test with Welch's correction for unequal variances. Error bars denote standard deviation.

Nutritional data

As shown in table 3, there was no significant between-group differences in the dietary intervention in the two groups. Energy intak was reduced by 826 ± 800 kcal in the LC+cTEMS group (from 2625 kcal ± 873 kcal to 1798 ± 417 kcal; $p < 0.01$) and by 850 ± 592 kcal (from 2560 ± 472 kcal to 1709 ± 362 kcal; $p < 0.001$) in the LC group. The percentage share of total energy intake of carbohydrates, fat and protein respectively, changed with -43 ± 14 % ($p < 001$), +37 ± 12% ($p < 0.001$) and +6 ± 7 % ($p = 0.020$) in the LC+cTEMS group and with -40 ± 10 % ($p < 0.001$) , +31 ± 8 % ($p < 0.001$) and +9 ± 8 % ($p < 0.01$) in the LC group. The total intake of fat in the increased significantly by 46 ± 52 g from 103 ± 38 g to 149 ± 41 g ($p < 0.05$) in the LC+cTEMS group. In the LC group there was a trend toward a increase in total fat intake by 26 ± 53 g (from 97 ± 38 g to 12 ± 29 g; $p = 0.134$). While carbohydrate intake was strongly reduced in both groups (from 315 ± 178 g to 16 ± 7 g in the LC+cTEMS-group [$p < 0.0001$] and from 262 ± 108 g to 19 ± 6 g in the LC-group [$p < 0.0001$]) dietary fiber intak was reduced (for LC+cTEMs -17 ± 9 g and for LC -11 ± 7 g ; both $p < 0.001$). There was no absolute changes in the intake of protein (97 ± 26 g vs. 95 ± 21 [p=0.814] in LC+cTEMS and 93 ± 34 g vs 147 ± 152 g [p=0.237] in LC).

Lipids and lipoproteins

As shown in table 4, we found no significant between-group differences in lipids or lipoproteins.

Blood chemistry and biomarkers

In the LC+cTEMS group there was significant reductions in plasma glucose (6.0 ± 0.8 to 5.4 ± 0.7 mmol/l; $p<0.001$), serum insulin (14.0 ± 16.3 to 9.8 ± 13.1 pmol/l; $p < 0.01$, HOMA-IR (4.0 ± 5.3 to 2.6 ± 3.7; $p < 0.05$) and c-peptide (0.89 ± 0.32 to 0.69 ± 0.27 nmol/l; $p < 0.01$). Corresponding findings were not found in the LC group (5.5 ± 0.7 to 5.4 ± 0.4 mmol/l; p=0.677 for plasma glucose, 7.4 ± 7.0 to 5.1 ± 3.4 pmol/l; p=0.237 for insulin, 1.8 ± 1.6 to 1.2 ± 1.6; p=0.223 for HOMA-IR and 0.72 ± 0.26 to 0.64 ± 0.14 nmol; p=0.255 for c-peptide). HbA_1C was significantly reduced in both groups, from 5.8 ± 0.7 to 5.5 ± 0.5 ($p<0.01$) in LC+cTEMS and 5.5 ± 0.3 to 5.4 ± 0.4 ($p<0.05$) in LC. We observed a significant fall in gamma-glutamyl-transferase (GT) from 36.7 ± 36.9 to 30.7 ± 33.4 U/l ($p<0.001$) in the LC+cTEMS group but not in the LC group, 54.9 ± 96.7 to 31.2 ± 45.9 (p=0.156), while alanine-amino-transferase (ALAT) was unchanged in both groups.

Table 3 Nutritional data

		Low-carbohydrate + cTEMS group			Low carbohydrate group			
Parameter	**Time**	**Mean**	**Absolute change**	**Within group**	**Mean**	**Absolute change**	**Within group**	**Comparison of groups**
Energy intake (kcal)	Baseline Follow-up	2625±873 1798±417	-826±800	p<0.01	2650±472 1709±362	-850±592	p<0.001	p=0.939
Carbohydrate E%	Baseline Follow-up	47±13 4±2	-43±14	p<0.0001	45±10 5±2	-40±10	p<0.0001	p=0.575
Protein E%	Baseline Follow-up	16±5 23±5	6±7	p<0.05	17±5 26±4	9±8	p<0.01	p=0.395
Total fat E%	Baseline Follow-up	37±10 74±6	37±12	p<0.0001	38±7 69±5	31±8	p<0.0001	p=0.180
Fat intake total (g)	Baseline Follow-up	103±38 149±41	46±52	p<0.05	97±38 123±29	26±53	p=0.134	p=0.450
Carbohydrate (g)	Baseline Follow-up	315±178 16±7	-299±180	p<0.001	262±108 19±6	-242±109	p<0.0001	p=0.387
Dietary fiber (g)	Baseline Follow-up	24±8 7±6	-17±9	p<0.001	17±6 6±5	-11±7	p<0.001	p=0.109
Protein (g)	Baseline Follow-up	97±26 95±21	-2±27	p=0.814	93±34 147±152	54±143	p=0.237	p=0.237

Nutritional composition of diet at baseline and follow-up expresses as mean±SD with mean absolute change±SD
p-values for within group differences by two-sided paired t-test.
p-values for comparison of groups by two-sided independent sample t-tests with Welch's correction for unequal variances.

E%: Percentage of total energy intake

Table 4 Lipids and lipoproteins

Parameter	Time	Low-carbohydrate + cTEMS group			Low carbohydrate group			Comparison of groups
		Mean	Absolute change	Within group	Mean	Absolute change	Within group	
Total Cholesterol (mmol/l)	Baseline Follow-up	4.9±0.8 5.5 ±1.9	+0.6±1.2	p=0.146	5.0±0.9 5.6±1.7	+0.5±3.0	p=0.171	p=0.918
HDL-Cholesterol (mmol/l)	Baseline Follow-up	1.2±0.3 1.3±0.3	+0.1±0.2	p<0.05	1.3±0.4 1.3±0.4	0.0±0.2	p=0.437	p=0.251
LDL-Cholesterol (mmol/l)	Baseline Follow-up	3.3±0.7 3.9±1.7	+0.6±1.2	p=0.153	3.3±0.7 4.0±1.6	+0.6±1.2	p=0.101	p=0.885
TC/HDL [1]	Baseline Follow-up	4.4±1.2 4.3±1.3	-0.1±1.0	p=0.672	4.3±1.6 4.4±1.5	+0.1±1.3	p=0.722	p=0.584
Non-HDL Cholesterol (mmol/l)	Baseline Follow-up	3.7±0.7 4.2±1.8	+0.5±1.2	p=0.252	3.8±0.9 4.3±1.6	+0.5±1.2	p=0.216	p=0.946
ApoA1 (mmol/l)	Baseline Follow-up	1.31±0.20 1.33±0.16	+0.02±0.14	p=0.652	1.41±0.27 1.39±0.22	-0.02±0.15	p=0.630	p=0.504
ApoB (mmol/l)	Baseline Follow-up	0.94±0.16 1.03±0.33	+0.09±0.23	p=0.245	0.96±0.21 1.10±0.35	+0.15±0.23	p=0.060	p=0.549
ApoB/ApoA1	Baseline Follow-up	0.74±0.17 0.78±0.23	+0.04±0.21	p=0.520	0.70±0.20 0.81±0.27	+0.11±0.17	p=0.054	p=0.398
Triglycerides (mmol/l)	Baseline Follow-up	1.2±0.5 0.9±0.4	-0.4±0.3	p<0.01	1.3±0.9 0.9±0.4	-0.4±0.7	p=0.087	p=0.780
VLDL+CM particles (nmol/l) [2, 3]	Baseline Follow-up	66.7±23.8 51.3±32.0	-15.4±21.4	p<0.05	56.4±32.1 54.2±38.6	-2.2±42.8	p=0.869	p=0.374
VLDL size (nm) [3]	Baseline Follow-up	47.4±7.1 41.9±6.2	-5.4±4.0	p<0.01	48.4±6.4 43.1±5.4	-5.3±7.5	p<0.05	p=0.924
LDL particles (nmol/l) [3]	Baseline Follow-up	1356±392 1267±367	-89±421	p=0.498	1415±444 1386±422	-29±345	p=0.786	p=0.648
LDL size (nm) [3]	Baseline Follow-up	20.8±0.9 21.6±0.9	+0.7±1.0	p<0.05	20.9±0.7 21.4±0.8	+0.5±0.6	p<0.05	p=0.579
Lp(a) (nmol/l)	Baseline Follow-up	39.1±27.2 30.2±26.2	-8.9±9.8	p<0.05	42.1±66.2 37.6±62.5	-4.5±18.3	p=0.438	p=0.717

Lipid and lipoprotein measurements at baseline and follow-up examination expresses as mean±SD and with absolute change±SD.
p-values for within group differences by two-sided paired t-test.
p-values for comparison of groups by two-sided independent sample t-tests with Welch's correction for unequal variances.
[1]Ratio between total cholesterol and HDL-cholesterol
[2] Total VLDL and Chylomicron particles
[3] Measured by Nuclear Magnetic Resonance

There was no significant between-group difference for any of the measured blood chemistry and biomarker variables.

Activity level measurement

Activity level was measured for one week during the intervention period using a triaxial accelerometer. LC+cTEMS spent 79.4 ± 3.8 % with sedentary activity, 18.3 ± 3.8 % with light, 2.2 ± 1.6 % with moderate and 0.0 ± 0.1 % with heavy activity while the LC group spent 77.2 ± 3.8 % with sedentary, 21.1 ± 3.9 with light, 1.6 ± 0.4 % with moderate and no time with heavy activity. There were no significant differences between the groups for any of the activity levels.

Discussion

In this study we investigated potential additive effects of cTEMS in dieting obese subjects. In both treatment groups the low-carbohydrate-high-fat diet resulted in a significant weight loss, and a subsequent reduction in body mass index, fat mass and fat percentage, but without significant between-group differences. The reduction in visceral fat accumulation however, was significantly higher in the group receiving both electrical stimulation and diet, corresponding to a 26% reduction in LC+cTEMS compared to 16% with LC alone. Furthermore, the larger reduction in respiratory exchange ratio, accompanied by unchanged peak oxygen uptake, indicates an increase in aerobic capacity per workload.

We have previously shown that regular electrical muscle stimulation in non-dieting obese subjects increases aerobic exercise capacity [13]; an effect most likely caused by improved aerobic-oxidative capacity in skeletal muscle and not by improved hemodynamics [14]. In this respect, EMS differs somewhat from the effects of endurance training where there is a close link between changes in $VO_{2\ max}$ and changes in cardiac stroke volume [15].

When a low carbohydrate high fat diet is followed over time, a fall in RER indicates an adaptation to the diet, with a shift in substrate utilization towards a increased fatty acid oxidation [16]. This is the most probable explanation for the RER-reduction in the control group at maximal workload. In the LC+cTEMS group however, we observed a further reduction in RER in addition to the dietary effect. Although we did not observe any increase in $VO_{2\ peak}$ in this group, the difference in RER is likely to be explained by EMS

induced training effects in skeletal muscle. Metabolic flexibility, the ability of the skeletal muscle to switch between fat and carbohydrate oxidation is known to be increased by regular exercise [17,18]. Our data indicates a similar effect induced by EMS.
Electrical muscle stimulation did not confer extra weight loss. This is however not contradictory to our finding of increased aerobic capacity.

In order to compare possible changes in metabolic rate at a specific workload, we analysed respiratory gases at a workload of 100 W during treadmill testing. Using the Weir equation[19] we calculated the energy expenditure at baseline and follow-up examination. cTEMS induced a significant 5 % efficiency improvement in the energy consumption at the 100W workload, indicating an improved muscular efficiency.
This finding point out one possible explanation for the lack of weight loss induced by increased physical activity in general. A premise to avoid the often-observed regain of weight after a diet+exercise intervention[20] is to maintain a higher activity level, and thus energy expenditure, to compensate for the improved energy efficiency.
Jabekk et al. found that in untrained women, a combination of a ketogenic low-carbohydrate diet and resistance exercise reduced body fat significantly without a loss in lean body mass [5]. Women, who did resistance training alone however, increased their lean body mass without any significant change in body fat. Further, in older adults, a combination of weight-reducing diet and regular exercise results in a greater improvement in physical function than either intervention alone combined with a greater reduction in body weight and a smaller reduction in lean body mass [21]. In this population, there was no decrease in body weight by exercise alone.

Our findings indicate an additional reduction in visceral fat accumulation induced by the electrical muscle stimulation. There is an on-going debate about exercise and visceral fat reduction [22] and the hypothesis that physical activity interventions preferentially is targeting VAT compared to dietary interventions has gained support in previous studies [8,23,24]. However, in a 2008 review from Chaston et al. they found no evidence of a greater relative reduction in VAT from energy deficit associated with increased physical activity [25]. Further, Ohkawara et al. found evidence of a relationship between the time and intensity of the exercise and the reduction of visceral fat [26] in their review of randomized controlled trials examining the effect of exercise on visceral fat. At least 10 MET hours per week were suggested to enable visceral fat reduction. We have

previously found the maximum response induced by cTEMS to be around 2.8 MET's, indicating a maximum of 6.8 MET's per week from cTEMS in our study. Thus, it is still unclear if the exercise effect of cTEMS fully or only in part induced the increased reduction of intraabdominal fat. Visceral fat measurements may not be comparable between studies as methods may differ. Although Computed Tomography (CT) remains the "gold standard" for measuring visceral fat, new, more convenient and non-radiating methods are now available. Estimating visceral fat area with multi-frequency bioelectrical impedance analysis has shown to correlate significantly with CT[12] and was used in our study.

The diet was initially restricted only in carbohydrates, but as seen in studies with a similar dietary intervention [5,27-29], despite ad libitum feeding, the subjects also restricted their caloric intake during the intervention period. cTEMS did not influence the caloric intake, as there was no difference in the energy reduction between the two groups. Whether this caloric reduction is a result of reduced nutritional options, reduced appetite due to dietary ketosis [30], central insulin-leptin interaction [31] reduced food-reward [32] or a combination of all these mechanism, still remain unanswered.

There were no differences between the treatment groups in blood lipids. However, the LC+cTEMS group displayed a significant increase in HDL-cholesterol, a significant reduction in triglycerides and a significant reduction in the number of VLDL and chylomicron particles, all changes that did not occur with significance in the control group. We do not know if this indicates a possible cardiovascular risk reduction. The finding is however interesting, also in the context of the visceral fat reduction, and should be studied further.

In conclusion, the present study showed a greater reduction of visceral fat in dieting obese subjects, when electrical muscle stimulation was used as an adjunct to the weight reducing intervention. Further, our data suggest that electrical muscle stimulation confers more efficient muscle work resulting in improved energy efficiency during physical activity. Our findings may have significant implications for weight reducing interventions, especially in subjects not able to take part in regular physical exercise. Further studies are necessary to investigate the underlying mechanisms of the present findings.

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