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Assessing the Induced Adipose Tissue Browning Effects of Hypoxic Swimming and Cold-Water Exposure in Healthy Human Participants Using Contrast-Enhanced Ultrasound (CEUS)

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Abstract

Introduction: The prevalence of obesity in Canada has increased in recent years. Due to this, studies have been conducted on brown adipose tissue (BAT) as it consumes energy to regulate thermal homeostasis rather than storing it as fat. Particularly, studies have shown that exercise regimens combined with a cold-temperature setting elicit the most "browning" of white adipose tissue (WAT). Hypoxic exercise has been seen to prevent obesity and some metabolic disorders, but the browning of WAT via hypoxic exercise has yet to be studied. Thus, this study aims to determine the independent and synergistic effects of cold-water exposure and hypoxic exercise BAT activation and adipose tissue browning in healthy adult males.

Methods: Moderately active healthy adult males will be randomized in three experimental groups (i.e., Cold-water exposure group (CG), Hypoxic exercise group (HG), and combined cold-water and hypoxic exercise group (HC)) and one control group. Familiarization sessions will be done prior to the three testing sessions. Cold conditions will be induced via exercise in 15ºC water. Hypoxic conditions will be induced via the endogenous-hypoxic breathing technique. Contrast-Enhanced Ultrasound (CEUS) scans of regions of interest (i.e., supraclavicular, axilla, anterior abdominal wall, and the inguinal fossa) will be obtained before and after familiarization and testing sessions and will be further utilized for analysis.

Results: It is anticipated that all experimental groups will demonstrate BAT activation and adipose tissue browning as reflected by the Acoustic Intensity (AI) curve and color-flow doppler intensity. Particularly, more pronounced in the supraclavicular region for CG, inguinal fossa for HG, and both for HC.

Discussion: This research protocol anticipates evidence that suggests independent cases of BAT activation and adipose tissue browning, reflected in the CG and HG. This protocol also anticipates synergistic effects of cold water and hypoxic exercise in inducing BAT activation and adipose tissue browning, reflected by HC.

Conclusion: Thus, hypoxia and cold-water interventions may have potential clinical use for managing the rising cases of obesity. Future implications may include public health initiatives that inform the population on ways to mitigate the effects of obesity and provide a potential therapeutic avenue to treat the disease.

Keywords: adipose tissue; browning; hypoxia; cold-water; swimming; contrast-enhanced ultrasound (CEUS); endogenous-hypoxic breathing technique

Introduction

Obesity

Obesity is described as the excessive distribution of adipose tissue (AT) in the body, and the prevalence of obesity in Canada has increased in the past years, resulting in a higher risk of developing comorbidities, type-2 diabetes, high blood pressure, stroke, cardiovascular diseases, and cancer [1–3]. Obesity is described as a condition that requires more rigorous diagnosis and proposed a diagnostic tool that considers four domains to get a holistic diagnosis [4].

Adipose Tissue

AT is an endocrine organ that plays a major role in cellular reactions and metabolic homeostasis like hormone secretion, energy storage, thermogenesis, and other immune and hematopoietic functions of the body [5–7].

White Adipose Tissue (WAT)

WAT is responsible for energy storage, as triglycerides, with additional metabolic (i.e., lipogenesis, fatty acid oxidation, and lipolysis) and endocrine functions (i.e., Adipokines secretion) [8]. This is an efficient storage method due to its anhydrous property and tendency to break bonds easily during lipolysis [9]. Accumulation and

dysfunction of the WAT may lead to inflammation observed in obesity via the production of inflammatory mediators under metabolic stress like the dysregulation in adipogenesis and WAT lipolysis, which is attributed to cardiovascular and metabolic complications like type-II diabetes [8, 10-11].

Brown Adipose Tissue (BAT)

BAT is responsible for thermoregulation that was observed abundantly in infants that was assumed to disappear as humans age but was later reported in adult humans which has a negative correlation with body mass index (BMI) and glucose concentration [12–14]. Subcutaneous brown fat can be found in several areas of the body including the area between the anterior neck and supraclavicular fossa, under the clavicles, axilla, the anterior abdominal wall, and the inguinal fossa [15]. It can also be found as a visceral brown fat along the cardiorespiratory structures and organs as well as around other solid organs like the pancreas, kidney, liver, adrenal, and hilum of the spleen [15]. Additionally, BAT possesses protective factors against comorbidities of obesity such as cardiovascular disease, type II diabetes, and cancer [16–18]. Acute cold-temperature exposure has been shown to promote BAT activity that results in enhanced temperature acclimation and improved insulin sensitivity [17, 19]. Non-invasive imaging techniques like Computed Tomography (CT), Positron Emission Tomography (PET), and Magnetic Resonance Imaging (MRI), have been used to determine BAT distribution [12]. Whereas invasive techniques like biopsies use needles or surgical procedures to obtain AT samples for more rigorous investigation of AT for better understanding of its metabolism and other diseases attributed to AT [20]. The uncoupling protein 1 (UCP-1) gene is one of the most common BAT biomarker that has been studied through AT biopsies and studies found that the UCP-1, when activated by heat loss, allows energy use of BAT to generate heat during thermogenesis and contributes to heat generation by interfering with proton leakage during the mitochondrial oxidative phosphorylation, resulting in heat dissipation instead of ATP production and storage [11, 12, 21–23].

Beige Adipose Tissue

Moreover, several studies have explored the phenomenon of WAT 'browning', which led to the discovery of beige AT that arises from WAT but reacts to stimuli like BAT, particularly with cold and exercise-induced hormones [13, 24]. Browning can induce energy expenditure in AT that can prevent the negative effects of obesity and promote innate immune responses [25–26]. Additionally, chronic suppression of thermogenesis can lead to increased fat mass and body weight, which can lead to obesity and other complications [27]. Further, beige AT was reported to be rich in mitochondria and efficient in energy dissipation, making it an intriguing research interest for its protective characteristics against illnesses and conditions [24].

Exercise-Induced Browning

Exercise-induced browning of AT has been studied over the years and the variation in testing procedures resulted in conflicting results to the effects of exercise in AT browning to human participants [28]. Moreover, there is a lack of evidence regarding hypoxic exercise induced AT browning in human participants. However, studies have reported weight loss effects of hypoxic exercise, as well as its inducing WAT browning capabilities in rats, particularly with the upregulation of Irisin and Leptin levels, adipokines associated with AT browning by promoting adipose tissue transdifferentiation into beige adipose tissue as well as direct activation of WAT lipolysis and fatty acid oxidation [29–33]. Moreover, studies exploring the effects of hypoxic physical activity (PA) were usually replicated in a hypobaric environment using chambers or conducting studies in mid-mountain areas, which poses risks and limitations like organ structural changes, that can lead to collapse and fainting, as well as economic and technical difficulties [34]. Consequently, the endogenous-hypoxic breathing technique utilizes the Endogenik-01 apparatus and have been shown to successfully induce hypoxic effects at normobaric conditions, as well as inducing improved performance in young oarsmen and swimmers [35]. To date, no studies have been conducted using the endogenous-hypoxic breathing to investigate AT browning.

Furthermore, swimming has shown to promote energy expenditure, weight loss, and improve cardiovascular health but lack extensive research due to its limitations in data collection, resources, and safety [36–37]. AT browning induced through swimming protocols have been investigated in rats. Subcutaneous AT browning has been investigated in rats where incremental swimming training of 30 minutes of individual swimming for over 2 weeks can induce AT browning independently, but not showcase additive browning effects when paired with mild-cold (20^{o}C) water exposure [38]. However, cold water (15^{o}C) swimming with resistance promoted synergistic AT browning effects of cold-water exposure and swimming program in rats [39].

Cold-Induced Browning

During cold exposure, BAT is activated by the sympathetic nervous system leading to lipolysis and UCP-1 activation by utilizing the free fatty acids for thermogenesis [40]. Studies have reported the presence of BAT activity for healthy and obese male participants with acute cold-exposure (16^{o}C) using PET scan, as well as WAT browning during acute cold-temperature (4^{o}C) exposure to piglets [22, 41–42]. Short-term cold exposure promotes WAT gene expression and lipid metabolism alterations that induce WAT browning, whereas chronic exposure has been shown to induce BAT hyperplasia in small rodents and human participants [43–45]. Further, acclimation to cold environments showed increased activity and capacity to contribute to thermogenesis

[19,44]. However, cold water (≤5ºC) exposure may cause physiological changes to the body like cold shock, hypothermia, and progressive decline in swimming ability that can harm people new to cold water swimming, so it is recommended for inexperienced individuals to undergo cold-water acclimatization under an expert's supervision [47]. There is no existing literature that tests the effects of cold water (15ºC) on humans, hence this study will be a pilot protocol that would test the feasibility of the protocol of cold water (15ºC) exposure to induce AT browning on experienced cold-water swimmers.

Detection Techniques

Moreover, Mu et al. highlighted the need for improved procedures investigating AT browning in human participants, particularly focusing on controlling testing temperature and utilizing less invasive techniques [28]. Two issues regarding invasive techniques include unexpected side effects and AT browning rate variations across the body [28]. Adipose tissue biopsies involve a process that requires more experts and scanning procedures as well as raising the issue of safety and sample quality [48–49]. Adipose tissue biopsies can be done through a needle-aspiration or surgical biopsy to obtain AT samples for analysis [48]. These procedures involve either injection and/or incision to the body to gain access to the AT samples of interest [48]. Thus, participants are usually advised to prevent participating in water sports like swimming to prevent infection [49]. On the other hand, there are several imaging techniques that have been used to investigate BAT activity, such as PET/CT scan, near-infrared spectroscopy, and MRI but are all indicated to have issues with radiation exposure, inaccessibility, and high cost [48]. Consequently, Contrast-Enhanced Ultrasound (CEUS) was found to be a feasible non-invasive and non-ionizing imaging technique in detecting BAT, since BAT contains a profound network of vessels and its activation can be reflected with increased blood flow in these tissues, especially for young and healthy individuals [50–51]. It is done with the use of ultrasound contrast agents (UCA) which are reported to have very low incidence of adverse side effects and are generally safe for use even with repeated UCA injections when observing multiple areas of the body [52]. CEUS have been used to estimate BAT activity in mice which was further supported in human participants where BAT perfusion was reflective of BAT activity [53–55]. Hence, this study will utilize CEUS to detect BAT perfusion since hypoxic exercise can induce vasodilation to increase blood flow in tissues to maintain oxygen levels [56–57].

Research Question and Objectives

Studies have shown that cold-temperature water exposure induces BAT activation and WAT browning in animal and human participants. However, swimming training programs have only been shown in rats so as proof of principle, the study aims to determine the independent AT browning inducing capabilities of hypoxic and cold-water exposure and investigate their synergistic effects on BAT activity and AT browning. The researcher hypothesizes that (1) hypoxic swimming exercise, and cold-water (15ºC) exposure will induce AT browning independently and (2) hypoxic exercise and cold-water exposure would reflect a synergistic AT browning inducing properties.

Methods

Participants

Ethics board review application will be submitted and will have to be approved prior to conducting this research study protocol.

Healthy adult men (18-45 years old) will participate in a 6-week supervised swimming training program after an orientation [39]. Participant screening completed before study participation, and use the Dietary Quality Score (DQS) and the International Physical Activity Questionnaire (IPAQ) for reliable and valid tools for the assessment of the participants guided by the inclusion-exclusion criteria for both the study and control groups [55].

Inclusion criteria includes participants within the BMI range of 18.5-24.9 kg/m^2 with an active lifestyle and good general health. Good general health will be assessed via the Short Form 36 (SF-36) health survey questionnaire [58]. Active lifestyle refers to 150-300 minutes of moderate intensity or 75 to 150 minutes of vigorous intensity of physical activity [59]. Exclusion criteria include participants with metabolic or cardiovascular diseases, such as hypertension and diabetes, as well as participants on medication (i.e. hypertensive) to remove the possible influences in response to exercise. Cold-water exposure may induce physiologic changes that might put the participants with comorbidities at risk [47]. People under ketogenic diets are also excluded in the study since it has been reported to have AT browning inducing effects [56]. Participant recruitment will account for a 20-30% dropout rate.

Study Design

The participants will be randomized into study groups: cold-only group (CG), hypoxia-only group (HG), hypoxia-cold group (HC), and control. Testing and familiarization sessions will be done under the supervision of a professional.

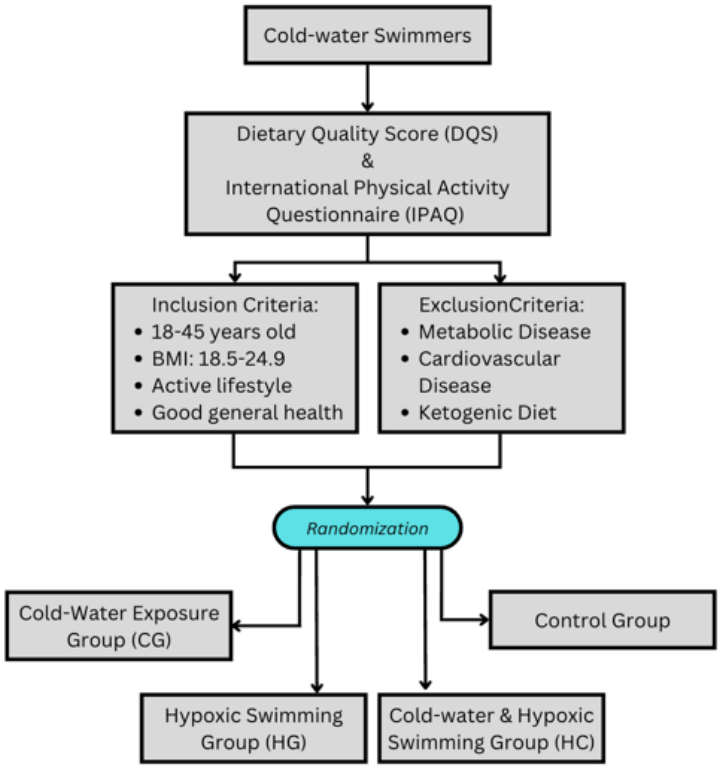

Figure 1. Overview of the sampling method. Selected cold-water swimmers will undergo prior screening via the DQS and IPAQ. Data gathered from the two screenings will be used to select qualified participants with the aid of the inclusion-exclusion criteria. Selected participants will then be randomized and designated in one of the experimental groups (CG, HG, and HC) or in the control group. This figure was made using Canva.

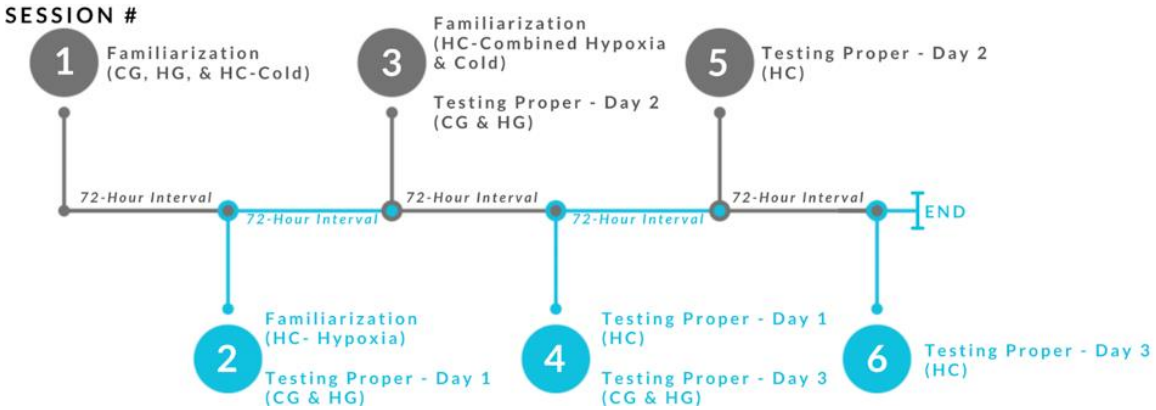

Figure 2. Timeline of the six (6) sessions for the testing protocol. Includes one day of familiarization session for HG and CG groups followed by three (3) days of testing proper. Further, the HC group will follow familiarization sessions for three (3) days, then followed by testing proper sessions for the succeeding three (3) days. This figure was made using Canva.

The entire protocol will run for four (i.e., CG and HG) or six (i.e., HC) sessions. All testing groups will undergo three testing sessions from 14:00 to 17:00 to reduce the diurnal effects on muscle [57]. Additionally, sessions will be done with a 72-hour interval to promote body recovery. Heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), and weight will also be monitored.

CG participants will perform 7 immersions for 3 minutes –maximum duration of 21 minutes– in a plunge tank filled with 15^{o}C cold water [60]. The familiarization will utilize a purpose-built hydraulic winch with a harness to gradually drop the participants in the water, where they will tread the water for 3 minutes or until intolerance to cold water is noted. On the other hand, the HG

participants will be doing 6 sets of 2-to-3-minute freestyle swims –total of 22 to 28 minutes or less– in a 25ºC pool (25m length) with 2 minutes of rest per set, or until exhaustion. Hypoxic conditions will be monitored using the endogenous-hypoxic breathing technique in normobaric conditions using the Endogenik-01 apparatus, a tool that can increase performance at the aerobic energy supply zone and physical preparedness, which promotes respiratory tract adaptations and abdominal breathing muscle strengthening to increase resistance to hypoxia [34–35, 61]. For HC, the first session will be familiarized with the cold-water protocol, then familiarization with hypoxia protocol for the second session. A third familiarization session will be done to combine cold and hypoxic protocols. HC protocol is expected to take 22 to 28 minutes maximum. Swimming protocols will be the same across six weeks without progression to minimize the variability of training effect. By the end of the swimming protocol, HG and HC groups would be expected to have 1 hour and 24 minutes of swimming training. Scans will be obtained before and 2-hours after each familiarization session [50]. Lastly, the control group is to remain sedentary for the whole testing process. A member of the research team will conduct weekly phone calls with participants of the control group to maintain study engagement and collect information on participant's activity levels.

Number of laps swam, and participants' HR will be monitored through each testing session. To keep the intensity of exercise consistent, participants will be asked to swim at an intensity that corresponds to ~60% of their age-calculated maximal HR (age predicted max HR = 220 - age) using a waterproof wearable heart rate monitor throughout the testing protocols [62].

Ultrasound Scanning

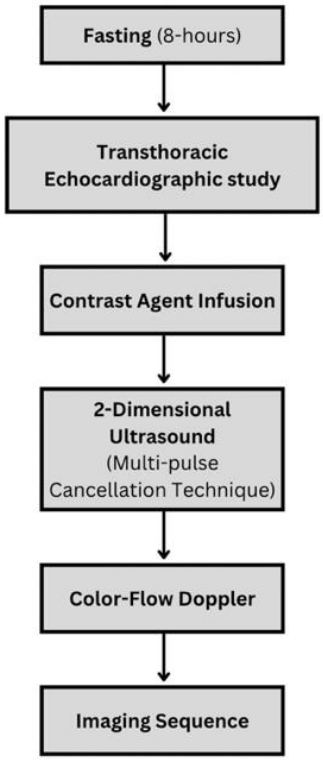

Figure 3. Contrast Enhanced Ultrasound Scanning Protocol. Starts with an 8-hour fasting prior to the testing day then proceeds to a transthoracic Echocardiographic study to identify the stroke volume and the potential contraindications of the contrast agent. Then, the contrast agent will be administered followed by the 2-Dimensional ultrasound using multi-pulse cancellation technique. Then, a color-flow doppler will be done to keep out of the vascular structures in order to perform the imaging sequence (3 images per pulse intervals)) with pulse intervals of 0.06, 0.25, 0.5, 1, 2, 3, 4, 5, 7, 8, and 10 seconds of the areas of interest (i.e., supraclavicular, axilla, anterior abdominal wall, and inguinal fossa). This figure was made using Canva.

The study will utilize a similar protocol done to human participants using CEUS [50]. The participants will undergo 8 hours of fasting prior to the testing day to control for the possible effects of food intake to the exercise swimming protocol and to increase adipose tissue blood flow observed during fasting and moderate-intensity

exercise [63]. Participants will undergo a limited transthoracic echocardiographic study to examine the contraindications of intravenous echocardiographic contrast. For better contrast enhancement, Perflutren gas-filled lipid microbubbles (Definity) will be used, where 1.3 mL Definity suspended in 30 mL 0.9% saline, infused over 5 min. Further, the CEUS scanning will be done in the supine position.

The participants will undergo multiple scans: (1) before familiarization, (2) 2-hours after each familiarization, (3) before testing protocol, (4) 2-hours after testing protocol, and (5) after 48 hours of the protocol [50, 64]. CEUS scanning protocol will be done by a trained professional using a two-dimensional ultrasound [50]. CEUS scans will be obtained in the regions of the supraclavicular, axilla, anterior abdominal wall, and the inguinal fossa as described by Sacks and Symonds [15].

Data Analysis

Ultrasound Analysis

Blinded from the stage of the protocol, an observer will analyze the ultrasound image where the region of interest will be traced to estimate the amount of blood flow for CG, HG, HC, and Control groups [50]. Acoustic intensities (AI), measured in acoustic units (AU), will be plotted in a curve using the formula, $y = A(1- e^{\beta t})$ and the estimated blood flow will be the product of A and β [50].

Statistical Analysis

All data will be presented as mean ±SD . Before conducting any statistical analyses, the normal distribution of the dataset (Shapiro-Wilk test) will be checked. A repeated measures ANOVA will be used to compare response for all participants at each CEUS scan time point. If there are significant differences found between any groups, a Bonferroni *post hoc* test will be conducted. Student's paired t tests will be used to analyze differences of collected anthropometric measures (i.e., BMI, HR, SPB, DPB, and Weight), collected before and after the study protocol. All statistical analyses will be conducted using R programming.

Results

As this is a research protocol, no data was generated but the expected primary outcomes are observations reflecting BAT activation and AT browning across all experimental groups, and secondary outcomes regarding HR, SBP, and DBP changes. All measures are expected to remain constant in the control sedentary group. Weight is expected to not result in a significant change for all study groups across the protocol.

BAT Activation and AT Browning

The anticipated results are expected to be observed across the four regions of interest: supraclavicular, axilla, anterior abdominal wall, and the inguinal fossa.

A higher AI peak is anticipated for CG and HG when compared to the control group. Particularly more pronounced in the supraclavicular region for CG and inguinal fossa for HG [28, 58–59]. Lastly, the HC group is anticipated to have the greatest peak of AI and the highest value of estimated blood flow when compared from the other experimental and control groups, particularly more enhanced effects for both supraclavicular and inguinal fossa regions. Hence, indicating all experimental groups to induce BAT activation.

All experimental groups are anticipated to have significantly increased AI value from session 1 to 3, where HC is anticipated to show the greatest AI increment. Additionally, the ultrasound images obtained after the testing protocol from sessions 1 to 3 is expected to reflect increasing saturation and intensity of the color-flow doppler, which reflects AT browning induced by acute exposure to cold and hypoxic environments. HG is anticipated to have the greatest color-flow doppler intensity in the inguinal fossa ultrasound image [28]. Additionally, HC is anticipated to reflect an ultrasound image with the greatest intensity of color-flow doppler across all four regions of interest, with the greatest intensity in the inguinal fossa ultrasound image [28, 50]. Thus, these anticipated results indicate the independently induced AT browning by cold and hypoxia, as well as their synergistic effects with AT browning.

Vital Signs – Heart Rate and Blood Pressure

Control group is expected to reflect the normal baseline values for HR ($\bar{x}$ = 60 -100 bpm), SBP ($\bar{x}$ = 120 mmHG, SD = 10), and DBP ($\bar{x}$ = 80 mmHg, SD = 10) at rest [67]. Comparing the vital signs monitored for the experimental groups from the control group, it is anticipated that CG will result in decreased HR, as well as increased SBP and DBP after each testing session [50]. For HG, HR and DBP are anticipated to increase with a slight decrease in SBP [68–69]. Lastly, HC is anticipated to have increased HR, SBP, and DBP [47].

Discussion

The anticipated results were established after consulting the present scientific literature. It has been well-established that cold-exposure can induce BAT activation and AT browning in rats and humans [22, 41–42, 44–45], which is consistent with the anticipated findings regarding the cold-water (15ºC) protocol. Moreover, hypoxic exercises have also been shown to induce BAT activation and AT in rats [29–31], particularly in the inguinal area [28, 66], which is consistent with the anticipated findings for the hypoxia protocol, as proof of principle. The anticipated result for the hypoxic protocol is also consistent with the hypothesis by Mu et al., which highlighted the relationship of hypoxic exercise with better AT browning capabilities due to its ability to upregulate irisin and leptin levels [28]. Irisin and leptin levels were shown to be positively correlated with

BAT activity and AT browning [28]. Moreover, the effectiveness of CEUS in detecting BAT activity and browning by observing BAT blood flow using AI to describe the intensity of the color-flow doppler [50]. The authors found that activated BAT and AT browning is attributed to increased AI peak, which shows a more intense color-flow doppler in the ultrasound scan [50]. Thus, it is anticipated that HC group would have the highest peak AI and most saturated color-flow doppler since it is expected that there will be synergistic effects of cold-water exposure and hypoxic exercise in BAT activation and AT browning. The anticipated effects of hypoxia, as well as the expected synergistic effects of cold-water exposure and hypoxia will be the expected novel findings of this research protocol. With the anticipated results, swimming could potentially provide an avenue to maximize the effects of both cold-water and hypoxia as the nature of the sport allows for both conditions to be applied. Furthermore, it has been shown that lap swimming is the most enjoyable form of exercise for those with obesity, as compared to walking or stationary cycling [69]. This is important when considering a potential therapeutic tool for sustainability and adherence.

The study investigated the AT browning inducing capabilities of cold-water exposure and hypoxic exercise on active and healthy participants. Future studies may aim to investigate these effects on people affected with obesity to explore the relationship of hypoxia and cold-water exposure in relation to obesity. Additionally, the study only focused on male participants due to the changes in the hormonal levels of women throughout the menstrual cycle. Thus, the study protocol may also be extended to female participants but ensuring the control of the effects of hormone levels as it determined that sex hormones affect BAT activity where estrogen has been shown to have direct stimulatory effects on BAT whereas progesterone has limited and indirect pieces of evidence regarding BAT activity [70]. Additionally, women have been shown to have similar or greater detection of BAT using PET, hence, exploring the significance of CEUS in BAT detection for women would also be beneficial [70]. Future studies will need to investigate the effects of this swimming and hypoxic protocol in the female population

It is also important to note that there are some limitations within the study. Particularly, the scanning technique utilized in the study was CEUS, which can only assume whether the increase of the color-flow doppler was due to BAT hyperplasia or WAT browning. More invasive techniques, such as biopsies, would be more appropriate for classifying the morphology of the AT of the areas of interest [71]. Additionally, the research protocol is costly and demands material resources and experts for data collection and analysis. The research protocol may potentially have a high dropout rate due to the nature of the protocol. The study requires repeated CEUS infusions which can impose limitations with participant compliance as well as staff, expert, and equipment availability as CEUS require trained individuals in order to conduct and analyze the ultrasound imaging. The study would also require 5 CEUS which may need multiple injections of the UCA to the participants and it may potentially lead to participant drop-out despite UCA being safe for use in healthy individuals [52].

Despite these limitations, this research protocol also presents many strengths. It is the first of its kind to investigate the effects of both hypoxia- and cold-induced WAT browning. If successful, this protocol will also set the groundwork for using non-invasive imaging techniques to assess BAT activity. Lastly, the potential results of this proposed protocol may highlight the importance of swimming training in promoting BAT activity and could explore therapeutic avenues that AT browning may bring.

Conclusions

This research proposal aims to demonstrate the cold-water and hypoxic exercise induced AT browning on healthy and regular cold-water swimmers. Using three testing protocols and CEUS imaging, the objective of this study is to reveal the AT browning capabilities of cold-exposure and hypoxic exercise, both independently and synergistically. Moreover, all experimental groups are expected to promote BAT activity and AT browning, mostly pronounced for the combined cold water and hypoxia protocol especially in the supraclavicular and inguinal regions of interest. Moreover, this study aims to demonstrate the capabilities of cold-water and hypoxia to induce AT browning to provide information on mitigating the effects of obesity and provides a potential therapeutic avenue to treat the disease.

List of Abbreviations Used

AT: adipose tissue
WAT: white adipose tissue
BAT: brown adipose tissue
CT: computed tomography
PET: positron emission tomography
MRI: magnetic resonance imaging
UCP-1: uncoupling protein 1
HIIT: high-intensity interval training
PA: physical activity
CEUS: contrast enhanced ultrasound
UCA: ultrasound contrast agents
DQS: dietary quality score
IPAQ: International Physical Activity Questionnaire
AI: acoustic intensity
HR: heart rate
SBP: systolic blood pressure
DBP: diastolic blood pressure

Conflicts of Interest

No conflicts of interest to declare.

Ethics Approval and/or Participant Consent
No ethics approval or consent was collected as this study is just a research proposal.

Authors' Contributions
KMAS: conducted the literature search, wrote, drafted, edited, and gave final approval of the manuscript.

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