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Non-invasive cooling wear as an effective means of reducing subcutaneous adipose tissue mass: an *in-vivo* study

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Abstract

Objective: The increasing prevalence of obesity is a major health problem worldwide. Several non-surgical treatments are now available that reduce body and subcutaneous fat mass. We aimed to determine the efficacy of mild cold for body mass reduction.

Methods: Novel cooling wear, which induces mild cooling *via* evaporation, was worn by 29 women with overweight for 4 weeks. Specifically, the participants wore a cooling waist belt and chaps for 1 hour per day. Non-invasive lipometry was used to determine their subcutaneous adipose tissue thicknesses, and the total weight loss, abdominal circumference, and body mass index (BMI) of the participants were measured.

Results: The participants achieved a significant total weight loss of 0.7 kg (0.9%), and significant reductions in BMI (0.2 kg/m^2) and abdominal circumference (1.9 cm, 1.7%). Furthermore, there was a trend towards a reduction in abdominal subcutaneous fat thickness and a significant reduction in thickness of the anterior thigh was noted. A questionnaire-based evaluation indicated high usability and comfort of the cooling wear.

Conclusion: There is a high and growing demand for non-invasive treatment strategies for obesity. Cooling wear represents a novel and promising approach that may be of particular use for individuals who do not require bariatric surgery.

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Introduction

The increasing prevalence of obesity represents a major worldwide health problem in adults, children and adolescents.[1] In patients with severe obesity, bariatric surgery is used as the ultimate means of achieving sufficient long-term weight loss.[2] However, despite the reduction in the incidence of postoperative wound healing disorders associated with the development of minimally invasive techniques, the incidences of peri- and postoperative complications of bariatric surgery should not be underestimated.[3] For patients aiming to achieve a moderate reduction in adipose tissue mass, a number of non-surgical modalities have become available in recent years, including cryolipolysis, radiofrequency, low-energy laser therapy and high-intensity focused ultrasound.[4] These techniques cause apoptosis or necrosis of the targeted adipocytes through different mechanisms of action. Of these, cryolipolysis has demonstrated great potential for the reduction of subcutaneous fat mass, without causing damage to adjacent tissues.[4] However, although it is a non-invasive technique, the sub-zero temperatures used can be associated with adverse events, such as pain, a stinging sensation and swelling or bruising at the treatment site, and therefore it is not well-tolerated by every patient.[5] To date, the mechanisms whereby subcutaneous fat reduction occurs following cryolipolysis have not been completely elucidated. However, the application of less extreme temperatures has been shown to either activate brown adipose tissue (BAT)[6] or stimulate subcutaneous adipose tissue (SAT) browning to form so-called "beige" adipocytes.[7] Both processes are associated with weight loss and several previous studies have investigated the potential for the use of cold-induced BAT activation or adipose browning in the treatment of obesity.[7,8] These studies have demonstrated that exposure to mild cold may stimulate subcutaneous fat tissue but have fewer systemic side effects than the use of excessive cold in humans.[9] Thus, modalities that mimic the activation of SAT by mild cold may provide a promising, non-invasive approach to the treatment of obesity that minimizes the incidence of complications.[9]

In the present study, we aimed to assess the effect of mild cold on SAT mass in human volunteers. Novel cooling wear, which generates mild cold *via* evaporation and was originally created for use by people working jobs at high ambient temperature, was used to apply mild cold to the belly and thigh regions of women, in an attempt to activate BAT and induce adipocyte browning.[10,11]

Methods

Participants

Female participants were enrolled according to the following inclusion criteria: body mass index (BMI) between 25 and 30 kg/m^2 and age 18 to 65 years. The exclusion criteria were: current pregnancy,

previous surgery in the abdominal region and inability to fully understand the study procedures or to provide informed consent. The study was conducted between August 2020 and March 2021 at the Medical University of Graz, Austria, in collaboration with Joanneum Research Forschungsgesellschaft mbH, Graz, Austria. The study design and protocol were approved by the ethics review committee of the Medical University of Graz (EK: 32-464 ex 19/20). Written informed consent was obtained from all the participants.

Cooling-wear

The tested cooling-wear, "SlimCOOL", which comprised waist belts and chaps, was provided by Pervormance International GmbH (Ulm, Germany).[12] The cooling effect of the waist belt and chaps is activated by holding them under tap water, which has a subsequent evaporative cooling effect while being worn. The high-tech material holds water in the interior of the garment or directly on the non-woven fibres, while the outer fabric remains dry. The normal body heat causes the water molecules bound in the material to evaporate, causing comfortable cooling to a surface temperature of approximately 18°C to 20°C.[12]

Experimental Design

The participants were instructed to wear a cooling waist belt and chaps for 1 hour each day for 4 weeks at any convenient time. Non-invasive lipometry was performed to determine the SAT thickness [mm] in the abdominal region at the start of the study, and 2 and 4 weeks later. Body mass was measured to define the total weight loss (TWL) and abdominal circumference was measured at the beginning of the study and 4 weeks later. The participants were also asked to complete questionnaires at the end that evaluated their well-being and abdominal circumference measurements during the study. Appendix Figure 1 shows a schematic overview of the study design.

Measurements

Lipometry. Non-invasive assessments of the abdominal SAT thickness [mm] and whole-body fat topography were conducted using a patented optical device (a Lipometer; European Patent No.: EP: 0516251).[13–15] Figure 1 provides a schematic diagram of the 15 anatomically defined measurement points across the entire body and within the abdominal region. Body fat percentage (BFP)

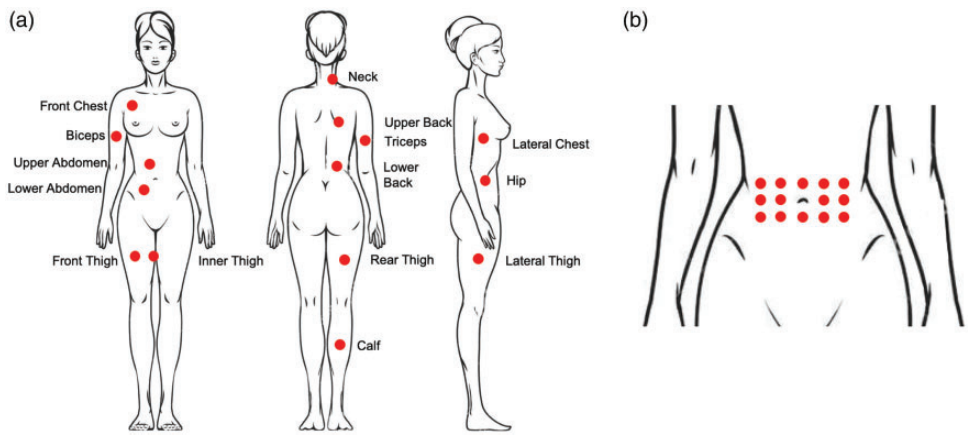

Figure 1. Schema of the anatomically defined whole-body lipometry measurement locations (a) and specific measurement locations within the abdominal region (b).

was calculated using these 15 SAT thickness measurements, together with age, height, body mass and BMI as independent variables.

Abdominal circumference measurement. Abdominal circumference was measured using a flexible measuring tape every third day by the participants themselves. To ensure the reliability of the data obtained, the participants were carefully instructed by the study team regarding the method of measurement. Their abdominal circumferences were also measured by a study physician at each outpatient visit. For each measurement, the tape was placed horizontally directly above the umbilicus, between the lower costal arch and the iliac crest.

Questionnaire. The participants completed a questionnaire regarding their well-being, progress and management during the study at the last study visit. They were also encouraged to not drastically alter their lifestyle, specifically with respect to their diet and activity, so as not to interfere with the assessment of the efficacy of the cooling wear. The exercise, nutrition and hydration of the participants were documented in a diary, which was checked at each study visit.

Statistical analysis

The data were analysed using Prism software (version 9.0.2; GraphPad Software, Inc., San Diego, CA, USA). The paired Student's *t*-test was used to compare the body mass and BMI of the participants at baseline and after 4 weeks of the intervention. One-way ANOVA with repeated measures was used to compare continuous datasets among the three time points of baseline, 2 weeks and 4 weeks, and Tukey's *post-hoc* test was subsequently used when significant main effects were present. All the statistical tests were two-tailed and differences were considered to be statistically significant when $p < 0.05$.

Results

To investigate the effect of the cooling wear on the body mass, abdominal circumference and SAT of women with overweight, 29 women with a mean age of 39.5 years (standard deviation (SD) 13.4) and a mean BMI of 27.1 (1.8) kg/m^2 were recruited. None of the participants said that they significantly altered their lifestyle during the study and no side effects were reported. One patient was excluded after 4 weeks of the study because of COVID-19 infection, and therefore her data were excluded from the statistical analyses.

Four weeks of daily cooling wear application for 1 hour induced significant weight loss ($p = 0.005$) and a significant reduction in BMI ($p = 0.005$) *versus* baseline. The mean TWL after 4 weeks was 0.7 kg, which represents a reduction in body mass of 0.9%, and the mean BMI decreased by 0.2 kg/m^2 (0.8%) (Figure 2a, b). The mean abdominal circumference decreased by 1.9 cm (1.7%; $p < 0.001$) over the 4-week study period (Figure 2c). Only abdominal circumference ($p < 0.001$) and total BPF ($p = 0.03$) demonstrated significant reductions, of 0.8 cm (0.9%) and 0.5%, respectively, during the first 2 weeks of the study. These data are summarized in Table 1.

To determine the share of the TWL that could be accounted for by a loss of body fat, we used a hand-held lipometer to measure the thickness of the fat layer below the cooling wear. The BFP was significantly lower after 4 weeks of daily cooling wear application (1.5%; $p = 0.02$) (Figure 3a). There was no significant reduction in the thickness of the abdominal SAT layer at the specific measurement points during the 4 weeks of the study (Figure 3b), but there was a trend towards a reduction (Figure 3).

A detailed analysis of the body fat distribution over the covered areas (abdomen, hips and thighs) was performed. Table 2 shows the mean SAT thicknesses over the

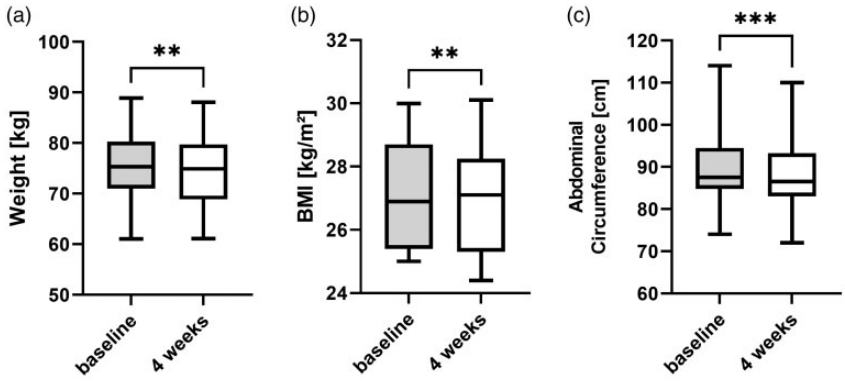

Figure 2. Cooling wear treatment for 1 hour/day for 4 weeks reduces body mass (a), body mass index (BMI) (b) and abdominal circumference (c). n = 29. $^{**}p < 0.01$, $^{***}p < 0.001$ (one-way ANOVA with followed by Tukey's post-hoc test or Student's *t*-test, as appropriate).

Table 1. Overview of the body mass, BMI, abdominal circumference, BFP and abdominal SAT thickness after 2 and 4 weeks of cooling wear application for 1 hour/day.

N = 29	Baseline Mean (±SD)	2 weeks Mean (±SD)	4 weeks Mean (±SD)	*P*-value for baseline *vs.* 2 weeks	*P*-value for 2 weeks *vs.*4 weeks	*P*-value for baseline *vs.* 4 weeks
Age [years]	39.5 (13.4)					
Body mass [kg]	75.2 (±7.6)		74.5 (±7.5)			0.005**
BMI [kg/m^2]	27.1 (±1.8)		26.9 (±1.7)			0.005**
Abdominal circumference [cm]	90.0 (±8.0)	89.2 (±7.9)	88.1 (±7.6)	<0.001***	<0.001***	<0.001***
Total BFP [%]	33.4 (±6.9)	32.9 (±6.5)	31.9 (±5.9)	0.41	0.03*	0.02*
Abdominal SAT thickness [mm]	12.7 (±3.4)	12.6 (±3.9)	12.5 (±3.5)	0.8535	0.5750	0.9342

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.
SD, standard deviation; BMI, body mass index; BFP, body fat percentage; SAT, subcutaneous adipose tissue.

abdomen, hips and thighs. These areas tended to show reductions in thickness, but only the anterior thigh SAT thickness significantly decreased (by 0.8 mm; 8.5%) between weeks 2 and 4 ($p = 0.03$).

Questionnaire data

Because comfort and ease improve compliance with weight-reducing interventions, the participants were asked to complete a questionnaire at their last visit regarding the comfort and use of the cooling wear. After 4 weeks of wearing it, 63% of the participants stated that the activation of the cooling wear with water was easy, 57% that the cooling wear was comfortable, 87% that the temperature was comfortable and 80% that the protocol was manageable during their everyday life. Regarding their progress, 70% reported a 1- to 2-cm decrease in abdominal circumference and 27% described a change in the appearance of the skin over the covered areas, such as an apparent strengthening

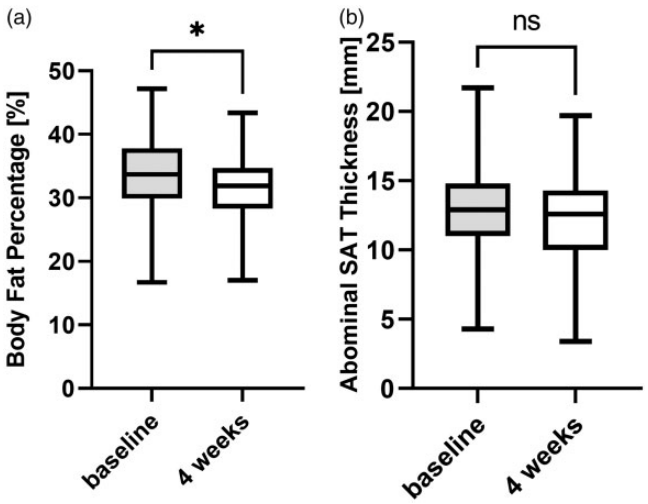

Figure 3. (a) Significant reduction in total body fat percentage induced by 1 hour/day cooling wear application for 4 weeks and (b) No significant reduction in abdominal subcutaneous adipose tissue (SAT) thickness was detected after cooling wear treatment for 4 weeks. *$p < 0.05$ (one-way ANOVA with followed by Tukey's post-hoc test or Student's *t*-test, as appropriate).

Table 2. Overview of the SAT thickness of the abdomen, hip and thigh regions after 2 and 4 weeks of cooling wear application for 1 hour/day.

N = 29	Baseline Mean (±SD)	2 weeks Mean (±SD)	4 weeks Mean (±SD)	*P*-value for baseline *vs.* 2 weeks	*P*-value for 2 weeks *vs.* 4 weeks	*P*-value for baseline *vs.* 4 weeks
Upper abdominal fat thickness [mm]	12.4 (±3.6)	12.2 (±3.4)	12.4 (±3.7)	0.8714	0.8544	0.9925
Lower abdominal fat thickness [mm]	12.2 (±3.7)	12.4 (±3.9)	11.8 (±3.9)	0.84	0.41	0.65
Hip fat thickness [mm]	14.3 (±5.5)	14.1 (±4.5)	13.7 (±4.6)	0.91	0.44	0.50
Anterior thigh fat thickness [mm]	9.3 (±2.1)	9.4 (±1.9)	8.6 (±2.0)	0.97	0.03*	0.13
Lateral thigh fat thickness [mm]	9.4 (±2.4)	9.2 (±2.5)	9.2 (±2.1)	0.85	0.99	0.89
Medial thigh fat thickness [mm]	10.0 (±2.5)	9.5 (±1.8)	9.0 (±2.3)	0.62	0.50	0.19
Posterior thigh fat thickness [mm]	6.8 (±1.8)	7.1 (±1.8)	6.7 (±1.8)	0.62	0.31	0.87

SD, standard deviation; BMI, body mass index; BFP, body fat percentage; SAT, subcutaneous adipose tissue. *$p < 0.05$ (one-way ANOVA with followed by Tukey's post-hoc test or Student's *t*-test, as appropriate).

of the tissue and a reduction in the appearance of cellulite. Figure 4 displays the results of the questionnaire completed at the final study visit.

Discussion

The worldwide increase in the prevalence of obesity represents a major health problem

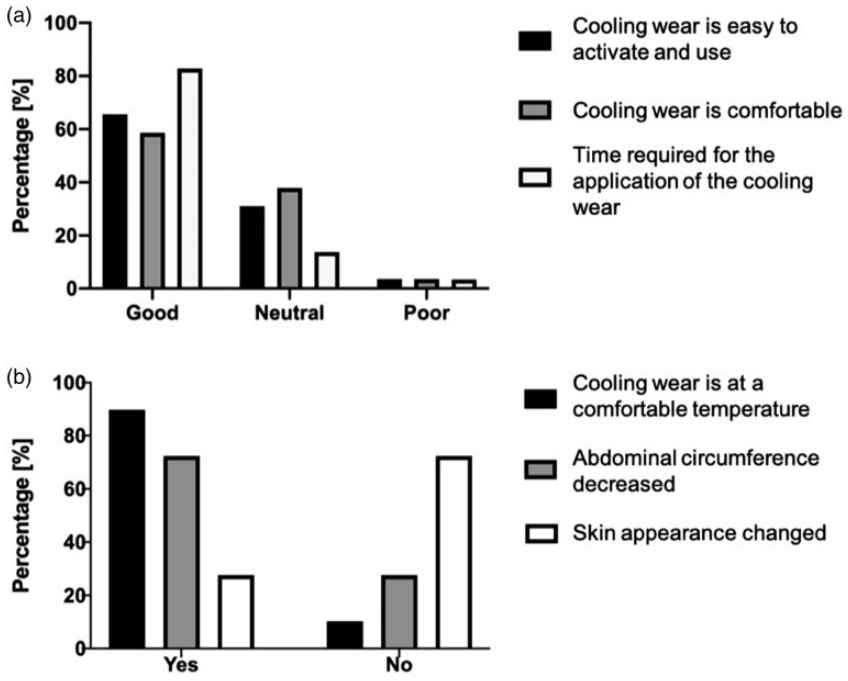

Figure 4. Results of the questionnaire regarding well-being, and the progress and management of the cooling wear application.

and has led to a large amount of research and the development of a variety of treatment strategies.[1,4] During recent years, great progress has been made in our understanding of the complexity of brown and beige adipose tissue, as well as their roles in metabolism and body mass regulation.[16] To date, several clinical studies have demonstrated reductions in the activity of BAT in individuals with overweight or obesity and shown that it can be activated through exposure to cold temperatures.[17] In the present study, we aimed to stimulate BAT and/or induce the browning of adipocytes using cold as a possible means of treating moderate obesity. We investigated the effects of mild cold, generated by evaporation, in the form of cooling wear, on the SAT of 29 women with moderate obesity. After 4 weeks of the daily application of mild cold for 1 h to the abdomen and upper thighs, a TWL of 0.9% was induced ($p = 0.005$), along with a significant reduction in BMI of 0.2 kg/m^2 (0.8%; $p = 0.005$).

A major goal of the treatment of obesity is the induction of sufficient weight loss, including when bariatric surgery is performed.[2] Surgical weight loss procedures also improve body composition and shape, and the physical function and fitness of patients with severe obesity over the long term.[2] However, for patients with moderate obesity, we have shown that a significant change in body composition can also be achieved using the non-invasive approach of the application of mild cold *via* cooling wear. After 4 weeks of regular application, a significant total BFP reduction of 1.5% ($p = 0.02$) and a trend towards a reduction in abdominal SAT thickness were identified. A statistically significant reduction of BFP was only detectable after 4 weeks,

which implies that BAT activation may have measurable results in the medium term. Longer term use of cooling wear might have more significant effects, and more pronounced changes in body shape within the treatment area may be noticed. After 2 weeks of regular application of the cooling wear, a reduction in abdominal circumference of 0.8 cm was induced, and this increased to 1.9 cm (1.7%) after 4 weeks ($p < 0.001$). Reductions in abdominal circumference of 1.34 cm to 6.86 cm have been achieved using other non-surgical fat-loss procedures, such as cryolipolysis, low-energy laser therapy, radio frequency, and high-intensity focused ultrasound.[18] However, to achieve satisfactory results using these techniques, several time-consuming sessions and substantial expenditure are often required.

In addition to the cosmetic aspects of obesity, it also underpins the metabolic syndrome, which also includes type 2 diabetes mellitus, hypertension and dyslipidaemia, and represents a major therapeutic challenge in the treatment of overweight and obesity.[19] The metabolic syndrome predisposes towards cardiovascular dysfunction and disease and is considered to be a major risk factor for perioperative morbidity and mortality in patients undergoing bariatric surgery.[19] Non-invasive techniques for weight- and fat reduction are popular alternatives to surgery for many patients who have moderate obesity or metabolic syndrome, because of the risks and financial expense associated with this.[20] However, non-invasive approaches can be associated with several unpleasant side effects.[5] Kennedy *et al.* reported a near 100% incidence of side effects, including erythema, oedema and dysaesthesia, connected with the treatment sites of cryolipolysis.[18] In contrast to the other non-invasive modalities that aim to reduce adipose tissue mass, the cutaneous side effects in the treatment area and adjacent tissues are less marked when mild cold is applied using cooling wear, while comparable results are achieved. In the present study, no side effects for connected with the application of mild cold were identified, and furthermore, the participants reported that high levels of usability and comfort are associated with this strategy.

Effective fat mass reduction may be feasible using this novel intervention. Patients with overweight or moderate obesity often have large subcutaneous fat deposits, but do not necessarily require bariatric surgery. This target group may particularly benefit from the further development and investigation of such cooling strategies, which are associated with well-tolerated weight and fat reduction.

Limitations

In the present study, we aimed to determine the effect of mild cold on the thickness of the SAT layer, which may be mediated through BAT activation or the induction of browning. Owing to the larger amount of BAT in women,[21,22] female participants with a BMI range of 25 to 30 kg/m^2 were enrolled. Thus, conclusions regarding the efficacy of mild cold for the treatment of patients with BMIs $> 30\,kg/m^2$ cannot be drawn. Further studies should be conducted to determine its effects in individuals with both lower and higher BMIs and in men. Even though all the participants reported that they had not changed their lifestyle during the study, there remains a potential bias in the study, because inclusion may have altered their health awareness. In addition, a placebo effect cannot be ruled out. Finally, it cannot be concluded with certainty that the effects were attributable to BAT activation or the induction of adipocyte browning. Further studies

of the roles of brown and beige adipose tissue in body mass regulation are required.

Conclusion

There is a high and growing demand for non-invasive treatment strategies for obesity. The use of cooling wear to activate BAT and induce adipocyte browning represents a novel and promising approach. This approach avoids the risks and costs associated with other non-surgical techniques, while inducing significant and comparable improvements in body mass, BFP and abdominal circumference. In patients that do not require bariatric surgery, such an effective method of reducing subcutaneous fat mass may be of particular interest. However, further clinical trials of the long-term efficacy and satisfaction associated with this strategy are required.

Author Contributions

HL and SPN conceived and designed the trial. HL, AH, CM and PK supervised the conduct of the trial and data collection. HL, MK and AD undertook the recruitment of the participants, managed the data, and performed quality control. SPN, MS and PK chaired the data oversight committee. HL drafted the manuscript, and all the authors contributed substantially to its revision. PK and L-PK take responsibility for the paper as a whole.

Declaration of conflicting interest

The cooling wear and funding were provided by Pervormance International GmbH (Ulm, Germany). The authors declare no other conflicts of interest.

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Appendix

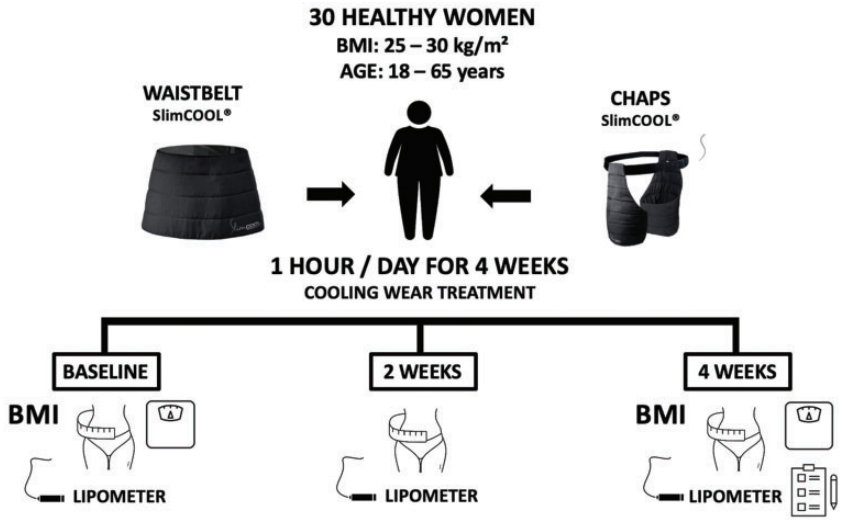

Figure A1. Schema of the study design. At baseline, BMI, body mass, abdominal circumference and subcutaneous fat thickness were measured. After 2 weeks of daily application of the cooling wear, abdominal circumference and subcutaneous fat thickness were measured. After 4 weeks, the participants' BMI, body mass, abdominal circumference and subcutaneous fat thickness were measured, and they completed a questionnaire.
BMI: body mass index; BFP: body fat percentage.