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RESEARCH ARTICLE

The effect of cold exposure with shivering on glucose tolerance in healthy men

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Abstract

Muscle glycogen use and glucose uptake during cold exposure increases with shivering intensity. We hypothesized that cold exposure, with shivering, would subsequently increase glucose tolerance. Fifteen healthy men (age = 26 ± 5 yr, body mass index = 23.9 ± 2.5 kg·m^{-2}) completed two experimental trials after an overnight fast. Cold exposure (10°C) was applied during the first trial, via a water-perfused suit, to induce at least 1 h of shivering in each participant. For comparison, a thermoneutral (32°C) condition was applied during the second trial, under identical conditions, for the same duration as determined during the cold exposure. After the thermal exposures, participants rested under a duvet for 90 min, which was followed by a 3-h oral glucose tolerance test. Skin temperature (means ± SE) decreased at the end of the cold exposure compared with that before (26.9 ± 0.3 vs. 33.7 ± 0.1°C, $P < 0.001$). Total energy expenditure during the 1 h of shivering was greater than that during the time-matched thermoneutral condition (619 ± 23 vs. 309 ± 7 kJ, $P < 0.001$). Cold exposure increased the areas under the glucose and insulin curves by 4.8% ($P = 0.066$) and 24% ($P = 0.112$), respectively. The Matsuda and insulin-glucose indices changed after cold exposure by −21% ($P = 0.125$) and 30% ($P = 0.100$), respectively. Cold exposure did not subsequently increase glucose tolerance. Instead, the Matsuda and insulin-glucose indices suggest insulin resistance post shivering.

NEW & NOTEWORTHY This is the first study to examine the effect of cold-induced shivering on subsequent glucose tolerance determined under thermoneutral conditions. Plasma glucose and insulin concentrations increased during the oral glucose tolerance test post shivering. Additionally, insulin sensitivity indices suggest insulin resistance following cold exposure. These results provide evidence for an acute post-shivering response, whereby glucose metabolism has deteriorated, contrary to the results from earlier studies on cold acclimation.

cold-induced thermogenesis; glucose metabolism; insulin sensitivity; oral glucose tolerance test; thermoregulation

INTRODUCTION

The global prevalence of diabetes mellitus continues to increase, with 439 million people estimated to have the disease by 2030 compared with 285 million people in 2010 (1). Type 2 diabetes is characterized by hyperglycemia and impaired insulin secretion, which are preceded by skeletal muscle insulin resistance (2). Exercise is one method to improve glucose homeostasis (3). Additionally, lifestyle changes incorporating physical activity and low-calorie diets reduce progression to type 2 diabetes in people at high risk (4). However, calorie-restricted diets are difficult to maintain in the long term owing to adaptations that promote weight regain (5). Also, as being overweight is a risk factor for type 2 diabetes (1), some individuals may be unable to exercise because of osteoarthritis (6). Therefore, alternative interventions for the prevention or treatment of type 2 diabetes are needed.

Cold exposure is a non–weight bearing and nonpharmacological intervention, which appears to be beneficial for glucose homeostasis. Thus, insulin sensitivity is increased during cold exposure in rats (7, 8). Moreover, cold acclimation prevents the decrease in glucose tolerance and insulin sensitivity in high-fat diet-fed rats (9). Human observational research also suggests that cold exposure may be beneficial, as type 2 diabetes prevalence is positively associated with ambient temperature and the explained variation is greater in US counties that have colder winter months (10). Furthermore, we showed that cold acclimation increased insulin sensitivity (11), and others have shown immediate reductions in plasma glucose concentration following cold exposure in individuals with type 2 diabetes (12).

The reasons why cold exposure increased insulin sensitivity have not been fully identified (11), although we found enhanced glucose transporter 4 (GLUT4) translocation after cold acclimation, which suggests the role of skeletal muscles. In response to cold exposure, the metabolic rate increases, which is accompanied by increases in heart rate and blood pressure and a reduction in plasma volume (13). Much research has focused on nonshivering thermogenesis, supported by the confirmed presence of brown adipose tissue in adult humans, which may be responsible for the increase in

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energy expenditure under such conditions (14, 15). During nonshivering conditions, cold-induced thermogenesis is proposed to reach up to 30% of resting metabolic rate (16), although the potential contribution of unmonitored skeletal muscles to cold-induced thermogenesis should not be disregarded. In contrast, the peak increase in metabolic rate when shivering is on average 400% of resting metabolic rate (17). To support the increase in metabolic rate during shivering, skeletal muscle glycogen is used (18–20), with the amount of muscle glycogen used increasing with the shivering intensity (18). Furthermore, muscles that shiver at greater intensities have larger glucose uptakes (21). Therefore, shivering thermogenesis may result in lasting improvements to glucose homeostasis.

The aim of this study is to determine the effect of an individualized cold exposure, with shivering, on glucose tolerance in healthy young men. Cold exposure (10°C) was applied, via a water-perfused suit, for an individually determined duration with the aim of inducing at least 1 h of shivering in each participant. The cold exposure, or thermoneutral exposure, was followed, 90 min later, by a 3-h oral glucose tolerance test (OGTT). We hypothesized that the cold exposure would increase glucose tolerance (reduce the glucose concentration during the OGTT) compared with the thermoneutral condition.

MATERIALS AND METHODS

This study was performed between January and November 2019 at Maastricht University, the Netherlands. The study was approved by the Medical-Ethical Committee of Maastricht University and performed in conformity with the Declaration of Helsinki (Fortaleza, Brazil, 2013). The study was registered at ClinicalTrials.gov (identifier: NCT03700164).

Participants

Sixteen healthy young men completed the study. In retrospect, one participant was excluded from analysis owing to being classified as having diabetes mellitus (2-h OGTT plasma glucose concentration was 14.8 mmol·L^{-1} during the thermoneutral trial). None of the participants regularly experienced cold exposure such as cold-water baths, cold-water swimming, or working in a refrigerated environment. Participants were white Europeans and their characteristics are shown in Table 1. Participants were healthy, had no known disease, were nonsmokers, were not taking any medication, had stable weight for at least 3 mo before the study, and were not dieting. All participants gave their written informed consent.

Table 1. *Participant characteristics*

Characteristic	Participants (n = 15)
Age, yr	26 ± 5
Body mass, kg	75.4 ± 7.2
Height, cm	178 ± 7
Body mass index, kg·m^{-2}	23.9 ± 2.5
Body fat, %	16.7 ± 1.9
Systolic blood pressure, mmHg	112 ± 10
Diastolic blood pressure, mmHg	67 ± 8
Heart rate, beats·min^{-1}	62 ± 9
Fasting plasma glucose, mmol·L^{-1}	5.07 ± 0.32

Values are means ± SD.

Participant Screening

Each participant completed a screening, two standardization visits, and two experimental trials. The screening session was performed in the morning with the participant fasting overnight for at least 10 h and involved a health screening and the collection of anthropometric data. Blood pressure and heart rate (M6 Comfort IT, Omron, Japan) were determined via the mean of three repeat measurements on the left arm with the patient in sitting position, after having sat for at least 10 min. Body composition was assessed by air-displacement plethysmography (Bod Pod, Cosmed, Italy). A resting electrocardiogram was performed, and a blood sample was collected via venipuncture to determine fasting plasma glucose concentration.

Standardization Procedures

At least 48 h before both experimental trials, participants attended the research center for standardization procedures. Participants were instructed to avoid exercise and minimize physical activity 48 h before the experimental trials. To discourage exercise and check adherence, a triaxial accelerometer (MOX1, Maastricht Instruments, The Netherlands) was attached to the participants' right thigh, above the patella. Participants were also asked to record their diet and activity 48 h before the first experimental trial. The diet and activity diaries were collected during the first experimental trial and returned to the participant during the second standardization visit, with instructions given to reproduce their recorded diet and activity 48 h before the second experimental trial. Alcohol and caffeine were not permitted 48 h and 24 h before the experimental trials, respectively. All participants were provided with and consumed the same pasta meal (2,015 kJ, 62 g carbohydrate, 26 g protein, and 12 g fat) on the evening before the experimental trials. Participants ingested a telemetric pill (CorTemp, HQ Inc.) before going to sleep on the evening before the experimental trials. Participants were asked to minimize physical activity in the morning of the experimental trials and to travel by motorized transport to the research center. Participants arrived at 08:00 AM after an overnight fast of at least 10 h.

Experimental Trial Protocol

The two experimental trials (Fig. 1) were separated by at least 10 days. At the start of each experimental trial, verbal confirmation of adherence to the standardization procedures was obtained. The first experimental trial involved cold exposure (10°C) via a water-perfused suit (ThermoWrap Universal 3166, MTRE, Israel), with individualized durations, to induce shivering for at least 1 h in all participants. The second experimental trial involved resting, for a time-matched duration, under thermoneutral (32°C) conditions in the same water-perfused suit. Owing to the potential effect of fasting duration on glucose metabolism, it was deemed necessary to time-match the cold exposure and thermoneutral exposure durations. Therefore, a counterbalanced design was not possible, as we anticipated inter-individual variation in the

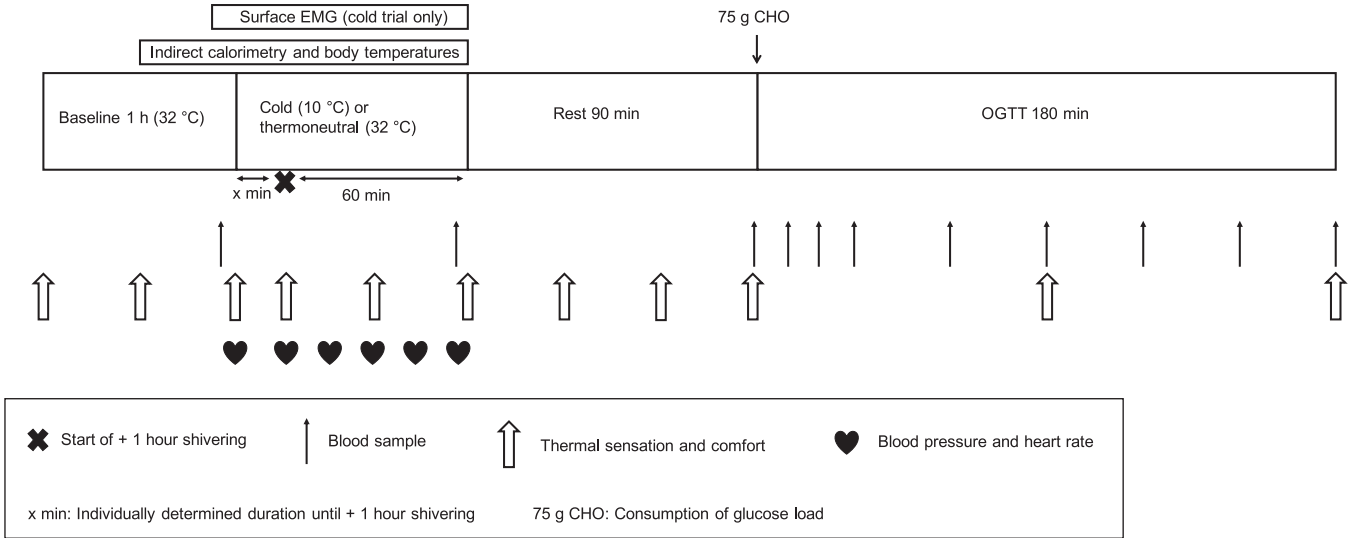


Figure 1. Experimental trial schematic.

metabolic response to cold exposure, and thus, a different duration until shivering was evident for each participant.

In participants wearing only their underwear, wireless skin temperature sensors (iButtons DS1992L, Maxim Integrated) at 14 ISO-defined body sites were attached (22). Following SENIAM guidelines (23), surface electromyography (EMG) electrodes (Trigno, Delsys Inc.) were attached to the skin, that was removed of hair and cleaned thoroughly with gauze and alcohol, above the following muscles on the right side of the body: m. pectoralis major, m. trapezius superior fibers, m. trapezius middle fibers, m. latissimus dorsi, m. vastus lateralis, m. vastus medialis, m. rectus femoris, and m. gastrocnemius medial. Participants were then fitted with the water-perfused suit, which was connected to two cooling towers (Blanket roll III, Gentherm). Participants, with their suits attached, were in the supine position, with their head slightly elevated. A 20-gauge cannula was inserted into their antecubital vein for repeat blood sampling. During both experimental trials, 32°C temperature water was perfused through the suit for a baseline period of 1 h. After 1 h at 32°C, the temperature of the water was reduced to 10°C during the first experimental trial (cold) or maintained at 32°C during the second experimental trial (thermoneutral). The temperature was reduced stepwise from 32°C to 24°C to 17°C to 10°C in 5-min intervals. Because the onset of shivering is variable (24), the duration of the cold exposure was individualized to induce at least 1 h of shivering, as this response indicates contractile activity, and is known to use muscle glycogen (18) and may thus be beneficial for glucose metabolism. Real-time EMG, increases in metabolic rate, and visual inspection combined were used to confirm shivering in each participant, and when shivering was confirmed, the cold exposure lasted an additional 1 h.

After the cold exposure or thermoneutral condition, the water-perfused suit was removed, participants put on a bath robe, and they rested in the same supine position under a duvet for 90 min for passive rewarming. Subsequently, participants consumed 75 g of glucose mixed in 200 mL of water (A23968, Novolab, Belgium). After consumption, participants were not allowed to move from the bed while blood samples were taken frequently for the next 3 h. We opted to perform the OGTT on the same day as the shivering session because glucose uptake increased when intravenous glucose tolerance tests were performed during cold exposure (7, 8, 25). Also, shivering muscles increase their glucose uptake (21). Furthermore, glucose tolerance or insulin sensitivity increased when the OGTT was performed immediately (26), and at 1 h (27), following a single exercise session. Thus, we expected a lasting improvement in glucose metabolism when we performed the OGTT 90-min post shivering.

Measurements and Calculations

Whole body oxygen consumption and carbon dioxide production were determined every 1 min during the final 30 min of the 1-h baseline period and throughout the entire cold exposure and thermoneutral period (Fig. 1) with an automated system using a ventilated hood (Omnical, Maastricht University, The Netherlands). Energy expenditure and rates of substrate oxidation were calculated with equations proposed by Weir (28) and Peronnet and Massicotte (29), respectively. The respiratory exchange ratio (RER) was calculated according to RER = $\dot{V}CO_2/\dot{V}O_2$.

During the final 30 min of the 1-h baseline period and throughout the entire cold exposure and thermoneutral period, skin temperature and core temperature were recorded at 1-min and 20-s intervals, respectively (Fig. 1).

Surface EMG was measured continuously (1926 Hz) starting from the last 10 min of the 1-h baseline period until the end of the cold exposure (Fig. 1). Raw EMG signals were processed by applying a fourth-order Butterworth filter (20–500 Hz) followed by full-wave rectification. The root mean square values (window = 50 ms, 50% overlap) were then determined in 10-min intervals.

Blood pressure and heart rate were determined, with the mean of three repeat measurements, with an automated blood pressure cuff (M6 Comfort IT, Omron, Japan) at the end of the 1-h baseline period and at the onset, the end, and

15, 30, and 45 min into the 1 h of shivering or time-matched thermoneutral condition (Fig. 1). Mean arterial pressure (MAP) was calculated according to: MAP = (1/3 systolic blood pressure) + (2/3 diastolic blood pressure).

Total area under the glucose and insulin curves (AUCs) during the OGTTs was calculated using the trapezoidal rule. Insulin sensitivity was estimated with the Matsuda (30) and insulin-glucose (31) indices.

The Matsuda index was calculated using the formula: $10{,}000/\sqrt{[(G_0 \times I_0) \times (G_{mean} \times I_{mean})]}$, where G_0 is the plasma glucose concentration ($mg \cdot dL^{-1}$) and I_0 is the plasma insulin concentration ($mU \cdot L^{-1}$) at 0 min of the OGTT. G_{mean} is the mean plasma glucose concentration ($mg \cdot dL^{-1}$), and I_{mean} is the mean plasma insulin concentration ($mU \cdot L^{-1}$) of time points 30, 60, 90, and 120 min during the OGTT.

The insulin-glucose index is the product of the total glucose and insulin AUCs during the 3 h of the OGTT, with the result divided by 100,000 for clarity.

Participants completed questionnaires on a laptop, with minimal hand movement, assessing their thermal sensation and comfort throughout the experimental trials (Fig. 1).

Blood Sampling and Biochemical Analyses

Blood was sampled during both experimental trials at the following time points: 5 min before the end of baseline (Pre), 5 min before the end of the additional 1 h at 10°C or 32°C (Post), immediately before the OGTT (BO), and 10, 20, 30, 60, 90, 120, 150, and 180 min after the start of the OGTT (Fig. 1). Blood samples for plasma glucose and lactate determination were collected into sodium fluoride tubes. Samples for plasma insulin determination were collected into EDTA tubes. Blood samples for serum free fatty acids (FFA) and triglyceride (TG) determination were collected into gel tubes and allowed to coagulate for 30 min at room temperature. Plasma and serum were collected after centrifuging for 10 min at 4°C or 21°C, respectively. Aliquots were frozen in liquid nitrogen and stored at −80°C until subsequent analysis. At all sample time points, hemoglobin concentration was determined immediately using an automated analyzer (ABL805 Flex, Radiometer, Denmark) and hematocrit was determined, manually, in triplicates after centrifugation of glass capillary tubes. Plasma volume changes during both experimental trials were determined according to Dill and Costill (32). The biochemical values reported are uncorrected for changes in plasma volume. Plasma glucose (HK CP, Horiba ABX, France), plasma lactate (Roche diagnostics, Switzerland), serum free fatty acids (Wako Chemicals, Germany), and serum triglycerides corrected for free glycerol (Sigma-Aldrich) were determined in duplicate with a Pentra C400 spectrophotometer (Horiba ABX). Plasma insulin was determined in duplicate by enzyme immunoassay (Crystal Chem). Samples from each participant were analyzed in the same run. The mean within-assay coefficient of variations were: glucose, 0.8%; lactate, 1.3%; FFA, 2.1%; TG, 2.8%; and insulin, 6.5%.

Statistical Analysis

All data were analyzed using IBM Statistical Package for the Social Sciences (SPSS) Statistics (v 25 for Mac, SPSS). Data are presented as means ± SE, with values as x ± y, unless stated otherwise. Trial differences in the activity performed before the trials, total energy expenditures, glucose and insulin AUCs, and the insulin sensitivity indices were assessed by two-tailed paired *t* tests or Wilcoxon signed-rank tests if the data were not normally distributed. Skeletal muscle electrical activity during the cold exposure was assessed by one-way repeated-measures ANOVA. The participants' thermal perception and comfort, body temperature, and cardiovascular and metabolic responses were assessed with two-way (intervention × time) repeated-measures ANOVAs. The Greenhouse–Geisser method was used if the sphericity assumption for repeated-measures ANOVA was violated. Holm–Bonferonni multiple-comparisons post hoc test was used to explore significant main or interaction effects. Effect sizes are presented as Cohen's *d* and interpreted as ≥0.2 small, ≥0.5 medium, and ≥0.8 large (33). Statistical significance was accepted when $P < 0.05$.

RESULTS

Physical Activity 2 Days Before the Trials

Mean physical activity durations, in the 2 days before the cold exposure and thermoneutral test days, were not significantly different [sedentary (including sleeping): 1111.2 ± 40.4 vs. 1096 ± 33.7 min, standing: 204.2 ± 28.2 vs. 214.2 ± 27.2 min, low physical activity: 15.3 ± 2.1 vs. 14.4 ± 1.4 min, moderate physical activity: 67.6 ± 9.1 vs. 67.8 ± 6.7 min, vigorous physical activity: 41 ± 7.5 vs. 43.8 ± 7.7 min, all $P \geq 0.571$, $n = 14$].

Skin and Core Temperatures

Mean skin temperature (Fig. 2*A*) showed an intervention, time, and interaction effect (all $P < 0.001$). Post hoc tests revealed that mean skin temperature decreased during the cold exposure (all $P < 0.001$) but not during the thermoneutral trial (all $P \geq 0.587$).

Core temperature (Fig. 2*B*) showed an intervention ($P = 0.009$) and interaction ($P = 0.019$) effect but no time effect ($P = 0.133$). Post-hoc tests did not identify any significant differences during the trials. No participant's core temperature decreased below 35°C during the cold exposure.

Skeletal Muscle Electrical Activity

During the cold exposure, the electrical activity of the m. trapezius superior fibers, m. trapezius middle fibers, m. pectoralis major, m. latissimus dorsi, m. vastus medialis, m. vastus lateralis, and m. rectus femoris increased over time (all $P \leq 0.044$) except for the m. gastrocnemius medial ($P = 0.133$). Post hoc tests identified that the electrical activity of the seven muscles significantly increased during the 1 h of shivering compared with that in resting at 32°C before the cold exposure (Fig. 3).

Cold Exposure Duration, Energy Expenditure, and Substrate Oxidation

The mean duration of the cold exposure was 76 ± 2 min (range: 70–95 min). Total energy expenditure during the entire cold exposure (time until shivering plus 1 h of shivering) was 717 ± 31 kJ (range: 539–948 kJ), which was larger ($P < 0.001$) than the 392 ± 17 kJ (range: 305–537 kJ) during the time-matched thermoneutral exposure. Total energy

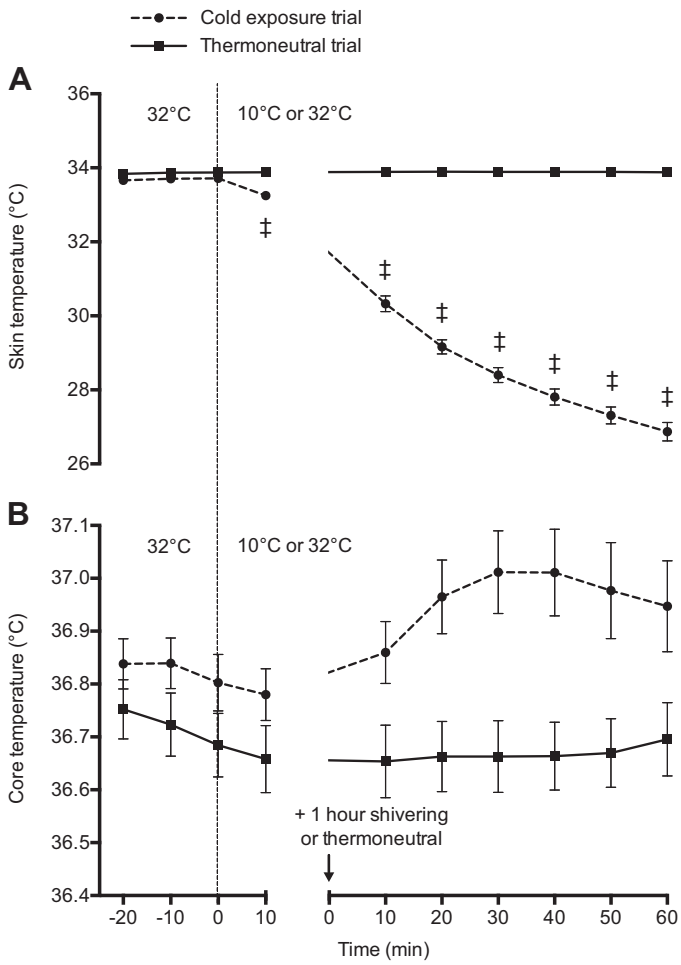


Figure 2. Mean skin temperature (*A*) and core temperature (*B*) during the thermal exposure of the experimental trials ($n = 15$). The break in the *x*-axis represents the varying time until the start of the 1 h of shivering or 1 h additional thermoneutral exposure. The first 10 min and last 60 min of the cold exposure and time-matched thermoneutral condition was common in all participants and is thus shown. Data were analyzed with a two-way repeated-measures ANOVA with Holm–Bonferonni post hoc testing used to locate differences. Significance is indicated by: ‡$P < 0.001$. Significance markers denote within-trial differences when compared with the last 10 min when resting at 32°C before the cold or time-matched thermoneutral exposure.

expenditure during the 1 h of shivering was 619 ± 23 kJ (range: 451–735 kJ), which was greater ($P < 0.001$) than the 309 ± 7 kJ (range: 266–360 kJ) during the time-matched thermoneutral exposure.

Energy expenditure (Fig. 4*A*) showed an intervention, time, and interaction effect (all $P < 0.001$). Post-hoc tests revealed that energy expenditure was larger at all time points during the cold exposure compared with that in resting at 32°C before the cold exposure (all $P \leq 0.001$). Energy expenditure did not change during the thermoneutral trial (all $P \geq 0.078$).

The respiratory exchange ratio (Fig. 4*B*) showed an interaction ($P < 0.001$) and time effect ($P = 0.002$), but no intervention effect ($P = 0.986$). Post hoc tests did not identify any significant differences during the trials.

Carbohydrate oxidation (Fig. 4*C*) showed an intervention ($P < 0.001$), time ($P = 0.018$), and interaction effect ($P = 0.010$). Post hoc tests did not identify any significant differences during the trials.

Fat oxidation (Fig. 4*D*) showed an intervention, time, and interaction effect (all $P < 0.001$). Post-hoc tests identified larger fat oxidation during the 1 h of shivering compared with that in resting at 32°C before the cold exposure (all $P \leq 0.001$). Fat oxidation did not change during the thermoneutral trial (all $P \geq 0.176$).

Heart Rate and Mean Arterial Blood Pressure

Heart rate (Fig. 5*A*) showed an intervention ($P < 0.001$), time ($P = 0.003$), and interaction effect ($P < 0.001$). Post hoc tests identified that heart rate was significantly higher during the 1 h of shivering than during resting at 32°C before the cold exposure (all $P \leq 0.002$). Heart rate did not change during the thermoneutral trial (all $P \geq 0.159$).

Mean arterial blood pressure (Fig. 5*B*) showed an intervention, time, and interaction effect (all $P < 0.001$). Post-hoc tests identified that the mean arterial blood pressure was significantly higher during the 1 h of shivering, except at time point 30 min, than during resting at 32°C before the cold exposure (all $P \leq 0.002$). Mean arterial blood pressure did not change during the thermoneutral trial (all $P \geq 0.519$).

Substrate Concentrations Before the Oral Glucose Tolerance Test

Plasma substrates were measured before and after the cold exposure (or thermoneutral condition) and just before the OGTT.

Free fatty acids (FFA) concentrations (Table 2) showed a time effect ($P < 0.001$), an interaction effect ($P = 0.015$), and tended to show an effect of intervention ($P = 0.064$). Post-hoc analysis identified that in the cold exposure trial, FFA increased after the cold exposure ($P = 0.004$) and remained increased before the OGTT ($P < 0.001$).

Glucose concentrations (Table 2) did not show an intervention or interaction effect (both $P \geq 0.237$), although glucose concentrations did change over time ($P = 0.034$). Post hoc tests did not identify any significant differences.

Insulin concentrations (Table 2) did not show any significant intervention, time, or interaction effect (all $P \geq 0.058$).

Triglyceride concentrations (Table 2) showed an intervention ($P = 0.014$) and interaction effect ($P = 0.048$), but triglyceride concentrations did not change over time ($P = 0.121$). Post hoc analysis revealed that triglyceride concentration increased after the cold exposure ($P = 0.008$).

Lactate concentrations (Table 2) showed an intervention and time effect (both $P \leq 0.004$), and there was a tendency for an interaction effect ($P = 0.055$). Post-hoc analysis did not identify any significant differences.

Oral Glucose Tolerance Test Responses

Prior cold exposure increased plasma glucose (Fig. 6*A*) and insulin concentrations (Fig. 6*B*) during the OGTT. The area under the glucose curve (Fig. 6*C*) tended to increase by 4.8% with cold exposure compared with that in the thermoneutral condition (1387.8 ± 37.6 vs. 1324.1 ± 33.3 mmol·L^{-1} 180 min, $P = 0.066$, $d = 0.46$). The area under the insulin

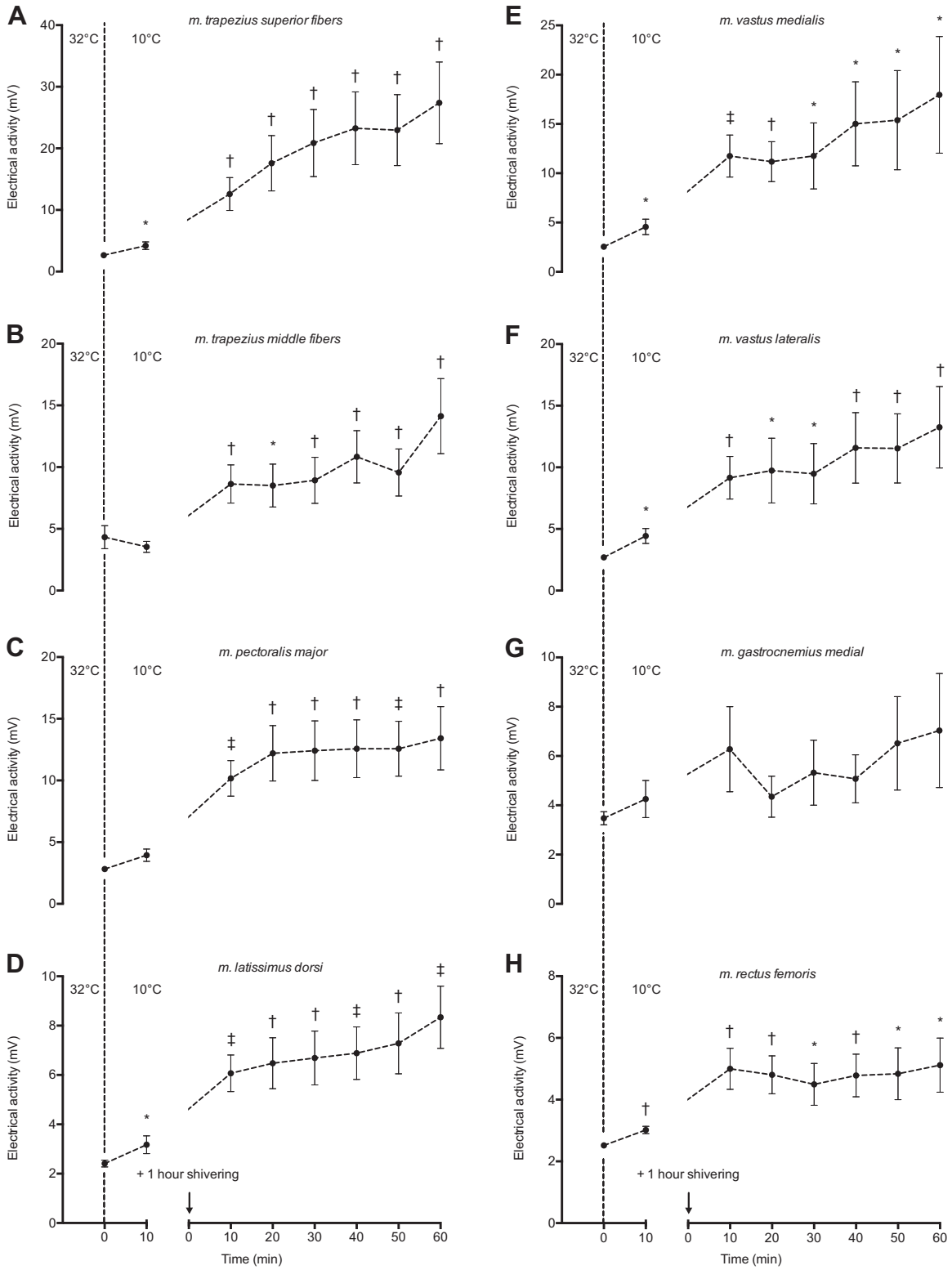
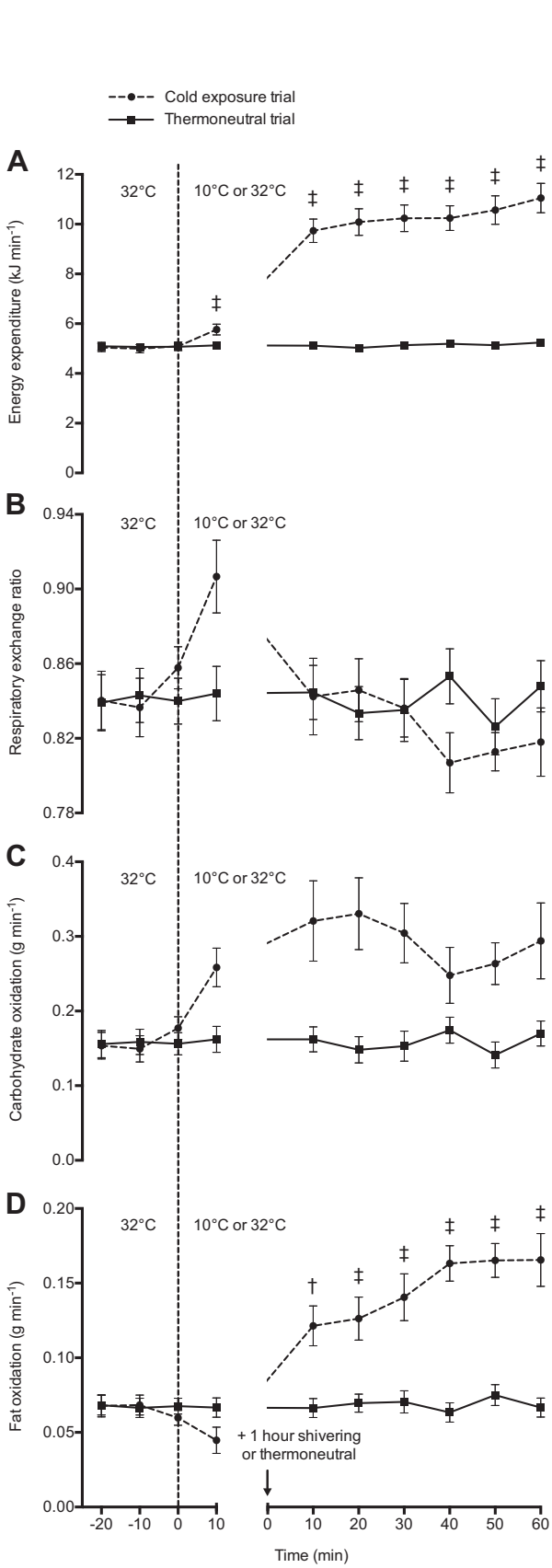


Figure 3. Mean electrical activity, measured 10 min before the cold exposure when resting at 32°C and during the cold exposure ($n = 14$), of the following muscles: m. trapezius superior fibers (*A*), m. trapezius middle fibers (*B*), m. pectoralis major (*C*), m. latissimus dorsi (*D*), m. vastus medialis (*E*), m. vastus lateralis (*F*), m. gastrocnemius medial (*G*), and m. rectus femoris (*H*). The break in the x-axis represents the varying time until the start of the 1 h of shivering. Data were analyzed with a one-way repeated-measures ANOVA with Holm–Bonferonni post hoc testing used to locate differences. Significance is indicated by: $^{*}P < 0.05$, $^{\dagger}P < 0.01$, and $^{\ddagger}P < 0.001$. Significance markers denote within-trial differences when compared with the last 10 min when resting at 32°C before the cold exposure.



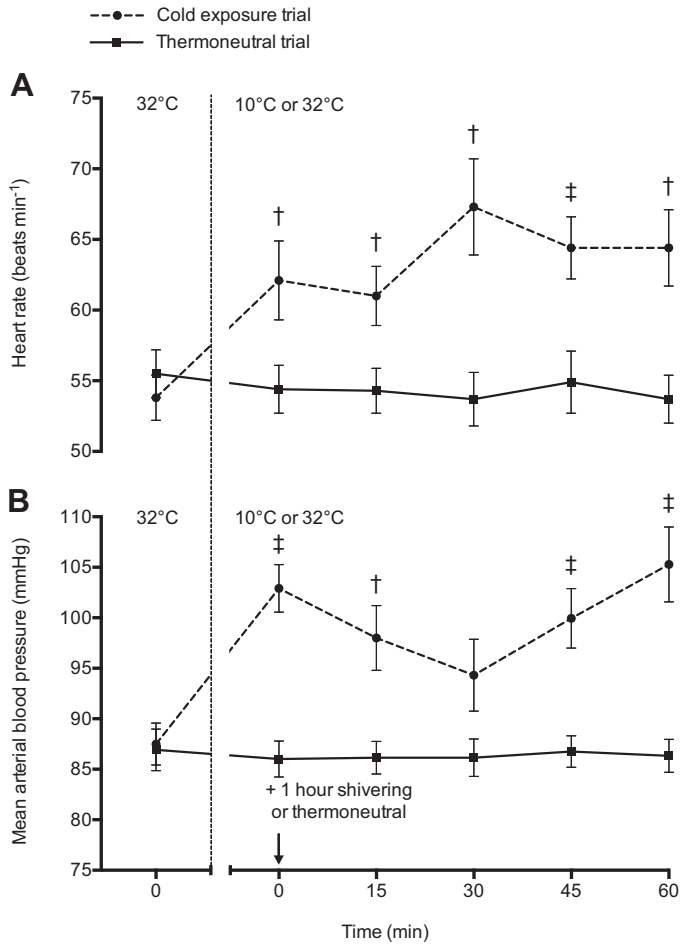


Figure 5. Heart rate (*A*) and mean arterial blood pressure (*B*) when resting at 32°C and during the 1 h of shivering or time-matched thermoneutral exposure. In some participants, shivering disturbed the measurement and resulted in missing values, therefore, $n = 12$. The break in the *x*-axis represents the varying time until the start of the 1 h of shivering or 1 h of additional thermoneutral exposure. Data were analyzed with a two-way repeated-measures ANOVA with Holm–Bonferonni post hoc testing used to locate differences. Significance is indicated by: †$P < 0.01$ and ‡$P < 0.001$. Significance markers denote within-trial differences when compared with resting at 32°C before the cold exposure or thermoneutral exposure.

curve (Fig. 6*D*) increased by 24% upon cold exposure compared with that in the thermoneutral condition (5,729 ± 902.9 vs. 4619.5 ± 818.3 mU·L^{-1} 180 min, $P = 0.112$, $d = 0.33$).

The Matsuda index (Fig. 7*A*) decreased by 21% after cold exposure compared with that in the thermoneutral condition (11.9 ± 1.4 vs. 15 ± 2.8 mg·dL^{-1}mU·L^{-1}, $P = 0.125$, $d = 0.38$). The insulin-glucose index (Fig. 7*B*) tended to be higher by 30% upon cold exposure than in the thermoneutral condition

Figure 4. Mean energy expenditure (*A*), respiratory exchange ratio ($\dot{V}CO_2/\dot{V}O_2$) (*B*), carbohydrate oxidation (*C*), and fat oxidation (*D*) during the thermal exposure of the experimental trials ($n = 15$). The break in the *x*-axis represents the varying time until the start of the 1 h of shivering or 1 h additional thermoneutral exposure. Data were analyzed with a two-way repeated-measures ANOVA with Holm–Bonferonni post hoc testing used to locate differences. Significance is indicated by: †$P < 0.01$ and ‡$P < 0.001$. Significance markers denote within-trial differences when compared with the last 10 min when resting at 32°C before the cold or time-matched thermoneutral exposure.

Table 2. *Substrate concentrations measured at 32°C (Pre), at the end of the 1 h shivering or thermoneutral condition (Post) and immediately before the OGTT (BO) during both experimental trials*

Parameter	Trial	Blood Sample Time Point		
		Pre	Post	BO
FFA, μmol·L^{-1}	Thermoneutral	357.7 ± 44.2	395.8 ± 55.1	491.8 ± 70.5
	Cold	364.6 ± 34	616.8 ± 88.4†	620.1 ± 49.4‡
Glucose, mmol·L^{-1}	Thermoneutral	5.44 ± 0.08	5.41 ± 0.09	5.32 ± 0.08
	Cold	5.37 ± 0.12	5.3 ± 0.09	5.22 ± 0.11
Insulin, mU·L^{-1}	Thermoneutral	1.9 ± 0.2	2 ± 0.3	2 ± 0.3
	Cold	2.5 ± 0.4	2.6 ± 0.5	2.1 ± 0.3
TG, mmol·L^{-1}	Thermoneutral	0.86 ± 0.16	0.86 ± 0.16	0.89 ± 0.17
	Cold	1.01 ± 0.17	1.14 ± 0.2†	1.08 ± 0.21
Lactate, mmol·L^{-1}	Thermoneutral	0.88 ± 0.07	0.85 ± 0.06	0.78 ± 0.07
	Cold	0.94 ± 0.06	1.22 ± 0.13	0.96 ± 0.06

Owing to the inability to collect a blood sample in one participant after the cold exposure, n = 14. Data were analyzed with a two-way repeated-measures ANOVA with Holm–Bonferonni post hoc testing used to locate differences. Significance is indicated by: $^{\dagger}P < 0.01$ and $^{\ddagger}P < 0.001$. Significance markers denote within-trial differences compared with baseline (Pre). FFA, free fatty acids; TG, triglycerides.

(80.4 ± 13.7 vs. 62.1 ± 11.9 mmol·L^{-1} 180 min mU·L^{-1} 180 min 10^{-5}, P = 0.100, d = 0.37).

Plasma Volume Changes

Plasma volume changes (Fig. 8) showed an intervention, time, and interaction effect (all $P < 0.001$). Post hoc tests identified that, when compared with baseline, cold exposure reduced plasma volume at all time points up to and including 90 min into the OGTT (all $P < 0.001$). During the thermoneutral trial, plasma volume changes were not significantly different.

Comfort and Thermal Sensation During the Experimental Trials

Perceived comfort (Fig. 9*A*) showed an intervention (P = 0.002), time ($P < 0.001$), and interaction effect ($P < 0.001$). Post hoc tests identified that participants were more uncomfortable during the 1 h of shivering than during resting at 32°C before the cold exposure (all $P \leq 0.002$). Comfort did not change during the thermoneutral trial (all $P \geq 0.066$).

Thermal sensation (Fig. 9*B*) showed an intervention, time, and interaction effect (all $P < 0.001$). Post hoc tests identified that participants were colder during the 1 h of shivering than during resting at 32°C before the cold exposure (all $P < 0.001$). Participants reported to be warmer 90 min into the OGTT than during resting at 32°C before the cold exposure (P = 0.003). Thermal sensation did not change during the thermoneutral trial (all $P \geq 0.205$).

DISCUSSION

The present study aimed to determine the effect of an individualized cold exposure, with at least 1 h of shivering, on glucose tolerance in healthy men. The individualized cold exposure reduced skin temperature and slightly increased core temperature. During the last 1 h of cold exposure with shivering, the electrical activity of seven skeletal muscles increased, which was accompanied with an on average doubled resting metabolic rate. In contrast to our hypothesis, the cold exposure did not increase glucose tolerance during an oral glucose tolerance test performed, 90 min after the cold exposure, under thermoneutral conditions. Instead, plasma glucose and insulin concentrations increased with prior cold exposure, and the insulin sensitivity indices indicate insulin resistance. Plasma FFA concentrations were elevated at the end of the cold exposure and immediately before the OGTT, which may have influenced the glucose and insulin responses during the OGTT.

Initially, the cold exposure was expected to increase glucose tolerance and insulin sensitivity when compared with the thermoneutral condition. However, our results differ from the insulin sensitizing effect of cold acclimation in patients with type 2 diabetes (11). Along with the population, another notable difference between the two studies is the time when the glucose metabolism measurement was performed. In the present study, glucose metabolism was measured 90 min after the cold exposure, whereas in the study by Hanssen et al. (11), glucose metabolism was measured the day following the final cold exposure. In this context, it is interesting to compare our results with the effect of exercise on glucose tolerance. Thus, in moderately trained adults, glucose and insulin concentrations were also higher when an OGTT was performed 30 min after exercise than when the OGTT was performed 1 day after the exercise (31). This result suggests that some time period and/or event, such as feeding, between the intervention and subsequent improvement in glucose homeostasis is needed. However, it should be noted that glucose tolerance and insulin sensitivity still improved when the OGTT was performed immediately (26), or 1 h (27), post exercise in less-trained individuals or in adults with prediabetes, respectively. Thus, the results of the current study may not generalize to other, more insulin-resistant, populations.

During the present study, cold exposure significantly increased plasma FFA concentrations, which were still elevated immediately before the OGTT. The differences in FFA concentration were still significant even after correcting for plasma volume changes. Our finding agrees with those of other reports of cold exposure inducing lipolysis and increasing FFA concentration (34, 35). The increased FFA concentrations owing to cold exposure in our study could explain the increased glucose and insulin concentrations during the OGTT and possible insulin resistance. This is due to FFA inhibiting glucose uptake (36) combined with the finding that insulin secretion can be increased in response to counter acute FFA-induced insulin resistance (37).

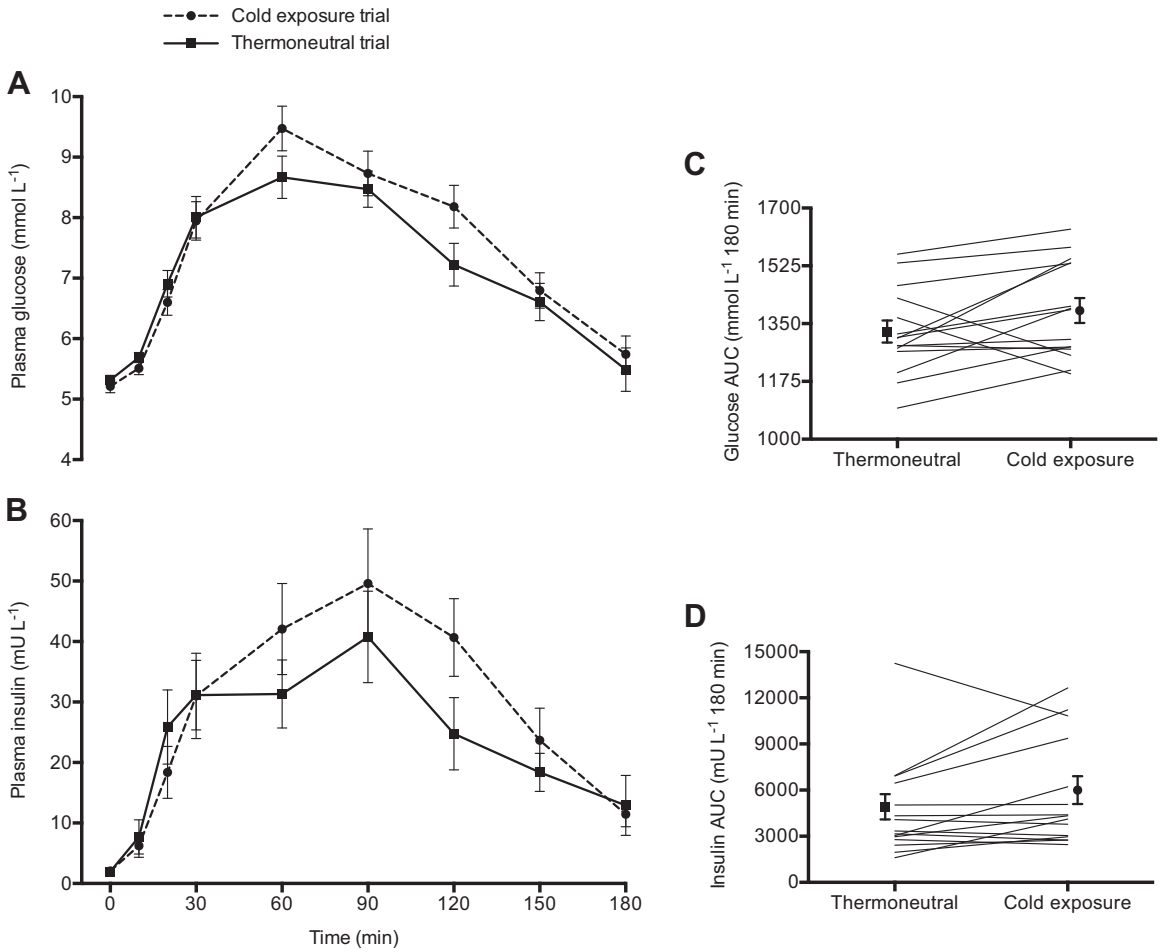


Figure 6. Mean plasma glucose concentrations (*A*), mean plasma insulin concentrations (*B*), and mean ± SE with the participant's ($n = 15$) individual responses of the area under the glucose (*C*) and insulin (*D*) curves during the 3-h OGTT after the thermal exposures. Area under the glucose curve data were analyzed with a paired *t* test. Area under the insulin curve data were analyzed with a Wilcoxon signed-rank test. OGTT, oral glucose tolerance test.

Our findings agree with those of other experiments, which also report decreased glucose metabolism, in healthy participants, in the recovery period after exercise. Specifically, when subjects cycled 2 h before an insulin clamp, at 70% maximal oxygen consumption ($\dot{V}O_{2max}$) for 82 min, glucose oxidation reduced and fat oxidation increased during insulin infusion 2–4 h after exercise (38). Additionally, without prior exercise, the insulin infusion increased forearm glucose uptake. However, cycling before the insulin infusion prevented the insulin-stimulated increase in forearm glucose uptake (38). Furthermore, exercising at 73% of $\dot{V}O_{2peak}$ for 45 min, followed by an OGTT 30 min later, resulted in larger insulinemia and glycemia during the OGTT compared with repeating the OGTT 1 and 3 days after the exercise session (31).

As the OGTT is a measure of whole-body glucose metabolism, it can only be suggested in which tissues insulin resistance may have occurred. Skeletal muscle groups do not contribute equally to cold-induced thermogenesis (39). Therefore, skeletal muscles that were the most activated could have been insulin sensitized, whereas inactive muscles may have become more insulin resistant when compared with the thermoneutral trial, a response similar to that suggested post exercise (38). Additional support for muscle-specific responses is provided in an experiment that combined euglycemic-hyperinsulinemic clamps with measures of individual leg glucose uptake, on days with and without one-legged exercise (40). Whole body glucose infusion rate reduced post exercise, which was accompanied by increased insulin action in the exercised leg, whereas insulin action reduced in the inactive leg (40). Thus, the shivering may have improved glucose uptake in some activated muscles, although this could have been masked by the greater overall decrease in insulin action in less active tissues.

In addition to the contribution of skeletal muscle, the liver may have been involved in the responses to the OGTT. During an oral glucose tolerance test, endogenous glucose production is not suppressed completely (41). Also, cold exposure increases hepatic glucose output (42), which may be explained by increases in plasma concentrations of norepinephrine (43, 44) and glucagon (45) during cold exposure. Moreover, when plasma FFA are elevated, endogenous glucose production and total glucose output after ingestion of a glucose load are increased (46). Therefore, if endogenous glucose production was still increased post shivering, which may be due to the continued elevation of counterregulatory

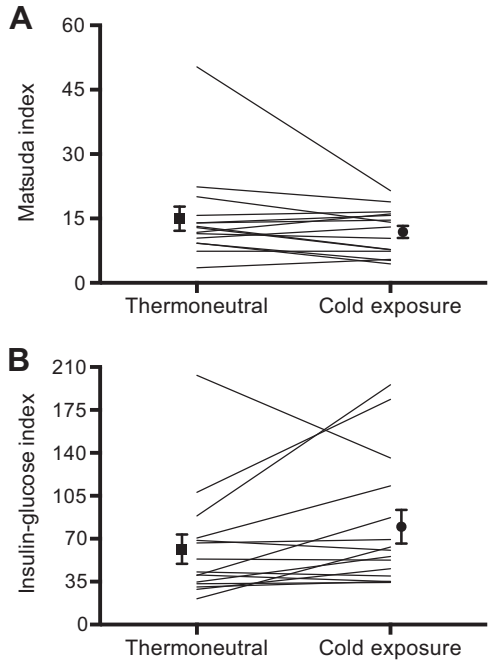


Figure 7. Means ± SE and the participant's ($n = 15$) individual responses of the Matsuda index (mg·dL^{-1}mU·L^{-1}) (A) and the insulin-glucose index (mmol·L^{-1} 180 min mU·L^{-1} 180 min 10^{-5}) (B). Data were analyzed with Wilcoxon signed-rank tests.

hormones, and/or the increased plasma FFA concentration, this may have increased total glucose output and thus insulin concentrations during the post-shivering OGTT.

As well as the possible insulin resistance induced by increased FFA concentrations, the role of muscle blood flow should be considered. Cold exposure alters skeletal muscle perfusion (47). Thus, as muscle blood flow is a determinant of insulin action (48, 49), it is unknown if the cold exposure influenced muscle blood flow during the OGTT, consequently altering glycemia and insulinemia.

Additionally, the gut should be considered in the context of the current findings. Glucose appearance in the systemic circulation following an oral glucose load is variable, but it has been determined to be 73% of the total ingested load 3.5 h after consumption (41). Thus, changes in the appearance of glucose could alter glycemia and insulinemia during the OGTT. The onset of gastric emptying does not appear to be altered post shivering because the glucose concentrations during the initial 30 min of the OGTT are similar between conditions. However, intestinal glucose absorption may have been altered. In the recovery period following 1 h of submaximal cycling, glycemia and the rate of oral glucose appearance during an OGTT increased in healthy men (50). It was unknown if this was due to increased intestinal absorption and/or reduced hepatic glucose uptake (50). Nonetheless, with the use of invasive techniques in dogs, it was determined that prior exercise enhanced the intestinal absorption of glucose (51, 52). It is unknown if the intestinal absorption of glucose increased following cold exposure.

Cold exposure reduces plasma volume (13); thus, it could be argued that the increases in plasma glucose and insulin during the OGTT, after cold exposure, are just reflecting the plasma volume changes. As expected, cold exposure decreased plasma volume by 12.9%, which was similar to a 12% reduction in plasma volume after 90 min of cold (5°C) air exposure (53). After correcting for plasma volume changes, average glucose and insulin AUCs during the OGTT were still larger following cold exposure by 1.2% and 20%, respectively. Notably, following cold exposure, we found that plasma volume was still lower after 90 min of rest, which is longer than the recommended 20-min rest to account for plasma volume changes after postural changes (54). Thus, care should be taken when interpreting changes in plasma parameters during and following cold exposure.

The reduced plasma volume immediately before the OGTT suggests that the dilution space for glucose was reduced. Therefore, this may have altered glycemia and insulinemia during the OGTT of the cold exposure trial. However, evidence does not support the idea that the reduced plasma volume is the main cause of the increased plasma glucose and insulin. Specifically, Dandanell et al. (55) determined glucose tolerance before and after 3 days of bed rest. Bed rest reduced plasma volume by 9.9% and increased glucose and insulin concentrations during the OGTT. On the fourth day, plasma volume was restored by albumin infusion, and even after restoring plasma volume, glucose tolerance was not improved (55). Additionally, when adults were dehydrated for 1 h in a heat tent with subsequent fluid restriction or rehydration, the participants' glucose and insulin responses to an OGTT the morning after were similar in the hypohydrated and rehydrated state (56). Thus, the reduced plasma volume before the OGTT is unlikely to have contributed to the increased glycemia and insulinemia following cold exposure.

A limitation of the research was the lack of objective criteria for determining the onset of the 1 h of shivering. We

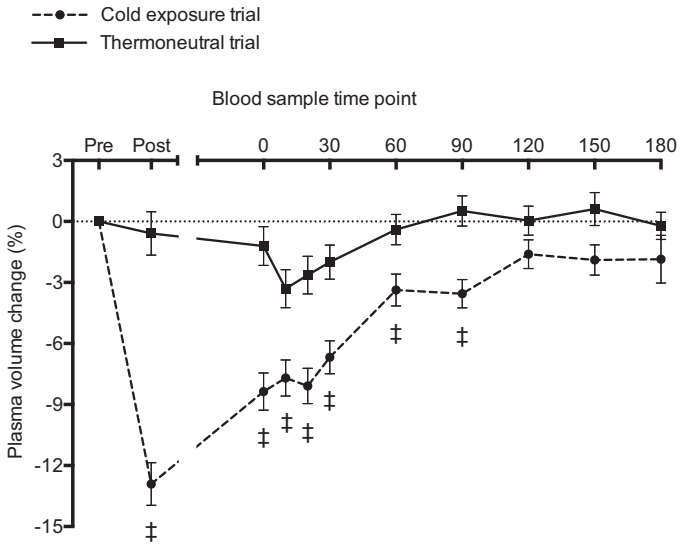


Figure 8. Plasma volume changes determined at 32°C (Pre), at the end of the 1 h shivering or thermoneutral exposure (Post), and immediately before and during the 3-h OGTT of both experimental trials ($n = 14$). The break in the x-axis separates the thermal exposure from the OGTT. Data were analyzed with a two-way repeated-measures ANOVA with Holm–Bonferonni post hoc testing used to locate differences. Significance is indicated by: ‡$P < 0.001$. Significance markers denote within trial-differences when compared with baseline (Pre).

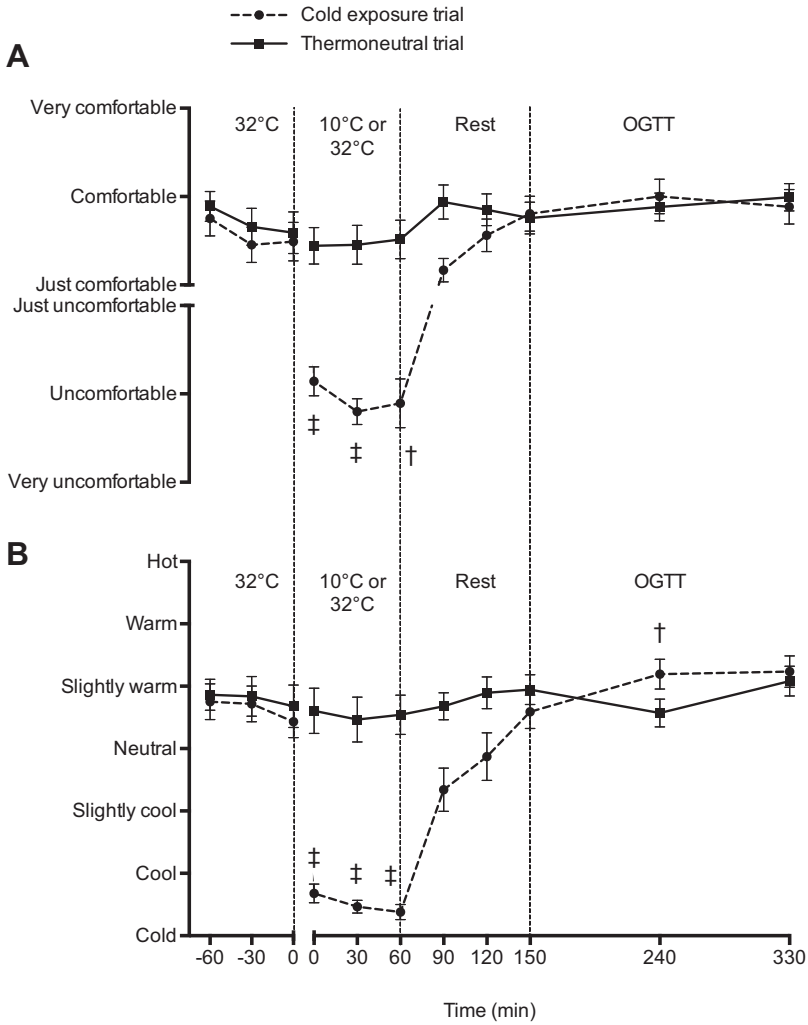


Figure 9. Perceived comfort (*A*) and thermal sensation (*B*) during the: 1 h rest at 32°C, 1 h of shivering (10°C) or thermoneutral (32°C) exposure, 90 min rest and the 3-h OGTT ($n = 13$). The break in the *x*-axis represents the varying time until the start of the 1 h of shivering or 1 h of additional thermoneutral exposure. Data were analyzed with a two-way repeated-measures ANOVA with Holm–Bonferonni post hoc testing used to locate differences. Significance is indicated by: $^{\dagger}P < 0.01$ and $^{\ddagger}P < 0.001$. Significance markers denote within-trial differences when compared with resting at 32°C immediately before the cold exposure or time-matched thermoneutral exposure. OGTT, oral glucose tolerance test.

confirmed that participants were shivering via increased energy expenditure, real-time EMG, and visual inspection. We decided to individualize the cold exposure based on an individual shivering, as shivering is an indicator of mechanical and metabolic stress and may be beneficial for glucose metabolism. However, the shivering response was diverse among individuals; thus, as previously discussed (57), what actually amounts to shivering could be debated. Future research remains to be done that will use more strictly defined objective criteria, specifically regarding predefined energy expenditure changes, to enhance reproducibility.

In conclusion, this study shows that cold exposure, with at least 1 h of shivering, 90 min before an OGTT, does not increase glucose tolerance in healthy men, which is in contrast to our hypothesis. Instead, insulin sensitivity indices indicate insulin resistance post shivering. As cold exposure stimulates lipolysis, it is possible that the mobilized FFA influenced glucose metabolism during the OGTT. It is unknown if changes in muscle blood flow or the appearance of glucose in the systemic circulation post shivering also contributed to the altered glucose metabolism. Future research will investigate the effect of single, and repeat, cold-induced shivering sessions on glucose metabolism the day following cold exposure.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

A.J.S., H.P., P.S., and W.D.v. conceived and designed research; A.J.S., P.R., and E.M-K performed experiments; A.J.S. and W.B. analyzed data; A.J.S., H.P., T.v., P.S., and W.D.v. interpreted results of

experiments; A.J.S. prepared figures; A.J.S. drafted manuscript; A.J.S., H.P., P.S., and W.D.v. edited and revised manuscript; A.J.S., H.P., P.R., W.B., T.v., E.M-K, P.S., and W.D.v. approved final version of manuscript.

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