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Table 1. Training Program[a]

Day	Before Lunch	Training Intensity	After Lunch	Training Intensity
Monday	CRYO (9:30 AM)		CRYO (5:30 PM)	
	Training A (10:15–11:00 AM)	Low	Training D (7:00–8:00 PM)	Moderate
	Training B (12:00 PM–1:30 PM)	60% of 1 RM		
	Training C (1:45–2:30 PM)	Moderate		
Tuesday	CRYO (9:30 AM)		CRYO (5:30 PM)	
	Training A (10:15–11:00 AM)	Low	Training F (7:00–8:00 PM)	Moderate
	Training E (12:00 AM–2:00 PM)	Moderate		
Wednesday	CRYO (9:30 AM)		CRYO (5:30 PM)	
	Training A (10:15–11:00 AM)	Low		
	Training B (12:00 PM–1:30 PM)	60% of 1 RM		
	Training C (1:45–2:30 PM)	Moderate		
Thursday	CRYO (9:30 AM)		CRYO (5:30 PM)	
	Training A (10:15–11:00 AM)	Low	Training A (6:30–7:30 PM)	Low
	Training G (12:00 PM–1:00 PM)	Moderate		
	Training E (1:15–2:00 PM)	High		
Friday	CRYO (9:30 AM)		CRYO (5:30 PM)	
	Training A (10:15–11:00 AM)	Low		
	Training B (12:00 PM–1:30 PM)	60% of 1 RM		
	Training C (1:45–2:30 PM)	Moderate		
Saturday and Sunday	Rest			

Abbreviations: CRYO, whole-body cryostimulation; RM, repetition maximum.

[a] Training A: Stretching exercise, hold-relax technique.
Training B: Strength training for local strength endurance (8 basic tennis exercises, each at 60% of 1 RM, involving arms and shoulders as follows: bench press, dumbbell pullovers, T-bar rows, reverse curls; legs as follows: squats, lunges; trunk as follows: crunches, dumbbell side bends).
Training C: Agility games with tennis balls on small court (main stress on coordination, agility, accuracy).
Training D: Conditioning exercise, team sports; volleyball: short games with short periods (few seconds) with high intensity, average heart rate at 80% to 90% maximum. Most vital elements during games were appropriate mechanical performance of exercises and scoring maximum number of points.
Training E: Conditioning exercise, team sports; soccer: short games with short periods (few seconds) with high intensity, average heart rate at 80% to 90% maximum. Most vital elements during games were appropriate mechanical performance of exercises and scoring maximum number of points.
Training F: Ice skating with main focus on balance and free style; average heart rate 60% to 70% of maximum.
Training G: Endurance, continuous distance running for 60 minutes, average heart rate 70% to 80% of maximum.

Exposure to extremely low temperatures may initiate theromogenesis, with or without shivering, to increase metabolic heat production to 2- to 5-fold resting levels.[13] Consequently, such cold exposure may accelerate the resting metabolic rate (RMR).

Our experiment was conducted in November and December, after the end of the competitive tennis season. This short break is considered both a regenerative period after the previous season and a preseason for the next year. Only a few authors have investigated this midseason period, and they mostly focused on the detraining effect.[14,15] Therefore, the purpose of our study was to examine the effects of whole-body cryostimulation in conjunction with a moderate-intensity training program on professional tennis players during the midseason break. We assumed that whole-body cryostimulation would influence the immunologic, hormonal, and hematologic responses; resting metabolic rate; and tennis performance and thus contribute to a more effective muscle-recovery process.

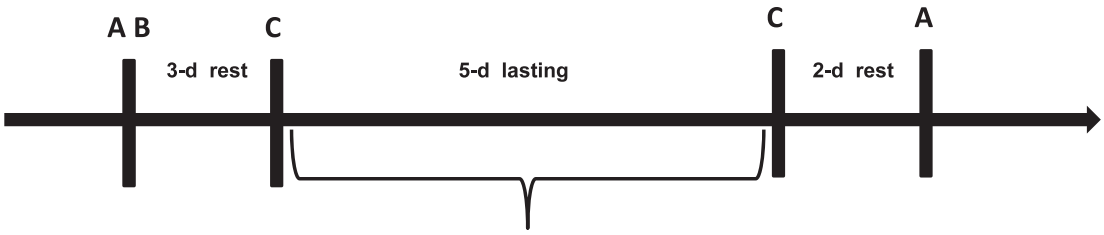
METHODS

Participants

Twelve high-ranking professional, national-level, male tennis players (ATP singles ranking = 150–900, ATP doubles ranking = 11–15) took part in our experiment during a 2-week tennis camp at the National Olympic Sport Centre in Cetniewo, Poland, during the November-to-December break in the season. All participants lived in the same accommodations and followed the same training schedule and diet. The daily energy content of food offered in the menu did not exceed 4200 kcal. The recommended protein dose varied from 1.3 to 1.5 g/kg of body mass.

We designed the experimentation schedule and collaborated on all details of the training program with the coaches (Figure, Table 1). The main portion of the study consisted of 5 days of moderate-intensity training and cryostimulation applied twice a day. Before this stage, we assessed body composition, resting energy expenditure, maximal aerobic capacity, and cytokine and hormone levels (Figure: time A,B). The assessment was followed by 3 days of rest; the tennis drill was performed on the fourth day (time C). After the training and cryostimulation program was completed, the tennis drill was repeated on the following day. The participants rested for the 2 following days, after which their cytokine levels and resting energy expenditure were assessed again (time A).

The course of the main experiment differed for the cryostimulation (CRS; n = 6) and control (CON; n = 6) groups, to which participants were randomly assigned. However, in 2 cases, we took the participants' known hypersensitivity to low temperatures into consideration and assigned them to CON. Both groups followed the same training schedule during the main stage of the experiment. All the details of the training program reported by the



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Tennis Drill

The movement used in the tennis drill was exactly the same as in the $\dot{V}O_2$max test described earlier except that intensity remained constant at the level of the individual's anaerobic threshold. The frequency of the ball machine was 16 to 18 balls per minute. The average oxygen uptake at the anaerobic threshold level corresponded to the average intensity of a tennis match.[19] The activity consisted of two 6-minute exercises separated by a 6-minute break; therefore, the work-to-rest ratio equaled 1:1. Breath-by-breath pulmonary gas measurement was also used to control exercise intensity. During the exercise, the stroke effectiveness was recorded. Additionally, in order to determine the physiologic cost of the exercise, blood lactate was assessed. Stroke effectiveness was expressed as the percentage of shots hitting the target zones, which consisted of 2 areas in the corners of the court, bordered by the boundary line, singles lines, and marking lines and 1 meter from the service and center lines.

Whole-Body Cryostimulation

The CRS group was exposed to cold twice a day (9:30 AM and 5:30 PM) in a cryogenic chamber at the National Olympic Sport Centre in Cetniewo, Poland. Before the experiment, each participant was examined by a physician for any contraindications to such treatment. Most participants had used whole-body cryostimulation previously. Each participant gave his written consent before the study, and the Bioethical Committee of the Regional Medical Society in Gdańsk approved the study according to the Declaration of Helsinki. Each cryostimulation session lasted 3 minutes at a temperature of –120°C. Entry to the cryochamber was preceded by a 20- to 30-second adaptation period in the vestibule at a temperature of –60°C. The participants were dressed in shorts, socks, gloves, and a hat covering their auricles. They did not participate in any other treatment after the whole-body cryostimulation to avoid obscuring the cryogenic effect.

Blood Analysis

Blood samples were collected for 2 main purposes. First, cytokines (interleukin 6 [IL-6] and tumor necrosis factor α [TNFα]), hormone levels (testosterone and cortisol), hematologic values, and creatine kinase (CK) were assessed. Measurements were taken at point A of the Figure at the initial and final stages of the experiment. Second, the lactate level (LA) at point C, before and after the test, was established. Measurements were taken before, immediately after, and after 5 minutes of rest following the tennis drill. The samples for determining the cytokine and hormone concentrations were always collected between 8:00 and 8:30 AM from the antecubital vein. After collection, the samples were immediately placed in ice at 4°C. Within 10 minutes, they were centrifuged at 2500*g* and 4°C for 10 minutes. Aliquots of plasma were stored at –70°C.

Hematologic measurements were determined by conventional methods using an LH 750 Hematology Analyzer (Beckman-Coulter Inc, Brea, CA). Plasma TNFα and IL-6 levels were determined by enzyme immunoassay methods using commercial kits (TNF: kit HSTA00D; IL-6: kit HS600B; R&D Systems, Minneapolis, MN). Detection limits for TNFα and IL-6 were 0.038 $pg \cdot ml^{-1}$ and 0.04 $pg \cdot ml^{-1}$, respectively. The intra-assay coefficients of variation were <2.5% for TNFα and <8.0% for IL-6.

Cortisol concentration was determined with electrochemiluminescence immunoassay (model Elecsys 2010; Roche, Nutley, NJ) and testosterone with the chemiluminescence immunoassay (COBAS 6000/E601, Roche).

Plasma creatine kinase activity was used as a marker of muscle damage and was evaluated by CK kit (Emapol, Gdańsk, Poland). The CK detection limit for the applied kit was 6 $U \cdot L^{-1}$, whereas its intra-assay coefficient of variation was 1.85%. Lactate concentration in the capillary blood was assessed before and 5 minutes after training (model LKM 140; Dr Lange, Dusseldorf, Germany), with an intra-assay coefficient of variation of 2.5%.

Statistical Calculations

Statistical analysis was performed using Statistica for Windows (version 8.0; StatSoft, Tulsa, OK). A 2 (group) × 2 (time) repeated-measures analysis of variance was calculated to determine the significance of the differences between groups and between precryostimulation and postcryostimulation sessions. The level of significance was set at .05 for all analyses. Additionally, in order to elaborate on the differential significance between groups before and after the 5 days of whole-body cryostimulation, the method of multiple comparisons (post hoc honestly significant Tukey test) was applied. Furthermore, due to the small number of participants, we also calculated the effect size (partial η^2), which ranges between 0 and 1. Using the Cohen rule of thumb as well as the conversion table for η^2, the interpretations of the partial η^2 value are unequivocal. However, the most restrictive interpretation method assigns values to the effect size as follows: 0.1 constitutes a small effect; 0.3, a medium effect; and 0.5, a large effect.

RESULTS

All participants completed the study, and no adverse events were reported. The combination of 5 days of whole-body cryostimulation and training had no effect on body weight or composition (Table 2).

In most participants, the hematologic values remained unchanged except for the white blood cell and basophil levels (Table 3). An increase in basophil percentage was observed in the CRS group, whereas the value decreased in the CON group. The effect size for this change (expressed as partial η^2) was 0.53. The levels of cytokines and hormones were noticeably different for the 2 groups after the main part of the experiment. Multiple-comparisons testing (post hoc honestly significant difference Tukey test) indicated that, in the CRS group, the cytokine TNFα concentration decreased by 60%, whereas in the CON group, it fell by 35%. At the same time, the concentration of IL-6 in the CRS group increased by 23%; in the CON group, a slight drop of 7.7% was recorded. These differences were statistically significant. Also, the effect sizes for these measurements demonstrated that the changes of 63% and 60% for TNFα and IL-6, respectively, were attributed to cryostimulation (Table 3).

In addition, we investigated the level of CK as a marker of damaged tissue. In both groups, this level was slightly

Table 2. Participants' Demographic and Anthropometric Characteristics (Mean ± SD)[a]

	Group			
	Cryostimulation		Control	
Characteristic	Before Study	After Study	Before Study	After Study
Age, y	23 ± 3.0	23 ± 3.0	20 ± 2.0	20 ± 2.0
Height, cm	185.1 ± 2.5	185.1 ± 2.5	182.5 ± 5.7	182.5 ± 5.7
Weight, kg	79.7 ± 6.2	79.3 ± 5.9	81.4 ± 7.1	81.2 ± 6.9
Fat-free mass, kg	72.6 ± 4.6	72.5 ± 4.5	73.6 ± 5.7	73.5 ± 5.6
Fat, kg	7.0 ± 3.2	6.7 ± 3.1	7.7 ± 3.0	7.6 ± 2.9
Fat, %	8.5 ± 3.2	8.3 ± 2.8	9.3 ± 3.0	9.2 ± 2.9
Body mass index, $kg \cdot m^{-2}$	23.2 ± 1.8	23.1 ± 1.7	24.4 ± 1.9	24.3 ± 1.9

elevated before the main part of the experiment, which could have resulted from insufficient recovery after the tournament season. After the 5-day training program, CK in the CRS group decreased from 305.0 to 241.4 $U \cdot L^{-1}$, whereas in the CON group, it remained almost the same (286.7 $U \cdot L^{-1}$ before the main part of the investigation and 295.5 $U \cdot L^{-1}$ after). The partial η^2 for the observed changes was 0.49.

Although the cortisol and testosterone values corresponded to reference physiologic values, they were altered in very different ways. The baseline cortisol level was different for the groups ($P = .004$). However, in the CRS group, the cortisol concentration increased from 412.0 to 463.0 $nmol \cdot L^{-1}$, whereas in the CON group, it dropped from 513.7 to 417.6 $nmol \cdot L^{-1}$ (Table 3). The average values of cortisol for the groups after the end of the investigation were not different. Yet significant changes were noted within the relative levels of testosterone. In the CRS group at the end of the experiment, testosterone had increased (from 18.9 to 21.4 $nmol \cdot L^{-1}$); in the CON group, it decreased slightly (22.5 to 19.4 $nmol \cdot L^{-1}$). The partial η^2 for testosterone was 0.45 and for cortisol was 0.52, suggesting a considerable effect size (Table 3).

Analysis of the RMR using indirect calorimetry showed no statistically significant differences occurred, either between groups or with time. The percentage contributions of carbohydrate and fat in energy production remained constant, but the protein contribution increased in the CRS group by 2% and decreased slightly in the CON group (0.5%). Nonetheless, the relative values of the oxidized proteins did not differ in the statistical evaluation (Table 4).

To check the influence of the specific tennis drill on the physiologic cost, we assessed oxygen uptake during the exercise and heart rate and lactate level before and after the main experiment. We took into consideration the oxygen uptake in minutes 2 and 6 of each stage of the tennis drill. We also calculated the differences between those values. The larger the difference in oxygen uptake, the greater the physiologic cost of the exercise. In the CRS group after the main experiment, the lower value for the difference in the oxygen uptake was recorded in both stages of the drill. It dropped from 18.2 to 12.5 and 18.3 to 16.6 $mL \cdot kg^{-1} \cdot min^{-1}$ for the first and second parts of the tennis drill, respectively, whereas in the CON group, it remained at the same level throughout both parts of the experiment. The differences were statistically significant, and the effect size confirmed the influence of the cold exposure in the CRS group (Table 5). With repeated performance of the tennis drill after the 5-day program, stroke effectiveness improved in the CRS and CON groups by 6% and 5%, respectively. This high stroke effectiveness was maintained in the second phase of the exercise only by the CRS group; in the CON group, growing fatigue led to its decrease. Moreover, heart rate during the tennis drill and recovery periods was lower in the

Table 3. Effects of Whole-Body Cryostimulation on Hormonal Responses and Cytokine Concentrations in Tennis Players (Mean ± SD)

	Group								
	Cryostimulation		Control						
Variable	Before Study	After Study	Before Study	After Study	Differences	*P* Value	$F_{1,10}$	Effect Size, Partial η^2	Test Power ($\alpha = .05$)
Tumor necrosis factor α, $pg \cdot mL^{-1}$	2.5 ± 0.3	1.0 ± 0.1	2.6 ± 0.6	1.7 ± 0.1	Group × time	.002	17.0	0.63	0.959
Interleukin-6, $pg \cdot mL^{-1}$	1.3 ± 0.2	1.6 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	Group × time	.003	14.76	0.60	0.932
Testosterone, $nmol \cdot L^{-1}$	18.9 ± 1.6	21.4 ± 2.5	22.5 ± 3.8	19.4 ± 3.6	Group × time	.01	8.07	0.45	0.727
Cortisol, $nmol \cdot L^{-1}$	412.0 ± 43.2[a]	463.0 ± 77.5	513.7 ± 63.1	417.6 ± 35.6	Group × time	.008	10.7	0.52	0.838
White blood cells, $10^3/\mu L$	5.6 ± 0.9[a]	6.1 ± 1.0	6.7 ± 2.1	7.0 ± 1.5	Group × time	.002	16.25	0.62	0.951
Lymphoctes, %	35.0 ± 4.1	34.0 ± 3.2	31.5 ± 8.9	31.9 ± 5.4	None				
Basophils, %	10.1 ± 0.8	11.1 ± 0.5	10.4 ± 2.1	9.8 ± 2.1	Group × time	.007	11.31	0.53	0.857
Neutrophils, %	53.7 ± 3.5	53.9 ± 4.0	57.9 ± 13.0	58.2 ± 10.0	None				
Red blood cells, $10^3/\mu L$	5.1 ± 0.1	5.2 ± 0.3	5.1 ± 0.5	5.4 ± 0.7	None				
Platelets, $10^3/\mu L$	202.3 ± 24.4	220.5 ± 30.4	246.3 ± 33.0	238.2 ± 23.0	None				
Hemoglobin, $g \cdot dL^{-1}$	15.0 ± 0.3	15.3 ± 0.4	15.3 ± 1.0	15.2 ± 0.8	None				
Creatine kinase, $U \cdot L^{-1}$	305.0 ± 81.9	241.4 ± 48.9	286.7 ± 11.0	295.5 ± 65.5	Group × time	.01	9.63	0.49	0.798

[a] Differences between groups at baseline ($P = .004$).

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?E6C6DE?8 J H6 ? E65 4 ?D56C23 J :?4C62D65 DEC 6 67764E:C6?6DD 7 3 E9 8C FAD 2D 6I A6C6DD65 :? E96 A6C46?E286 7 32 D 9:EE?8 E96 E2C86E K ?6D H6C6C : AC C65 DEC 6 67764E:C6?6DD H2DD66? :? E96 (8C FA 5FC?8 3 E9 E96 LCDE2?5 D64 ?5 A6C7 C 2?46D 7 E96 5C H96C62D:? E96 % 8C FA :E H2D D66? ? J 5FC?8 E96 LCDE) 23 6

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) E96 36DE 7 FC ? H 6586 E9:D :D E96 LCDE DF5J D9 H:?8 E96 DJ?6C8:DE4 2?E :?M2 2E CJ 67764E 7 5 6C2E6 :?E6?D:E EC2:?:?8 2?5 H9 6 3 5J 4CJ DE F 2E ? :? AC 76DD ?2 2E9 6E6D %FC C6DF ED DF886DE E92E DF49 4CJ DE F 2E ? 2J 7F?4E ? 2D2AAC AC2E6 DFAA CE7 C2 EC2:?:?8 AC 8C2 E 6?DFC6 2? 67764E:C6 :5D62D ? 3C62 :?2 J 8:C6? E96 : :E65 ?F 36C 7 A2CE4:A2?ED :? FC 6I A6C 6?ED 49 D6? 7C 2 ?8 9:89 C2? :?8 2E9 6E6D H6 6?4 FC286 7FEFC6 2FE9 CDE F?56CE2 6 2C86CDEF5:6D ? E96 67764ED 7 H9 6 3 5J 4CJ DE F 2E ?

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4 6C EE & 2D2 2?: (6E2 4FE6 H9 6 3 5J 4 :?8 7 C6I6C4:D6 :?5F465 9J A6CE96C :2 2 DJ DE6 2E4 C6C6H . , N

2?L 32C5: 3:?: 6 682E , 9 6 3 5J 4CJ E96C2AJ :? 2E9 6E6D ,.- N

. 2 2F49:) : (82 : (2H2? I EC6 6 4 5 EC62E 6?E — ? E96 H9 6 3 5J :? C96F 2E:5 2CE9CED (23DEC24E *0* DFAA & 6 6E 64 6 . 2C(6 2 (6E2 7764E 7 42 4 5 A24 2AA:42E ? ? DJ DE6 :4 2?23 :4 2?5 :?M2 2E CJ C6DA ?D6 E DAC?E:?E6CC2 EC2:?:?8 2 AC DA64EC6 4 A2C2EC6 EC2 , 3- N

F7L6 5 (E6:?32496C 2:C49: 5)) 96 FD6 7 :I65 6E9 5 A2CE3 5J A6 6 4 :?8 AC 465FC6D7 CE62 DA CE2E9 6E6DEC2:?:?8 :? E96 962E ., . - N

:CG ?6? : 6 DD ? 2FE2:?6? & 9; 2:?6?) 6:CD2 6A 7764EC6?6DD 7 5:776C6?E4CJ E96C2A6D ? A2:? 2?5 5:D62D6 24EC:EJ :? 24EC6 C96F 2E :5 2CE9CED 2 C2?5 :D65 D?8 6 3 :?565 4 ?EC 65 EC2 . N

F3326 5) C ?D ? (6?682C 6D 4CJ E96C2AJ 92D6? C6FC? E A2CE4:A2E ? DJ DE6 2E4 C6C6H . , N

DE6) ??6 J CJ E96C2AJ 2?5 ; :?E A DE ? D6?D6 :? 962 E9J A2CE4:A2?ED 2 DJ DE6 2E4 C6C6H . , N

69 2?? DE6C 6EK6C 6E2 &9JD 8:42 C6DA ?D6D E D9 CE 2?5 ?8 E6C C6CEC2:?:?8 :? 6?5FC2?46 2E9 6E6D ? C6:56C CJ %) 6 65D *0* ,., ,. 92 A2:8? F 2? :?6E4D N

66D ? : 496 :42 2?5 : F? 8:42 2C 6CD 7 C6CEC2:? :?8 ,.- N

(:E9 JE :?6 9J A E96DD 7 C6CEC2:?:?8 2 A9J D 8:42 252AE2E ? E 6I 46DDC6 DEC6DD ,.- , N

(FKF : 2 2;: (. 2 252 6E 2 (J DE6 :4 :?M2 2E CJ C6DA ?D6 E 6I 92FDECC6 6I 6C4:D6 4JE :?6 :?6E4D , *0* N

2DE6 2?: , C6??6C 9:?5 (5 6I A DFC6 9F 2? : F?6 C6DA ?D6D 2?5 :?EC246 F 2C 4JE :?6 6I AC6DD ? ,.- , N

C?6CJ 2CC H F;: 2 . F?8 , 2E8F6 :? E6??:D 6492?:D D 7 72E8F6 2?5 67764E ? A6C7 C 2?46 ,.- N

C24D (&CE496EE , :4 H:C6 & C66? :D9 A & &9JD42 A6C7 C 2?46 492?86D27E6CF?DFA6CC:D65 EC2:?:?8 5FC?8 E96 2FE ? DAC?8 D6 6DE6C3C62 :? 4 A6EEC6 E6??:DA2J6CD , ,.- N

/ :6 2?? CKJH24K) FDK4KJ 6E2 6C 3:4 2?5 2?26C 3:4 492?86D H:E9 9:89 :?E6?DEH :?E6CC2 EC2:?:?8 :? 24EC6 4 686 2865 6? ., . - N

(49FEK .) 96 32DD 7 5:C64E 2?5 :?5:C64E 42 C 6KJ 2?5 E96:C A E6?E2 D . - . *0* N

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