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A randomized trial of cold-exposure on energy expenditure and supraclavicular brown adipose tissue volume in humans

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ABSTRACT

Objective. To study if repeated cold-exposure increases metabolic rate and/or brown adipose tissue (BAT) volume in humans when compared with avoiding to freeze.

Design. Randomized, open, parallel-group trial.

Methods. Healthy non-selected participants were randomized to achieve cold-exposure 1 hour/day, or to avoid any sense of feeling cold, for 6 weeks. Metabolic rate (MR) was measured by indirect calorimetry before and after acute cold-exposure with cold vests and ingestion of cold water. The BAT volumes in the supraclavicular region were measured with magnetic resonance imaging (MRI).

Results. Twenty-eight participants were recruited, 12 were allocated to controls and 16 to cold-exposure. Two participants in the cold group dropped out and one was excluded. Both the non-stimulated and the cold-stimulated MR were lowered within the group randomized to avoid cold (MR at room temperature from 1841 ± 199 kCal/24 h to 1795 ± 213 kCal/24 h, $p = 0.047$ cold-activated MR from 1900 ± 150 kCal/24 h to 1793 ± 215 kCal/24 h, $p = 0.028$). There was a trend towards increased MR at room temperature following the intervention in the cold-group ($p = 0.052$). The difference between MR changes by the interventions between groups was statistically significant ($p = 0.008$ at room temperature, $p = 0.032$ after cold-activation). In an on-treatment analysis after exclusion of two participants that reported ≥8 days without cold-exposure, supraclavicular BAT volume had increased in the cold-exposure group (from 0.0175 ± 0.015 l to 0.0216 ± 0.014 l, $p = 0.049$).

Conclusions. We found evidence for plasticity in metabolic rate by avoiding to freeze compared with cold-exposure in a randomized setting in non-selected humans.

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1. Introduction

Brown adipose tissue (BAT) can activate non-shivering thermogenesis which turns glucose and fatty acids into heat. In rodents the volume and activity of BAT increase following prolonged stimulation by cold [1]. In humans it has been demonstrated that there is a tendency for obese people to have less activity in BAT and also that high age is related to lower non-shivering thermogenesis activity after stimulation [2–4]. However, most studies on BAT activity in humans have relied on radioactive markers such as fluorodeoxyglucose-PET with or without concomitant computer tomography (CT) [3–6]. Both these imaging techniques subject the participants to radiation, which limits the use of such investigations in healthy subjects. Magnetic resonance imaging (MRI) is an imaging technique which is devoid of radiation. BAT has a water content of about 50% which is intermediate compared with that of fat with has 10% water and muscle tissue having about 90% of water. This makes it plausible to use MRI to study BAT volume in humans [7–11] based on water content. The iron content of a particular tissue is also detectable by MRI, which has been the basis for functional magnetic resonance imaging (fMRI) as a means to detect and measure regional blood flow in tissues such as the human brain [12]. Similar techniques could potentially also be used for non-invasive and non-radioactive detection of blood flow and hence the activation of human BAT.

Since there is an inverse relationship between BAT activity and measures of obesity [13], and since activation of BAT can increase the energy expenditure, there is a great interest in how to increase BAT in order to lose body weight [13–16]. However, almost all trials on this theme have been conducted in animals, and little is known about plasticity of human BAT regarding both volume and activity. Data from observational studies in humans are suggestive of changes in BAT activity. Saito et al. demonstrated that BAT activity determined by PET CT was higher during periods of low outdoor temperatures compared with warm periods [4]. More specifically, a recent study by Yoneshiro et al. demonstrated that in young men who had been selected for little or no BAT activity, cold stimulation for 2 h a day at 17 °C during 6 weeks promoted development of visible BAT activity in all these subjects and that this also resulted in increased cold-induced activation of BAT [17].

We aimed to study whether basal metabolic rate, with or without acute cold-stimulation, could be affected by repeated cold exposure for 6 weeks in a randomized study in non-selected healthy men and women. We also aimed to see whether such stimulation would affect BAT volume and activation when assessed by MRI-based techniques which also gave information about potential changes in subcutaneous and visceral fat depots.

2. Methods

2.1. Recruitment and Intervention

The participants were recruited by local advertising at the University of Linköping. The subjects had to be free from known significant diseases as judged by medical check-up and history. Subjects were randomly assigned to either the cold (intervention) or warm group (controls) by drawing ballots. Since a higher drop-out rate was anticipated in the intervention group, 16 subjects were randomized to the cold-intervention and 12 to become controls. The study was conducted from February to April 2013 in Linköping, Sweden. Thus the study start was during the coldest time of the year in Sweden, at late winter, and progressed through very early spring. Participants randomized to the cold group were instructed to stay cool but to avoid shivering, for at least one hour a day every day for the study duration of six weeks. Examples of how this could be done were provided. For instance to sit by an open window or on a balcony with light clothing. Prolonged cold showers or baths were also suggested. The subjects in the cold group were asked to keep a diary on how they accomplished the cold-exposure, and also to grade how cold they had felt on these occasions. Participants of the control group were conversely instructed to stay warm for the duration of the study and to not, at any time, feel cold. They were encouraged to use long-johns and caps.

2.2. Metabolic Rate

Blood samples and measurements of metabolic rate were obtained at baseline and at the end of the study in the fasting state in the morning. A ventilated hood technique (Quark RMR, Cosmed, Finland) was used to measure metabolic rate, as based on oxygen inhalation and carbon dioxide exhalation i.e. indirect measurement of metabolic rate. The registration started with the subject being warm, wearing only underwear but covered with a blanket in a room with a temperature of about 21 °C. After 8–14 min, depending on when the metabolic rate had individually stabilized, the subject drank a glass of ice cold water (3 dl) and a cold vest, that had previously been fitted, was activated by starting the internal flow of cold tap-water. The total duration of the registration, including the acute cold stimulation, varied between 18 and 28 min. The mean values of the last five minute-based readings of metabolic rates during regular body temperature (before acute cold stimulation) were calculated and also the corresponding readings during acute cold exposure (but without shivering) after drinking the chilled water.

2.3. Laboratory Tests

Blood was drawn in the morning after a 10 h over-night fast. Standard laboratory tests such as plasma glucose, thyroid hormones, apolipoproteins and insulin were analyzed according to routines at Department of Clinical Chemistry at the Linköping University Hospital. The Milliplex® MAP Gut Hormone Panel (Merck Millipore, Billerica, MA) designed for analysis with Luminex®-technique (Luminex, Austin, TX) was used for analysis of leptin, in concordance with the accompanying instructions. Total coefficient of variation (intra + inter assay) for leptin was 11%.

2.4. Magnetic Resonance Imaging

Muscle, visceral fat and total fat volumes were quantified based on water-fat whole-body MRI, acquired on a 3.0 T

Philips Ingenia MR-scanner (Philips Health Care, Best, Netherlands). The MRI protocol and muscle quantification procedure were identical to the one described by Karlsson et al. [18] at 3.0 T. The visceral fat volume and total fat volume were quantified based on the whole-body calibrated relative fat content [19,20] using multi-atlas segmentation [19] software provided by AMRA, Linköping, Sweden.

A series of supraclavicular multi-echo water-fat MRI was acquired after the whole-body acquisition. To induce an acute cold response during the MRI, the subject wore a cold vest and a second such vest was also wrapped around the legs. At an interval of 111 s a total of 10 image volumes were acquired, three prior to pumping ice cold water through the cold vests, and seven image volumes with water pumping. The images were acquired using a 8-channel head coil combined with integrated spine coils and a 8 channel anterior array, with FOV of 302.59 × 120.75 × 380.00 mm^3 (AP, FH, RL), a matrix size of 216 × 216 and 69 slices, a flip angle of 10°, a TR of 26 ms, TEs of 1.14 × n (n = 1 to 12) ms and a water fat shift of 0.259 voxels, and an acceleration factor of 2.5. Water and fat images were separated from the raw MRI during post processing using an in-house implementation of the Ideal algorithm [21] initialized by a first set of water and fat images based on the two first echoes and phase sensate reconstruction [22]. The fat images were calibrated to relative fat content images [19,20] for fat quantification. Fig. 1 shows an example of an MRI image in which BAT in the supraclavicular region has been marked out.

The volume of BAT in the supraclavicular depot was measured by two readers who segmented dense clusters of voxels with intermediate relative fat content using a semi-automatic tool [23]. A dense cluster was defined as a cluster of voxels with an intermediate relative fat content unlikely to be caused by partial volumes of white adipose tissue and lean tissue. The readers segmented the BAT in the pre- and post-images pairwise, but the order in which the images were presented to the readers was randomized. Thus the readers were blinded to the pre- or post-state of the images. After the segmentation, the readers reached consensus regarding which segmentations to use for each subject. The alternatives for each subject were to use the segmentations of reader one, two or both. This method for imaging BAT is a further development of the method as described earlier [11] in reference 11 which was used to detect BAT according to the methods presented in reference no 25 in which the findings were confirmed by biopsies [24,25].

The relative fat content and R_2^* of the supraclavicular depot were automatically measured in regions of interest (ROIs), by multi-atlas segmentation of the first time point using the consensus semi-automated BAT segmentations (as described above) as atlases. This was based on the methodology described by Karlsson et al. [18]. An atlas based on a specific subject was not used for segmentation of that subject. The segmentations were then extended to all time-points. The effect of lean tissue partial volume effects was minimized by removing voxels neighboring lean tissue. Lean tissue was defined as tissue with a relative fat content below 0.1.

2.5. *Statistical Analyses*

Statistical estimates were calculated using SPSS 19.0 software (IBM Corporation, Somers, NY). Comparisons within and

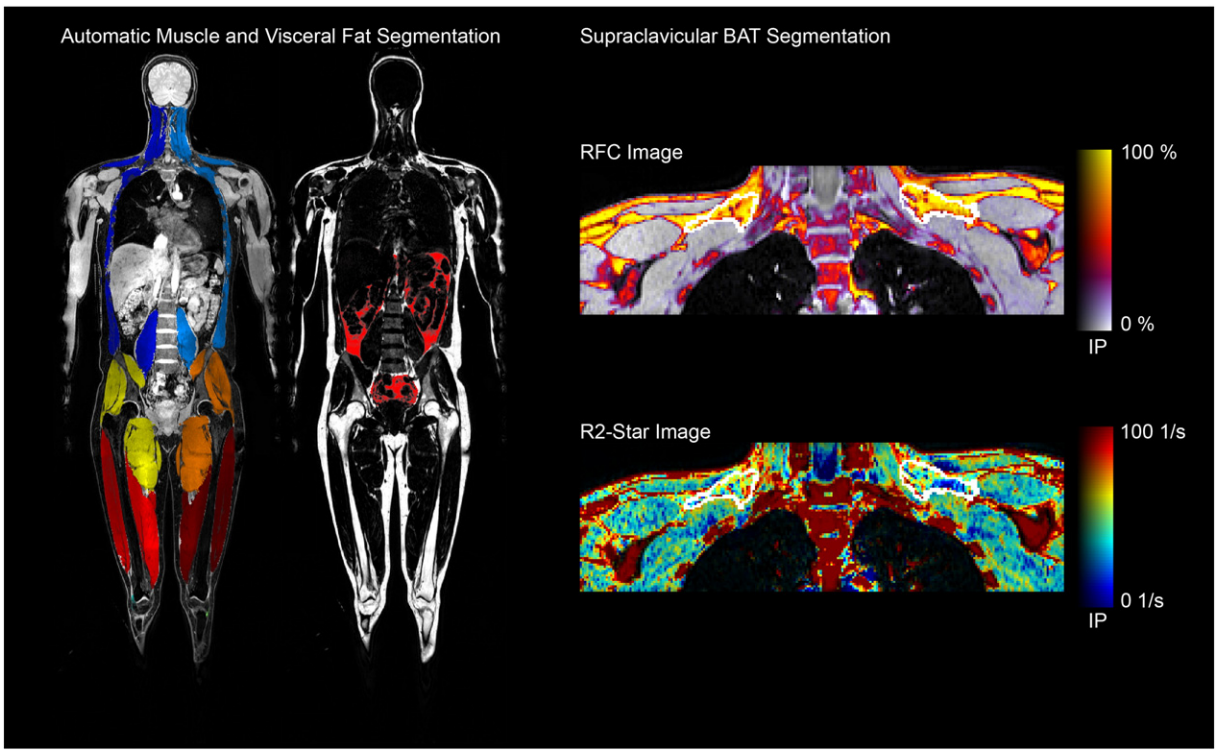

Fig. 1 – Automatic muscle segmentations are marked in red, yellow, orange and blue shades. The visceral fat volume segmentation is marked in red. The supraclavicular brown adipose tissue is lined out by white in the figures to the right.

between groups were done with Student's paired and unpaired 2-tailed t-test or as stated in the results section. Mean values and standard deviations are given. Statistical significance refers to 2-sided $p \leq 0.05$. Leptin values where log-transformed before analyses due to skewed distribution. Based on an earlier intervention study of changes in basal metabolic rate we had a precision of 3% for the basal measurement of MR [26]. This gave 80% power to detect a 5% change of unstimulated metabolic rate within any of the two groups.

2.6. Ethics

The study was approved by the Regional Ethics Committee of Linköping and performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from each participant after full explanation of the purpose and nature of all procedures. The study was registered at ClinicalTrials.gov (NCT01797328).

3. Results

3.1. Intervention

The 28 subjects (18 women and 10 men) in the initial cohort were successfully recruited by local advertisements at the University of Linköping Sweden. All participants except four were medical students. Two subjects randomized to the cold group dropped out for personal reasons (declining further participation) while all 12 participants randomized to the control group finished the study. The age of participants randomized to controls was 24.3 ± 2.9 years (range from 20 to 29 years) and participants in the cold group were 26.1 ± 3.9 years old (range from 20 to 33 years). The subjects in the cold group reported an accumulated period of cold-provocation of 3277 ± 2094 min (range from 2325 to 10,115 min) or 76.7 ± 41 min/day. Although participants were informed to report infections to make further investigations of cause of illness possible, no such contacts were made by the participants during the study. However, three episodes of minor upper airway infections were noted in log books from the cold group. Two subjects in the cold group reported prolonged periods when they had not followed the cold-exposure protocol, in one case a total of 8 days and in the other a total of 11 days without cold-exposure. Fig. 2 shows the flow-diagram of the study.

3.2. Laboratory Tests, Body Weight and Metabolic Rate

One female participant in the cold group was found to have thyroid stimulating hormone (TSH) levels above the reference interval in combination with low free T4 and consequently data from this participant were excluded which left a final cohort of 25 subjects (12 controls and 13 subjects in the intervention group) of completers. Baseline results and findings after 6 weeks of these 25 participants are shown in Table 1. There was a group imbalance regarding baseline fasting insulin levels, being lower in the group randomized to cold. There was no difference in supraclavicular BAT volume between men and women at baseline ($p = 0.78$ by independent samples test) or within the cold group ($p = 0.51$). At baseline there were no statistically significant correlations between age to either metabolic rate (at room temperature, $p = 0.9$, after cold-stimulation, $p = 0.18$) or supraclavicular BAT volume ($p = 0.99$). There were no statistically significant changes in thyroid hormone levels or TSH in any of the groups following the interventions. Body weight increased numerically in the control group but this was not statistically significant. However, the volume of visceral fat as determined by MRI increased in the control group only (Table 1). Both the cold-stimulated metabolic rate and the metabolic rate at room temperature were lowered within the control group, and there was a trend towards increased metabolic rate at room temperature following the intervention in the cold-group (Table 1, $p = 0.052$). The differences between the responses to the two interventions were statistically significant regarding both metabolic rate at room temperature ($p = 0.008$) and after acute cold-stimulation with cold water and cold vest ($p = 0.032$, Table 1). These findings were independent of gender (statistical significance after correction for gender regarding the change in metabolic rate by the interventions at room temperature: $p = 0.02$, or after acute cold-stimulation: $p = 0.036$).

The increase metabolic rate by acute cold-provocation correlated with T3 levels in the total material (level of s-free T3 in relation to metabolic rate after cold-activation at baseline: $r = 0.47$, $p = 0.02$, s-free T3 in relation to the increase of metabolic rate by acute cold at baseline $r = 0.50$, $p = 0.009$). The volume of supraclavicular BAT was not related to the increase in metabolic rate by acute cold stimulation, nor to levels of T3 at baseline (all $p > 0.3$). We found no interactions between fasting insulin levels to changes in basal metabolic rate induced by the interventions.

High sensitive CRP did not change within any of the groups nor were there differences in the changes between groups (all $p > 0.3$). Leptin levels did not differ between groups at baseline ($p = 0.22$) but serum leptin levels increased following the intervention in the warm group, as seen in Table 1.

3.3. MRI Scans and BAT Volumes

As seen in Table 2 an intention-to-treat analysis of the total cohort showed no statistically significant changes in the volume of supraclavicular BAT depots within the two groups. However, R_2^* levels, corresponding to iron content, increased following the intervention in the cold group. Assuming that changes in BAT volume might follow by uninterrupted daily cold-stimulation, the same analyses as in Table 2 were performed with the two participants who reported 8 or 11 days, respectively, without cold-stimulation excluded from the data (i.e. an on-treatment analysis). In this analysis of compliant completers, a statistically significant increase in supraclavicular BAT volume was found. The change in R_2^* was statistically significant between groups in the on-treatment analysis (Table 3) and so were differences between the changes in metabolic rate at room temperature following the interventions ($p = 0.011$). Figs. 3–5 show individual changes in supraclavicular BAT volumes in controls, in the cold group and also in the 11 compliant completers of the cold group.

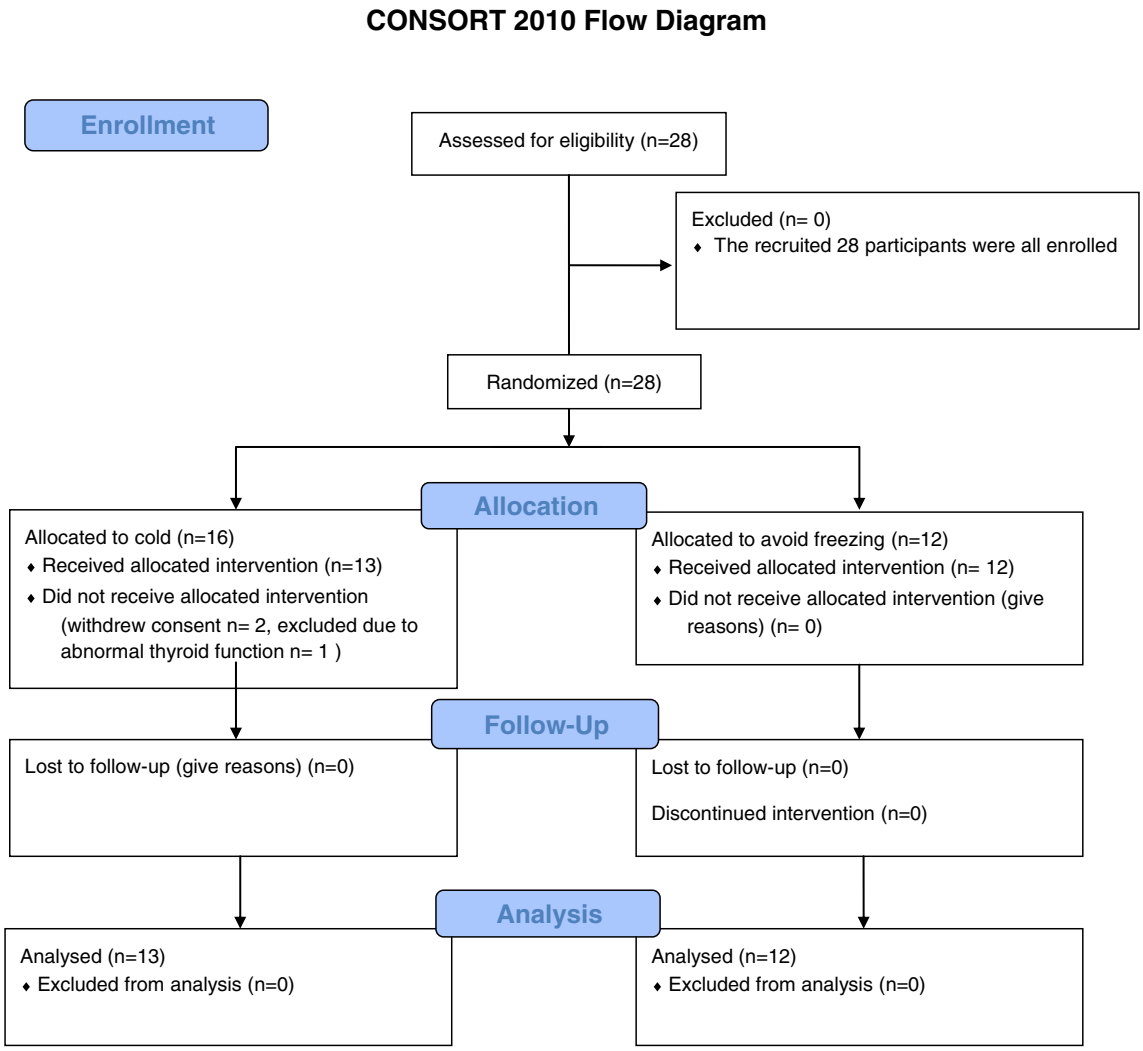

Fig. 2 – Flow diagram of the study.

There were no correlations between reported amount of total time of cold-exposure to change in metabolic rate or volume of supraclavicular BAT (all $p > 0.5$).

Acutely R_2^* was reduced by cold activation during the MRI when the cold vest was rinsed with cold water (comparing R_2^* at room temp with levels after cold-activation in Table 3) and this change was statistically significant at all time-points in both groups (all $p < 0.01$). The acute effects of cold exposure on blood flow (to reduce R_2^*) during MRI were not significantly affected by the interventions in any of the groups (change from baseline to the end of the study in the cold group $p = 0.10$, in controls $p = 0.86$, by paired t-tests. Corresponding calculation in the cold group of compliant completers, $p = 0.19$).

4. Discussion

4.1. *Main Findings*

In this randomized prospective trial of cold-exposure in healthy non-selected participants we found a decrease in metabolic rate in the subjects randomized to avoid feeling cold and a corresponding increase of metabolic rate in participants that subjected themselves to repeated cold exposure. To our knowledge this has not been demonstrated in unselected participants before. There was a weak trend towards an increase in body weight in the controls, and a statistically significant increase in visceral white adipose tissue as measured by MRI was indeed shown in this group. Major cardiovascular risk factors such as blood lipids, glucose and fasting insulin were however unaffected by either intervention. Our findings are thus in line with the idea that resting metabolic rate can be affected by ambient temperature in non-selected subjects. Our study started in February after a period of unusual cold winter in South-East Sweden, and it is plausible to assume that some adaptation to this could already had taken place in the participants. Conversely, we found data in line with that part of such cold-adaptation was lost in the controls as demonstrated by the decrease in metabolic rate after 6 weeks of avoiding cold exposure.

The cold group did not show deteriorations in markers of cardiovascular risk but they did make notes of a few instances with symptoms of infections. There were no reports of such symptoms in the controls and this might suggest that the

Table 1 – Effects of repeated cold exposure compared with avoiding to feel cold (controls) at any time during 6 weeks on metabolic factors, hormones and on metabolic rate in healthy subjects, *n* = 25 (8 women and 5 men in the cold group, 7 women and 5 men in controls).

Variable	Group	Baseline (M ± SD)	After (M ± SD)	P within group	P for change between groups
Weight (kg)	Controls	68.4 ± 8.8	69.2 ± 9.2	0.26	0.22
	Cold	66.04 ± 8.6	65.9 ± 9.3	0.72	
BMI (kg/m^2)	Controls	22.5 ± 2.1	22.7 ± 2.1	0.24	0.22
	Cold	21.6 ± 1.2	21.6 ± 1.5	0.73	
TSH (mU/l)	Controls	1.67 ± 0.53	1.60 ± 0.64	0.60	0.28
	Cold	2.13 ± 1.1	1.82 ± 0.92	0.10	
T4 (pmol/l)	Controls	14.0 ± 1.8	13.8 ± 1.5	0.66	0.41
	Cold	14.6 ± 1.5	13.9 ± 1.4	0.10	
T3 (pmol/l)	Controls	5.0 ± 0.60	5.0 ± 0.79	0.91	0.39
	Cold	5.2 ± 0.68	5.0 ± 0.79	0.14	
Fasting insulin (pmol/l)	Controls	8.3 ± 3.2*	9.5 ± 6.3	0.45	0.66
	Cold	5.8 ± 2.3	6.3 ± 2.4	0.52	
Fasting glucose (mmol/l)	Controls	5.11 ± 0.20	5.11 ± 0.73	0.97	0.84
	Cold	5.04 ± 0.40	5.10 ± 0.45	0.68	
ApoB/apoA ratio	Controls	0.630 ± 0.10	0.605 ± 0.11	0.29	0.87
	Cold	0.629 ± 0.18	0.599 ± 0.14	0.18	
Leptin (ng/ml)	Controls	3252 ± 2173	6399 ± 5034	0.041	0.70
	Cold	6602 ± 7396	7209 ± 5093	0.47	
Non-visceral fat (l)	Controls	18.4 ± 7.6	18.5 ± 7.8	0.40	0.74
	Cold	17.6 ± 4.4	17.8 ± 4.6	0.13	
Visceral fat (l)	Controls	0.837 ± 0.56	0.900 ± 0.57	0.031	0.52
	Cold	0.962 ± 0.70	0.995 ± 0.70	0.40	
Metabolic rate, room temp. (kcal/24 h)	Controls	1841 ± 199	1795 ± 213	0.047	0.008
	Cold	1737 ± 227	1811 ± 217	0.052	
Metabolic rate, cold stimulated (kcal/24 h)	Controls	1900 ± 150	1793 ± 215	0.028	0.032
	Cold	1848 ± 420	1894 ± 304	0.39	

*The difference in fasting insulin levels was statistically significant at baseline (p = 0.028).

cold-provocation induced a reduced resistance to infections. However, the controls were not instructed to keep a diary in the same manner as the cold group, since they did not perform any such measurable activity corresponding to the cold-provocation. Rather, they were instructed to never allow themselves to experience a sense of cold. Therefore, by means of the design of the trial, there might have been periods of infections that were not reported in controls since such reports would anyhow not affect the intervention. Indeed, there were no discernible effects on high sensitive CRP within either group.

Leptin levels increased in the control group. This could theoretically have been related to the increase in visceral white adipose tissue. It has earlier been shown that immersion into water with a temperature of 27 °C acutely reduces leptin levels in humans [27]. Hence, an opposite effect by avoiding low temperatures would also be in line with our findings of increased leptin levels in the controls.

Table 2 – Effects of repeated cold exposure (on an intention-to-treat basis) compared with avoiding to feel cold (controls) during 6 weeks on MRI determined supraclavicular brown adipose tissue (BAT) volume and response to acute cold exposure during the MRI. Total *n* = 25, cold group, *n* = 13, controls *n* = 12. Data are means ±SD. R_2^* corresponds to the amount of iron in a region of interest within BAT and changes in R_2^* when comparing room temperature with cold activated values reflect the changes in blood flow.

Variables		Baseline	After 6 weeks	P within group	P for changes between groups
BAT volume (L)	Controls	0.0168 ± 0.011	0.0174 ± 0.0065	0.75	0.57
	Cold	0.0204 ± 0.015	0.0228 ± 0.013	0.33	
BAT relative fat content	Controls	0.761 ± 0.084	0.818 ± 0.048	0.071#	0.43
	Cold	0.874 ± 0.074	0.883 ± 0.077	0.48	
BAT R_2^* room temperature	Controls	61.1 ± 6.7	60.3 ± 7.9	0.66	0.021
	Cold	61.8 ± 7.5	68.4 ± 9.1	0.014	
BAT R_2^* cold activated	Controls	49.0 ± 7.9	47.9 ± 5.9	0.61	0.29
	Cold	47.6 ± 6.9	49.9 ± 5.9	0.32	

#Data missing in two subjects.

Table 3 – On-treatment analysis of the effects of repeated cold exposure compared with avoiding to feel cold (controls) during 6 weeks on MRI determined supraclavicular brown adipose tissue (BAT) volume and response to acute cold stimulation during the MRI. Total n = 23, cold group, n = 11 (compliant completers), controls n = 12. Data are means ± SD. R_2^* corresponds to amount of iron in a region of interest within BAT and changes in R_2^* when comparing room temperature with cold activated values reflect changes in blood flow.

Variables		Baseline	After 6 weeks	P within group	P for changes between groups
BAT volume (l)	Controls	0.0168 ± 0.011	0.0174 ± 0.0065	0.75	0.20
	Cold	0.0175 ± 0.015	0.0216 ± 0.014	0.049	
BAT relative fat content	Controls	0.761 ± 0.084	0.818 ± 0.048	0.071#	0.43
	Cold	0.706 ± 0.12	0.737 ± 0.089	0.074	
BAT R_2^* room temperature	Controls	61.1 ± 6.7	60.3 ± 7.9	0.66	0.038
	Cold	62.2 ± 7.7	67.8 ± 9.3	0.030	
BAT R_2^* cold activated	Controls	49.0 ± 7.9	47.9 ± 5.9	0.62	0.35
	Cold	47.6 ± 6.1	49.6 ± 6.1	0.42	

#Data missing in two subjects.

We found an increase in R_2^* in the cold group. This suggests an elevated total iron content that could be a consequence of more mitochondria, or of a larger volume of blood in the vessels of the studied regions of interest. Since the relative fat volume of BAT was unchanged in the cold group, we would suggest the former interpretation to be more likely since a larger vessel volume would have been expected to decrease the relative amount of fat. From a perspective of potential mechanisms we judged it to be of interest also to perform calculations of the BAT volumes, and iron contents of BAT, in participants who reported uninterrupted cold-stimulation. In this on-treatment analysis, we found an increased supraclavicular BAT volume in the cold group. This suggests that even after a period of low ambient temperatures for several months during early winter time, an increase in supraclavicular BAT is possible by daily cold-exposure.

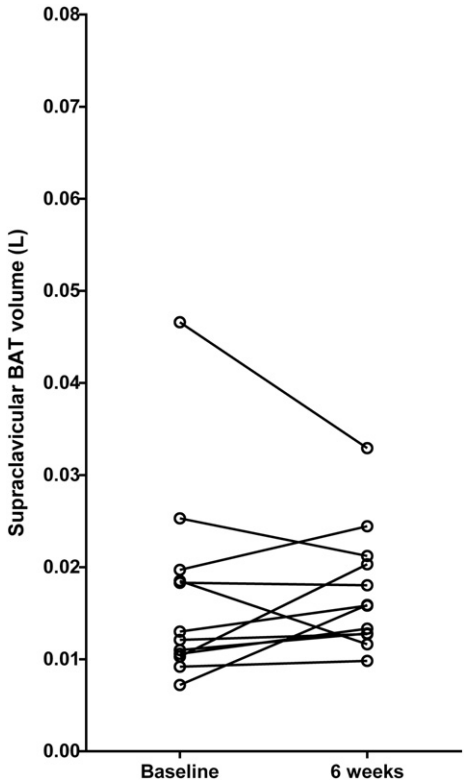

Fig. 3 – Individual changes in the volumes of supraclavicular brown adipose tissue before and after 6 weeks of either avoiding feeling cold or to at least 1 h/day of feeling cold. Fig. 3 shows effects in controls.

We found a significant decrease in R2* in response to acute cold stimulation by the cold vest in both groups at both scanning occasions. This decrease indicates an increased blood flow in the supraclavicular BAT depot. An increased blood flow gives an increased oxygenation which reduces R2*, an effect that is utilized in functional MRI (fMRI). The effect found in this study is in agreement with earlier reported BAT fMRI [28] and appeared numerically stronger in the intervention group after the intervention period, but this difference was not statistically significant.

We found no correlations between supraclavicular BAT volume and metabolic rate in the total cohort at baseline while levels of T3 did indeed correlate with the acute increase in metabolic rate triggered by cold-stimulation. The most likely explanation for this lack of correlation between supraclavicular BAT volume and measures of metabolism is that this particular portion of BAT merely represents part of the total BAT volume. The choice of supraclavicular BAT for volume-determination was based on the fact that this is a region that is generally considered to harbor BAT in humans. Although PET CT in this sense is likely better suited to measure activity in the total BAT volume in humans, it has been shown that BAT can be histologically demonstrated in the supraclavicular region in subjects having no BAT activity by PET CT in this region [5].

4.2. *Limitations*

We acknowledge several shortcomings of the study. We find it likely that our results would have been different had we performed the study under any other season of the year in Sweden. However, the randomized design allowed analyses of potential opposite changes in the groups subjected to the diverging temperature conditions of the study. Even though we did not specifically select participants with low amounts of BAT as has earlier been published [17], all our participants were young and non-obese. Also, by recruitment of

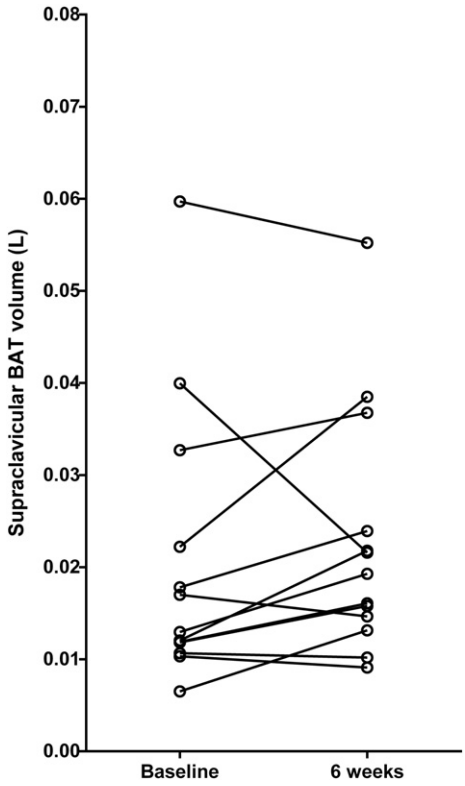

Fig. 4 – Individual changes in the volumes of supraclavicular brown adipose tissue before and after 6 weeks of either avoiding feeling cold or to at least 1 h/day of feeling cold. Fig. 4 shows the total cohort of subjects randomized to cold exposure.

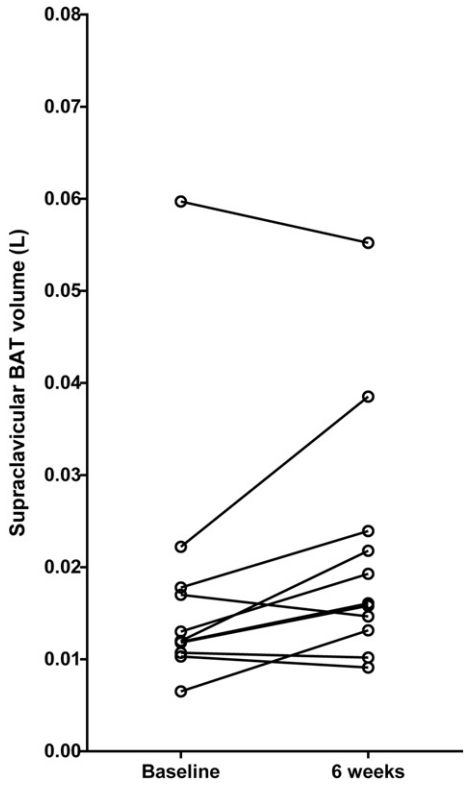

Fig. 5 – Individual changes in the volumes of supraclavicular brown adipose tissue before and after 6 weeks of either avoiding feeling cold or to at least 1 h/day of feeling cold. Fig. 5 shows effects in the compliant completers of the cold-exposure group.

participants by local advertisement at the university, mostly students were recruited. This was likely the explanation of the limited age-span of participants that, accordingly, diminished statistical power to search for interactions of our main findings with age. We do not know whether the results would apply to obese and/or older subjects, whom indeed, in observational studies tend to have little or no BAT [15,16]. We also acknowledge lack of data on diet and physical activity of participants. However, all participants were asked not to change dietary or exercise habits during the trial. The measurements performed after cold exposure could potentially have been affected by shivering, since we only estimated such energy consuming activity visually, and not by any more specific measurement of muscular activity. However, our measurements of metabolic rate, and also of R_2^*, showed changes at room temperature between groups by the 6 week intervention. Hence, these results were unaffected by potential shivering during the investigations. We acknowledge that BAT volume by MRI was only analyzed in the supraclavicular region. The reason for this was that this is the most common and accepted location of BAT in humans. To assess the volume with this new technique in other more controversial regions, confirmation by for example biopsies, would have been necessary. Finally we acknowledge that the study was of a limited size when taking the comparatively large inter-individual variations of measured variables, before and after both interventions, into account.

4.3. *Conclusions*

In summary we found indirect evidence for plasticity regarding metabolic rate in this randomized study of repeated cold exposure in non-selected humans. We also demonstrated that MRI can be used to detect BAT volume and changes in R2* in the supraclavicular region which potentially could be used for larger studies of BAT volume and BAT activation in adults.

Author Contributions

TR, CH-V, OD-L, JT, ND, MH and MEL ran the trial and collected the data. SE, AP, MB and FHN designed and obtained resources for the study. All authors participated in data analysis, drafting of the article and revised critically for important intellectual content. All authors contributed with their specific technical skills in the conduct of the study and approved the final article.

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Conflicts of Interests

There are no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported herein.

Appendix A. Supplementary Data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.metabol.2016.03.012.

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