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Brace yourselves, winter is coming: a pilot study of the effects of brief, infrequent cold water immersion upon body composition in young adult males

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Abstract

Background: The existence of functioning brown adipose tissue (BAT) in adult humans has brought into question the possibility of utilising the BAT mechanism as an obesity tackling strategy. This pilot study examined the effects of a short-term (6wk) cold water immersion (CWI) programme on the body composition of (n=10) healthy male adults. It was hypothesized that the thermal stresses would produce reductions in fat mass (FM) and body fat percentage (BFp) as a result of thermogenic activation of the BAT mechanism. *Methods:* Using a single arm prospective trial design, participants were subjected to singular acute (18min) cold water exposures (15±1°C) weekly for the duration of the intervention (6wk). *Results:* Non-significant decreases were observed in FM (-1.55±2.24kg; $p = 0.057$) and BFp (-1.62±2.46%; $p = 0.067$), and significant increases in fat free mass (FFM; 1.46±1.68kg; $p = 0.023$). *Conclusions: T*he results indicate that the intervention could be adopted as a plausible method to exert positive changes to body composition. These findings should stimulate follow up studies to examine the interventions efficacy in a larger more representative sample and examine its feasibility of implementation as a genuine obesity tackling strategy.

Keywords

Brown Adipose Tissue, Obesity, Overweight, Thermal Stress, Thermogenesis, Novel Therapies

Introduction

The prevailing epidemic of human obesity, instigated by the rise of easy access to high-calorie meals and the gradual growth of sedentarism, poses a serious threat to global health [1]. In order to fully appreciate obesities effects on public health it is possible to examine a comparison of international obesity rates, identifying obesity prevalent nations [2]. Seemingly, the US were recorded as having one of the highest obesity rates, with only Spain accumulating more [2]. More detailed examination of the US population 'National survey data' revealed the prevalence of obesity was as high as 35.8% and 35.5% amongst adult women and adult men, retrospectively [3]. Consequently, mounting interest now lays in finding novel weight loss strategies. Thus far however, very few (if any) approaches have proven efficacious on population levels [4]. Common obesity tackling strategies, such as engaging in exercise (physical activity), dieting, or both, have to some extent failed leaving a serious threat to global health [5]. The extent of this failure can be observed when examining the US 'National weight control registry' which found only a small minority (≈20%) of overweight (obese) individuals successfully achieved long-term weight loss [5].

In response, novel approaches to tackling obesity are emerging; for instance those founded upon the 'Metabolic Winter' hypothesis [6]. Cronise *et al*. [6] hypothesis centres on the belief that an evolutionary discordance concerning human biology and modern world human behaviours exists. Although Cronise *et al*. [6] recognised a *"…calorie excess in the modern world…"* may have contributed to the obesity pandemic, deviations to the human evolutionary path, where previously prehistoric humans would have been subjected to extreme seasonal (cold) stresses, may equally be responsible. Cronise *et al*. [6] identified that dramatic lifestyle changes to the generic modern day human, for instance the ability to make environmental and clothing adjustments when cold (thermal stress) [7], has led to a world with 'excess warmth' where humans are unintentionally eliminating essential thermogenically activated bodily mechanisms, such as the brown adipose tissue (BAT) mechanism. Summarized, Cronise *et al*. [6] stated *"…maybe our problem is winter never comes…"*

Two forms of adipose tissue are recognized in humans (and other mammals) with both tissues having opposing functions in whole body energy metabolism [8]. White adipose tissue (WAT) stores energy, taking the form as triacylglycerol, which can be expended (releasing energy) in the form of glycerol and free fatty acids [9]. Contrastingly, brown adipose tissue (BAT), or brown fat, has the unique capability of transitioning energy (food) into the production of heat [10]. BAT cells contain large volumes of mitochondria [1] which holds the uncoupling protein 1 (UCP1), the inner membrane mitochondrial protein only recognized (expressed) within brown fat [11]. The presence of protein UCP1 prevents the process of cellular ATP synthesis by dissipating the proton [1]. Instead, this allows UCP1 to increase mitochondrial membrane conductance allowing heat to be generated [12]. This particular process of heat production, where the generated heat (cold-induced) cannot be associated to shivering or any type of muscle activation is classified as 'Non-shivering thermogenesis (NST), and is primarily controlled by BAT activity [13].

Mounting interest surrounding the BAT mechanism is now emerging, with particular attention to how the tissue is stimulated. Current evidence suggests the BAT mechanism is predominately activated when an individual is exposed to a temperature close to where shivering (shivering threshold) occurs [9]. It is generally accepted that this bodily response (BAT mechanism) assists in the prevention of any serious harm (hypothermia) and also to maintain an average body temperature [14]. It is believed that the BAT mechanism is directly regulated through the sympathetic nervous system (SNS) which, upon activation from a cold stress, will trigger the BAT mechanism and in-turn increase body temperature [11]. As noted, the BAT mechanism possesses a unique ability of transferring energy, obtained from food, into heat [10] made possible by UCP1. This unique process provides an alternative to storing excess energy (food) as fat, which ultimately could reduce an individual's body fat mass and potentially prevent obesity [10]. BAT's energy (fat) burning capacity has led to discussion on its capabilities and potential uses. If the mechanism can be thermogenically triggered then it is plausible that a thermal stress inducing intervention could be developed, and specifically aimed at tackling the current obesity epidemic.

Unfortunately, until recently the accumulated data of BAT's metabolic processes and its effects to body composition (fat mass) have largely been obtained through studies using rodents [15] who due to their small surface-to-mass ratio rely heavily on the BAT mechanism throughout their lives [16] making them ideally suited. Human testing for BAT had been, until recent times, non-existent. Previously it was believed that BAT was exclusively present (active) in new-born infants and would be lost during human maturity (adulthood) when other bodily mechanisms would take over [14]. Yet radiologists, whilst attempting to detect tumours using computed tomography (PET/CT) and radiotracer F-flurodeoxyglucose (F-FDG), discovered active BAT in their patients - adult humans [9]. Detection of functioning BAT in adult humans lead to a significant number of follow up studies examining its capabilities and purpose. Using varying thermal treatments, with many studies adopting cryotherapy methods, such as cold water immersion (CWI) [17] the tissue eventually received universal recognition as an active tissue in adult humans [9].

Arguably the most significant BAT connected findings were reported by Saito *et al.* [8]. Their protocol (phase 1) involved exposing 56 volunteers to either a cold (19˚C air temperature) or mild environment (27˚C air temperature) lasting for exactly 2 hours (8). The participants who were exposed to the cooler temperature (19˚C) experienced a significant uptake of F-FDG, the parameter used to assess BAT activity, when compared to those undertaking the mild (27˚C) environment [8]. These results were followed up by a phase two. Using an identical protocol to phase 1 the experiment was re-tested intermittently over a year (summer, autumn and winter) using 8 participants [8]. Through measuring F-FDG amongst participants, seasonal differences, concerning BAT recruitment, could be examined. F-FDG uptake was found in 6 of the 8 participants in winter when compared to only 2 of the 8 participants in summer [8]. The somewhat 'mild' temperatures used in the protocol suggest BAT could be activated with even the mildest of temperature modifications. This is accompanied with the seasonal variation, rising and falling in accordance to seasonal temperatures, that BAT activity demonstrated [8]. These outcomes suggest manipulating the BAT mechanism through thermal adjustments is possible.

Following on from this work Yoneshiro *et al*. [18] used comparable protocols, with 17˚C air temperature for 2h, yet over an intervention period lasting 6 weeks. The authors reported a decrease in fat mass (FM) and an increase in energy expenditure (EE), with BAT being a principle contributing factor. Van der Lans *et al.* [19], used a 10 day intervention which incorporated mild (15˚C-16˚C) conditions, but over substantially longer exposure periods (6h) and attained comparable results (increased EE).

Taken together, the results of these studies suggest that, not only is BAT present and active in adult humans, even with varying protocols used, but may play a significant role in regulating body composition. Nevertheless, existing literature fails to provide a real-world ecologically valid (practical) intervention programme that could be used to combat the obesity epidemic. Therefore, in contrast to the existing BAT studies, whom incorporated excessively long and frequent cold exposures, the current study sought to examine a practical low frequency and low duration CWI protocol. The aim of this pilot study was to examine the effects of such an intervention upon body composition over a 6wk intervention. Using body composition measurements, pre and post intervention, changes to body mass (BM), body mass index (BMI), fat mass (FM), fat free mass (FFM), and body fat percentage (BFp) were

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examined. In accordance to the work of Yoneshiro *et al.* [18], Saito *et al.* [8], and Van der Lans *et al.* [19] it was hypothesized that a reduction in FM and thus BFp would occur.

Materials and Methods

Study Design

The current pilot study adopted a single arm prospective trial design with pre- and post-intervention' measurements taken. The study examined the effects of a short-term CWI intervention programme on the body composition of university students. The study took place during November and December of 2015 in Southampton, UK. Approval of the study's design and ethical considerations was granted by the ethics committee at the author's institution (ID No: 420).

Participants

Ten healthy male participants (age 22.8 ± 2.61 y; stature 180.8 ± 5.3 cm; body mass 84.03 ± 15.01 kg) took part in the current study. A sample size estimate for the purpose of this pilot study was made based on the work of Yoneshiro *et al*. [18]. Using a within participant Cohen's d ($d = \mu_{change}/ \sigma_{change}$; [20]) an effect size of 4.77 for change in BFp was calculated. G-Power software (G-power 3.1.9.2) was subsequently used to calculate a sample size estimate of ~3 participants for the current study [21]. However, it was felt that this was unrealistically low and thus in order to avoid the risk of type II error and disruptions caused by attrition, the number of participants was increased to 10.

Before participation in the study's protocols, participants were required to complete a 'Physical Activity Readiness Questionnaire' (PAR-Q) and measurement of resting blood pressure. Resting blood pressure was required to fall below 160/90 according to lab guidelines in order to allow participation. Experimental procedures were clarified during pre-study consultations through verbal and written clarification before written informed consent was obtained from each of the participants.

Study Procedures

Participants visited Southampton Solent University's Sport and Exercise Science laboratory to conduct the following examinations and interventions. Participants were exposed to repeated full body immersions (up to the neck) in iced water (15±1°C) for 18 min over a 6 week intervention period with a single immersion per week (6 immersions in total). In weeks one and six participants' underwent measurement of body composition using a BOD-POD instrument (Gold standard, COSMED, Italy). Following the manufacturer's protocol BM, BMI, BFp, FM, and FFM of each participant was measured. Body composition assessments were taken pre and post intervention within ~48 h of the first and the final immersion.

Cold Water Immersion

Participants were required to adhere to strict CWI protocols. Prior to each immersion the participants would be guided through a detailed 'CWI instructions' handout providing clear direction. A platform (secure bench - height 800 mm) was positioned beside the tub and used during the entering and exiting stages of the CWI procedure. When entering the CWI tub (width 580 mm, depth 720 mm, height 1063 mm) participants undertook a multi-stage gradual exposure (feet, knee, waist, and chest) preventing 'cold shock' [22]. When fully immersed participants were asked to remain motionless, holding a supine position immersed to the neck for the full 18 min. During immersions each of the participants' core temperature was measured using an aural thermometer (TH8 Infrared Ear Thermometer) every 60 s, ensuring their core temperatures did not drop significantly below the average 37°C (98.6°F) baseline [23]. A pre-set core temperature (37±1°C) was used as the safety cut-off point for CWI cessation [24]. During alternate minutes water temperature (15±1°C) was measured using a handheld thermometer, with temperature adjusted using ice and water (warm/cold) accordingly. Whilst exiting the tub participants were provided with an individual large towel and were instructed to 'dry off'. Participants wore minimal clothing during the immersions and were told to bring a complete set of dry clothing (trousers, t-shirt, socks, underwear) to get changed into post immersion. The environment the participants exited the tub into was room temperature (~20-22°C).

Nutritional & Lifestyle habits

Participants were asked, and frequently reminded throughout, to maintain their typical physical activity routines and nutritional habits. In addition, participants were required to record their nutritional intake (3 d food diaries) in weeks one and six. Post intervention, data from individual food diaries were analysed using the software package DietPlan7 to produce nutritional statistics (total kcal, carbohydrate, protein, and fat intake). This was in order to attempt to differentiate body composition changes that may have occurred through spontaneous dietary manipulation from participation compared with those resulting from the intervention.

Statistical Analysis

All ten participants completed the intervention in full. Assumptions of normality for data were checked using a Kolmogorov Smirnov test [25]. Data that met assumptions of normality were compared between pre- and post-intervention measurements using a two-tailed paired sample *t*-test. Alternatively, data that violated assumptions of normality of distribution was compared between pre- and post-intervention measurements using a Wilcoxon signed ranks test [25]. Observed power (β) was computed *post hoc* using G-Power (G-Power 3.1.9.2) for each paired comparison. In addition, effect sizes were calculated using a within participant Cohen's *d* ($d = \mu_{\text{change}}/\sigma_{\text{change}}$; [20]). This allowed a comparison of the magnitude of the effect for each measure and was interpreted as small (0.20-0.49), moderate (0.50-0.79), or large ($\geq$0.80). Lastly, individual data were visualised through use of individual response plots using Graph Pad (Graph Pad Prism 6; Fay Avenue, California, USA). Statistical analysis was performed using the software IBM SPSS Statistics (version 22; IBM Corp, Armonk, New York, USA) with $p \leq 0.05$ set as the limit for statistical significance. As this was a pilot study no corrections were made for multiple comparisons to control for experiment-wise error as it as felt this was be too conservative and inflate the type I error rate too considerably.

Results

Body Composition

Paired sample *t*-test revealed that changes in the following variables were non-significant; BM (-0.31±1.89kg, $t_{(9)} = 0.517$, $p = 0.618$, β = 0.75), BMI (-0.09±0.58kg.m^2, $t_{(9)} = 0.511$, $p = 0.622$, β = 0.74), BFp (-1.62±2.46%, $t_{(9)} = 2.086$, $p = 0.067$, β = 0.46), and FM (-1.55±2.24kg, $t_{(9)} = 2.185$, $p = 0.057$, β = 0.50). However, paired sample *t*-test revealed a significant change in FFM (1.46±1.68kg, $t_{(9)} = -2.748$, $p = 0.023$, β = 0.69). Effect sizes (ES) for absolute change in the dependent variables were small for BM (d = 0.16) and BMI (d = 0.15), moderate for BFp (d = 0.65) and FM (d = 0.69), and large for FFM (d = 0.87). Figure 1 shows individual response plots for pre- and post-intervention body composition data for a) BFp, b) FM, and c) FFM.

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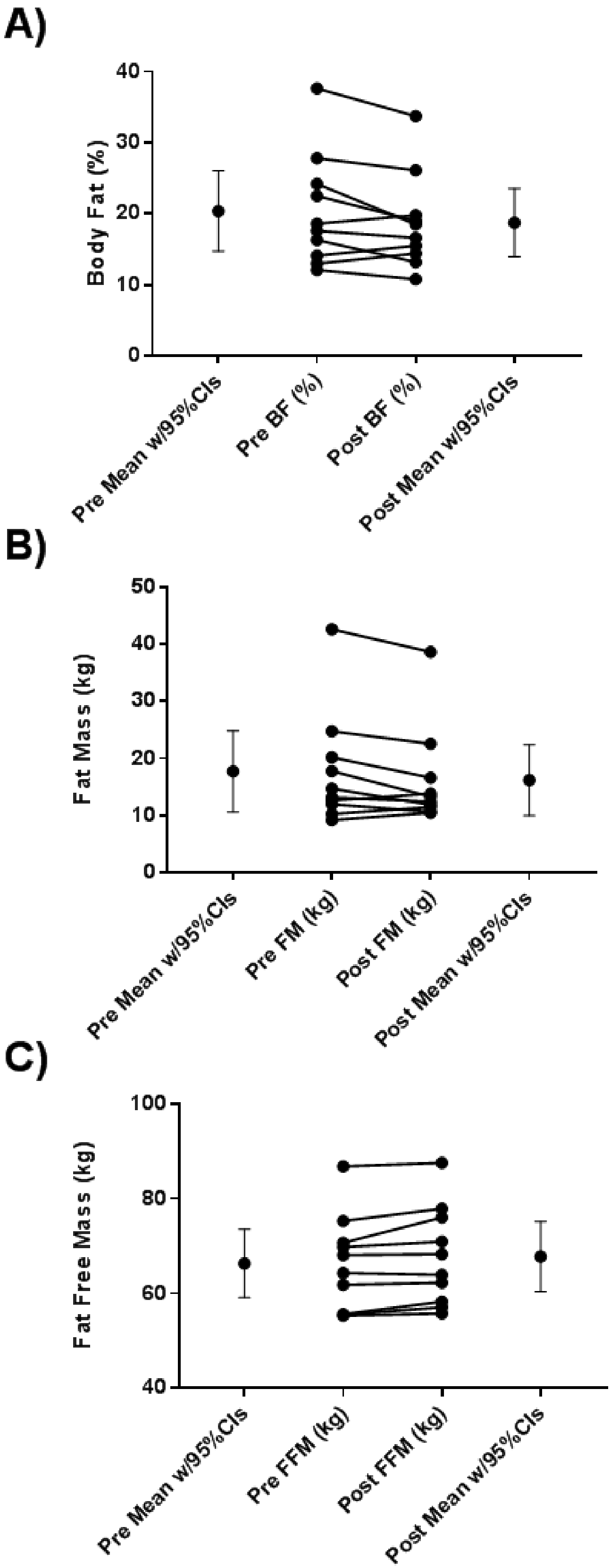

Figure 1. Mean (±95%CIs) and individual responses for pre- and post-intervention A) body fat percentage, B) fat mass, and C) fat free mass.

Dietary Data

Paired sample *t*-test revealed there were no significant differences between week one and week six for either Carbohydrates ($t_{(9)} = 0.16$, $p = 0.876$, $\beta = 0.52$), Protein ($t_{(9)} = 1.64$, $p = 0.135$, $\beta = 0.31$), or in Fat ($t_{(9)} = 2.23$, $p = 0.052$, $\beta = 0.51$). Individual dietary data components can be observed below in Table 1. Wilcoxon sign rank test revealed there was a significant reduction in total kilocalories (Kcal) from week 1 to week 6 ($Z = -2.090$, $p = 0.037$) as seen in Table 1 (below).

Table 1 - Pre and Post Nutritional intake in week 1 and 6

	Kcal*	**Carbohydrates (g)**	**Protein (g)**	**Fat (g)**
Week 1	2846.4 ± 533.8	403 ± 93.1	80.9 ± 20.4	100.8 ± 22.5
Week 6	2737.8 ± 434.0	401.7 ± 87.5	76.4 ± 15.4	91.4 ± 15.1

* indicates a significant change from week 1 to week 6. Data are means ± SD.

Discussion

The present pilot study examined the short-term effects of a CWI intervention on the body composition of healthy male student volunteers. The participants were subjected to CWI protocols once a week over the intervention period (6wk). Within the protocol participants were repeatedly exposed to thermal stresses (15±1°C) lasting 18 min (per immersion). Measurements of effect (changes to body composition) were measured before and after the intervention programme using a BOD-POD. Potential confounding effects caused by spontaneous dietary changes were monitored using individual three day food diaries which were completed in weeks one and six of the intervention by the participants. Results obtained from the BOD-POD (body composition data) and food diaries (dietary data) were subsequently compared allowing greater insight to be drawn from the study's results.

The findings from the present study contribute to the emerging examination of thermal programming being incorporated into human based studies to promote physiological and psychological responses [26]. Yet, the present study specifically examined changes to body composition. The most noteworthy discovery was the significant increase of FFM ($p = 0.023$). In addition, although they did not reach statistical significance ($p \leq 0.05$) the changes to FM ($p = 0.057$) and BFp ($p = 0.067$) (Figure 1a) could also be deemed likely (probable significance) when considering that this was a pilot study and using the qualitative probabilistic scale proposed by Hopkins *et al*. [27] for interpretation of *p* values. The magnitude of the size of change for FM and FFM can be fully appreciated in Figure 1b (negative trend) and 1c (positive trend), respectively. The paired sample *t*-test revealed no significant change for the remaining variables (BM or BMI). Effect sizes indicated a moderate effect for both BFp and FM, and a large effect for FFM. The remaining variables were either found to have small effect sizes. The data collected from the present study suggests a meaningful change is likely to have occurred despite some variables not achieving statistical significance.

One possible explanation for the changes in body composition could be the BAT mechanism. Otherwise known as the 'brown fat' mechanism (BAT thermogenesis) this bodily process is commonly identified for maintaining body temperature [28]. This unique bodily mechanism is powered through the transfer of food (fat) into body heat [10]. BAT's combustion of energy (food) inhibits excess energy being stored as fat and in-turn prevents obesity [10]. BAT and its thermogenic ability is made possible by UCP1 [12]. Following its activation from long-chain fatty acids the tissue (UCP1) increases mitochondrial membrane conductance, enabling heat to be produced [12]. Seemingly, this bodily mechanism acts as a response to thermogenic stimulation (cold environment) which is known to be regulated by

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the SNS [29]. The current study subjected its participants to cold conditions (15±1°C) repeatedly over the intervention period (6wk). Theoretically, it is possible that these CWI exposures stimulated the BAT mechanism within the participants which may explain the changes to FM (Figure 1b), FFM (Figure 1c), and BFp (Figure 1a).

Indeed, Yoneshiro *et al*. [18] used a protocol with distinct similarities to the present study. Yoneshiro *et al*. [18] utilised thermogenic stimulation via air temperature modification (17°C) for 2 h periods over an identical intervention period (6wk) to the present study. Comparable outcomes can be seen between the studies. Yoneshiro *et al*. [18] found that participants significantly decreased their BFp (-5.2% ± 1.9%) albeit to a greater degree than the present study (-1.62% ± 2.46%), possibly due to the greater durations of exposure. In addition, the present study was conducted during the months of November and December in Southampton, UK where average highs of 8.9-11.7°C and lows of 3.2-5.4°C occur according to MET Office data[1]. Thus, participants may already have experienced some degree of BAT effect from environmental conditions due to the season [8]. A principle strength of the Yoneshiro *et al*. [18] study was the availability of FDG/PET screening, a form of measuring technique used to visibly identify active BAT stores. This enabled a clear and verified trend that linked the reduction in BFp and BAT activity. Seemingly, as the levels of BAT increased the reductions of BFp followed [18]. Although the present study could not observe or verify BAT activity it would seem the results were aligned to the Yoneshiro *et al*. [18] study. Therefore, BAT activity is certainly a plausible reason when considering the cause of changes in body composition, specifically changes to FM, FFM, and BFp, to the participants in the present study.

The descriptive statistics of the present study demonstrated that on average the participants experienced positive body composition changes in FM, FFM, and BFp. Yet when assessing individual participant data (Figure 1a, 1b, 1c) there was considerable variability evident between participants. The data variations could be explained by participants altering their dietary habits or physical exercise which have the potential of modifying body composition [11]. Further, participant's baseline BMI classification may have impacted this whereby those with higher BMIs had the potential for greater losses. Indeed, *post hoc* examination of the correlations between baseline BMI and absolute change in BFp (r = -0.543, p = 0.105), FM (r = -0.633, p = 0.049), and FFM (r = 0.634, p = 0.049) suggested this may have been the case. However, as noted above it is also possible that BAT, active or inactive, may have played a role producing body composition changes. It has been noted that *"some individuals can remain lean and in energy balance for many years with no difficulty, while others do so only by conscious effort, and still others fail to do so and become obese"* ([31], p.1361). This could be explained by an individual suffering from defective BAT [31]. Found in rodents, diabetes and/or insulin resistance can prevent BAT mitochondriogenesis, reducing ones thermogenic capacity and in-turn the BAT mechanism from functioning [32]. Based on this it is conceivable that some participants may have defective BAT which resulted in less responsiveness with respect to changes to their body composition after cold exposures. Existing literature supports this theory with Saito *et al.* [8] examining the effects of repeated cold exposure on BAT activity in healthy adult humans. One phase of the study incorporated repeated cold exposures (19°C air) in both summer then winter [8]. Results indicated that BAT was stimulated at differing degrees dependent on the season, demonstrating seasonal variation [8]. The results also revealed that during the summer BAT was found active in 2 of the 8 participants whilst in winter it was found in 6 [8]. For one of the participants no significant effect was detected for either season [8]. The variability in change to participants

[1] https://www.metoffice.gov.uk/public/weather/climate/gcp185f25

body composition could be directly associated to whether a participant suffered from defective BAT. Further investigation would be needed to test this hypothesis.

As previously mentioned the most significant change in terms of body composition within the present study came in FFM (p = 0.023) as seen in Figure 1c. This finding was surprising in light of the hypothesised effects of BAT upon BFp and FM. Participants may have altered physical activity levels despite instruction not to which may have caused this increase in FFM. However, there are also plausible bodily mechanisms from cold exposure which may be responsible. Mineo *et al*. [33] observed the acute effects of cold exposure on the skeletal muscles of rodents. Using both a UCP-dta mice, a form of transgenic rodent which was deficient in BAT, and regular wild type mice, the two sample groups were subjected to varying degrees of cold exposure (4 - 23°C) [33]. Results indicated that despite their BAT deficiency the UCP-dta mice significant increases to both skeletal muscle oxidative capacity and citrate synthase activity occurred [33]. The absence of any significant volume of BAT within the UCP-dta mice suggests that muscle based mechanisms may be operating to provide body heat. Although unclear to what extent, it is possible that these muscular adaptations could impact on body composition, perhaps even within the present study which may explain changes such as the alterations to FFM, FM, and BFp.

Other causes may also have been responsible for the body composition changes in FFM of participants in the present study. Vaanholt *et al*. [34] investigated the effects of cold exposure (10 - 22°C) on the body composition and metabolic rate of rodents (mice). The results revealed significant changes to the organ mass of the mice [34]. This included significant increases in mass to both the heart and kidneys [34]. Although this study did use rodents it is possible similar organ adaptations may have occurred to the participants during the current study. It is therefore possible changes to FFM and other variables were not in fact BAT related and may have been due to other mechanisms such as increased energy expenditure as a result of increased organ masses.

Unfortunately, despite indirect evidence of BAT activity through surrogate variables, the lack of technology, such as FDG-PET/CT used in comparable studies [18], prevented its examination within the present study. In an attempt to reduce the confounding effects of external variables participants were asked to maintain their normal physical activity and dietary patterns, both of which have the ability to alter body composition [35]. Thus three day food diaries were examined for both week one and week six.

The paired sample *t*-test (Table 1) showed no significant change ($p \leq 0.05$) in carbohydrates (p = 0.876), protein (p = 0.135), or fat (p = 0.052). However, a Wilcoxon sign rank test (Table 1) did reveal a significant change in total Kilocalorie intake (Kcal) (p = 0.037). Based on this data there is an argument that the changes to body composition (Figure 1) could directly correspond to these dietary changes (Kcals) and not the BAT mechanism. To determine the magnitude of the effect the participants' diets had on body composition (FM), changes in body composition was compared to dietary data (reduction in Kcal). Based on the reduction of calories the participants would have been expected to have lost ~0.6 kg of FM. However, FM lost equated to ~1.6 kg. For participants to lose the ~1.6 kg they would have needed to reduce caloric intake by ~12,250 kcal over the six week intervention. However, the intake was reduced by only ~4561.2 kcal. When compared a considerable difference (~7689 kcal) can be seen between predicted and actual caloric intake. Based on these findings the dietary intake of the participants likely only partly account for the changes to body composition (FM) (Figure 1b). The unaccounted loss in mass (0.9kg) seemingly was derived through alternative mechanisms, potentially BAT. It should be noted these calculations are based on the assumption that ~3500 kcal is equal to ~0.45 kg fat [36]. In reality, this calculation does not account for a number of uncontrolled variables that could influence energy consumption and expenditure [37]. For example, this could occur if a participant had changed their lifestyle (increased exercise). Therefore, as these calculations have been based on an assumption the change in body composition cannot be entirely confirmed as due to BAT related activity.

However, it is also possible that the participants' dietary intake may have stimulated the BAT mechanism in tandem with cold exposure promoting the reduction of BFp and FM (Figure 1). This concept was first established by Rothwell and Stock [38] who fed rodents a 'Cafeteria Diet' and found an expanded and activated BAT within the rodents. In fact, rodents subjected to the cafeteria diet exhibited significant increases in NST alongside BAT [38]. These findings correspond to existing literature suggesting the increased BAT activity can be a thermogenic response to the energy excess caused by the high-caloric diet [39]. In reference to the current study table 1 suggests that nutritional intake of the participants maintained a relatively balanced level over the intervention. Therefore it is unlikely, though not impossible, that BAT, and consequently the changes to body composition, was directly affected by diet. However, it should be noted the current existing literature surrounding the nutritional effect on BAT activity uses predominantly rodents, such as Rothwell and Stock [38]. To fully appreciate these effects future experiments should attempt to involve human participants.

Although physical activity was not monitored during the present studies intervention (6 weeks) participants were asked to maintain their normal exercise routines. The known effect of exercise on the BAT mechanism is largely disputed and remains unclear [40]. However, its effects on body composition in isolation are better known. The American College of Sports Medicine suggests overweight individuals can lose significant body mass through 200 – 300 min of moderately intense exercise per week [41]. These effects have been demonstrated by Cullinen and Caldwell [42] who examined the effects of moderately intense resistance training on untrained individuals. Results showed participants had undertaken an increase in FFM and a reduction BFp, comparable to the results from the present study. It is conceivable that the participants of the present study may have altered their ordinary exercise routines and not reported this to the investigators which may have in turn influenced the study's results. Future studies should closely monitor physical activity to address the effects of spontaneous changes in physical activity behaviours.

The present study's protocol was specifically designed with real world application and practicality which could be easily replicated. The promising results from the current study, with particular attention to the reduction to FM and BFp, make it an appealing obesity tackling strategy. With practicality in mind the current study's protocol was altered, breaking the trend of prior studies employing longer and more frequent exposures, to the use of short immersions (18 min) and infrequent (once a week) exposures. As well as this, water temperature was dropped (15±1°C); a much colder level than comparable BAT studies such as the Saito *et al*. [8] study. This protocol differs significantly from existing BAT studies. Van der Lans *et al*. [19] observation of cold acclimation in healthy adults involved their participants undergoing comparable temperatures (15-16°C) but with extensive intervention periods (6 h over 10 consecutive days). Despite the present study using reformed intervention protocols, in terms of duration and frequency, similar favourable (decreased FM and BFp) results occurred compared to the previously mentioned studies [8][18]. This suggests that not only is the current study's protocol effective but practical and could be adopted by the public. One differing feature that could explain the success of the current study is the adoption of cold water instead of the more commonly used cold air. Until recently, the use of cold water may have been overlooked due to its potential to cause significant discomfort to participants [43]. Yet, there is evidence to suggest, (as seen in the present study), that cold water has unappreciated benefits. Armstrong *et al*. [44] research into thermal treatments found individuals who were subjected to ice water immersion (1 - 3°C) cooled approximately twice as fast when compared to those exposed to the air exposure (24.4°C). The results of the present study and the surrounding literature seem to advocate the use of CWI. Its ability to cool participants considerably quicker then air exposures may be more efficacious.

A fundamental issue regarding the present pilot study was recruiting an adequate sample size that would enable power for detection of any statistically significant changes in outcomes [45]. Unfortunately, only a small sample (n = 10) of volunteers were recruited to participate. This small sample size was appropriate for a pilot study of this nature but may have reduced the likelihood of statistically significant effects being found [46]. This possibly explains why no significant changes could be observed for many of body composition variables; BM, BMI, FM, or BFp, or dietary data; carbohydrates, protein, or fat. Although a lack of statistical significance could be seen as a negative, it is standard for pilot studies, like the present study, to acquire a small sample size [47]. This allows a signification (if any) of any noteworthy results to be further investigated by future and more definitive studies [47]. This is especially relevant for the present study where the protocol differed significantly to existing BAT studies [8][18]. Therefore the sample size of the present study could be deemed adequate under the circumstances.

Despite its novel results the present study did have a number of limitations. The low recruitment of volunteers may reflect an unwillingness to undertake the protocol. It is possible that individuals believed the CWI would cause discomfort and therefore were deterred. Even though the experimental design used more ‘user friendly’ cold exposures (shorter and less frequent immersions), the fact that only 10 participants volunteered for the study might suggest that people may not be willing to use it. This may present future problems if the current study’s protocol is to be employed as an intervention for obesity. Future work should not only examine further the efficacy of the intervention, but also the effectiveness under real world settings including uptake and adherence. This could include assessment of participant’s subjective discomfort, and even assessment prior to CWI exposure to determine willingness of participants. Finally, another criticism of the present study was the method used to assess body composition. Though the BOD-POD enabled a valid and reliable measurement of basic body composition variables [48] it could not detect BAT directly which is believed to be the fundamental cause of the decrease to FM and BFp in response to cold exposure. Future studies would benefit from incorporating FDG/PET screening, as seen to be used in Saito *et al.* [8] study, which could detect BAT and its activity.

Conclusions

In summary, this pilot study demonstrated that a short-term CWI intervention can significantly improve body composition in healthy male adults. A significant increase was observed in FFM in addition to non-significant reductions in FM and BFp. These results are in agreement with findings from existing studies of cold air exposure programmes [8][18] suggesting that the BAT mechanism may be the cause of the body composition changes. Future work should incorporate an examination of BAT activity in addition to more generic body composition changes. The findings of the present study need to be interpreted with caution as this was a pilot study. However, the results do indicate the potential for CWI to be a potentially efficacious intervention to promote positive body composition changes. As such, future studies should investigate the efficacy and effectiveness of this intervention in larger samples and ecologically valid settings.

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