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Running title: Metabolism and mild cold stress in SCI

Word count: 3179 Tables:3 Figures:3

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Metabolism and mild cold stress in SCI

ABSTRACT

Study design Experimental study

Objectives To compare lipid metabolism in individuals with a cervical spinal cord injury (SCI_C) and able-bodied (AB) persons in response to mild cold stress.

Settings Laboratory of Wakayama Medical University, Japan.

Methods Nine males with SCI_C and 11 AB wore a water-perfusion suit in a supine position. Following 30-min rest thermoneutrality, the whole body was cooled by perfusing 25°C water through the suit for 15-20 minutes (CS). Blood samples were collected before, immediately, and 60 (post-CS60) and 120 minutes after CS (post-CS120). Concentrations of serum free fatty acid ($[FFA]_s$), total ketone bodies ($[tKB]_s$), insulin ($[Ins]_s$) and plasma adrenaline ($[Ad]_p$), noradrenaline ($[NA]_p$) and glucose ($[Glc]_p$) were assessed.

Results $[Ad]_p$ in SCI_C were lower than AB throughout the study (p=0.0002) and remained largely unchanged in both groups. $[NA]_p$ increased after cold stress in AB only (p<0.0001; GxT p=0.006). $[FFA]_s$ increased by 62% immediately after cold stress in SCI_C (p=0.0028), without a difference between groups (p=0.65). $[tKB]_s$ increased by 69% at post-CS60 and 132% at post-CS120 from the start in SCI_C with no differences between groups (p=0.54). $[Glc]_p$ and $[Ins]_s$ were reduced in SCIc only (GxT p=0.003 and p=0.001, respectively).

Conclusion These data indicate that mild cold stress acutely elevates lipid and ketone body metabolism in persons with SCIc, despite the presence of sympathetic dysfunction.

Keywords: total ketone bodies; autonomic dysfunction; whole-body cooling; obesity; diabetes; energy homeostasis

INTRODUCTION

Individuals with a spinal cord injury (SCI) are strongly encouraged to participate in sport and physical activity to help optimize physical and mental health [1]. This may include outdoor activities, which exposes its participants to different environmental conditions and their associated risks. While the physiological and metabolic responses to cold environments are well characterized in able-bodied (AB) individuals [2], they remain understudied in the SCI population.

In Japan, the majority of SCI individuals have a lesion at the cervical level (SCI_C) [3]. A SCI_C is a debilitating condition that can lead to a loss of sensation and motor control of the tissue below the lesion. Depending on the level and completeness of the injury, it can result in sympathetic decentralization of the heart, adrenal medulla and peripheral tissue such as adipose tissue, skeletal muscle and the vasculature [4]. Many people with SCIc have a reduced catecholamine concentration at rest that does not increase in response to physiological stressors such as exercise [5]. Furthermore, cutaneous thermoregulatory responses are essentially absent, resulting in their core temperature being critically dependent on environmental temperature [6]. These processes are closely linked with acute changes in metabolism [7]. It may therefore be anticipated that metabolic responses in persons with SCI_c are divergent from AB individuals.

In AB individuals, exposure to cold stress increases lipid metabolism and energy expenditure [8]. Free fatty acid (FFA) turnover is increased after 3 h rest in 5°C and energy expenditure is elevated by up to 30% in response to mild cold stress [9]. The cold-stress induced metabolic response is thought to be driven by the sympathetic nervous system through sympathetic nerve innervation as well as an elevation in circulating catecholamine concentration [10]. In support, β-receptor knockout mice exposed to a cold environment do not show an increase in metabolism and energy expenditure, and rapidly develop hypothermia [11]. Preliminary data indeed point towards an altered physiological response to cold stress in persons with SCIc. For instance, a cold

pressure test induces an exacerbated blood pressure response in this population, likened to autonomic dysreflexia [12]. However, conflicting evidence exist, with a similar metabolic response to mental (i.e. arithmetic exercises) and physiological stress (i.e. isometric handgrip exercise) reported in persons with SCIc compared with AB persons, despite the sympathetic decentralization of metabolic tissue [13,14]. On the other hand, lipid metabolism in response to cold stress is yet to be investigated in persons with SCIc.

The lack of SCI_c-specific evidence includes knowledge on ketone body metabolism in response to cold stress [15]. The circulating concentration of ketone bodies, comprising of acetoacetic acid, 3-hydroxybutyric acid and to a lesser extent acetone, is the result of the balance between their release from the liver and uptake by peripheral tissue such as skeletal muscle [15]. As such, sympathetic dysfunction may attenuate the production and release of ketone bodies in persons with SCIc, while the smaller muscle mass and reduced oxidative capacity found in persons with SCIc may reduce the removal of ketone bodies from the circulation [16].

This study investigated the effects of mild cold stress on lipid and ketone body metabolism in persons with SCIc. This will provide mechanistic insight into the role of sympathetic activation in metabolic responses to cold stress in humans, while it will also have practical implications for health in persons with SCI_C.

METHODS

Participants

Twenty male participants volunteered for this study, of which nine persons with SCI_C (range of injury level between C5-C7; American Spinal Injury Association impairment scale A; time since injury: 19 (11) years and 11 AB individuals. All participants provided written informed consent and completed health questionnaires prior to participation in this study. The anthropometric

characteristics of the SCI_C and AB groups were similar, as shown in Table 1, with a respective mean (SD) for age of 37 (10) vs 37 (13) years, height of 176 (8) vs 173 (4) cm, and a body mass of 61.2 (13.1) vs 67.2 (8.7) kg. All SCIc were non-smokers; two AB reporting smoking but refrained from it during the 24 hrs prior to data collection. Participants were excluded if they were taking medication that directly affects cardiac function. The study conformed to the guidelines set by the Declaration of Helsinki and were approved by the Review Board on Human Experiments at Wakayama Medical University.

Study protocol

Experimental protocol is shown in Figure 1. All participants were asked to refrain from alcohol and caffeine for 24 hrs preceding the experiment. Following at least a 4 hr fast, participants arrived at the laboratory and voided their bladders prior to any experimental measures. They put on a water-perfused suit wear, while they did not wear any clothes underneath except for shorts, and took a supine position on a bed for 30 min before the start of the experiment. The water-perfused suit covered the entire skin surface except for the feet, hands, and head. During this period, 33°C water was perfused into the suit to keep the body at thermoneutrality. A Teflon catheter was placed in the antecubital vein for blood sampling. After a 10-min thermoneutral measurement period, the participant was exposed to whole-body mild cold stress through perfusing 25ºC water, for 15-20 min such that mean skin temperature decreased by approximately 2°C. The reason why the water temperature of 25 ºC was determined as the present cold stress that shivering was observed below this temperature in able-body participants preliminary and water at this temperature would not be supposed to induce autonomous hyperreflexia. DeGroot et al. [17] suggested in able-body persons that visible shivering was observed at T_{sk} of 29 ℃ (in the present study, no participants experienced less than 29 ℃ of T_{sk}, as shown in *Results*). After the cold stress, water temperature

was returned to 33°C for 30 minutes as part of a 'recovery' period. Thereafter, participants were allowed to take off the suit and rest in a seated position for 90 minutes.

Measurements

Body mass of SCI_C was measured using a wheelchair-accessible mounting scale (PW-630, TANITA Corporation, Tokyo). Oesophageal (T_{oes}) and mean skin temperatures (T_{sk}) were monitored with thermocouples. A copper-constantan thermocouple was inserted into the oesophagus via the nostril, up to the approximate level of the heart. Other thermocouples were placed over 7 locations on the skin; T_{sk} was calculated based on the weight of each site: forehead (0.07), chest (0.175), abdomen (0.175), upper arm (0.1), lower arm (0.1), thigh (0.2), and calf (0.16), as reported previously [18].

Systolic (SBP) and diastolic blood pressures (DBP) were measured every min from the brachial artery of the right upper arm placed at the heart level by inflation of the cuff with a sonometric pickup of Korotkoff's sound (Tango+, Sun Tech Medical Inc., Morrisville, NC). Mean arterial blood pressure [MAP=(SBP-DBP)/3 + DBP] was calculated. Heart rate (HR) was calculated by averaged R-R intervals of the electrocardiogram (BSM-2401; Nihon Koden, Tokyo) every minute. Oxygen uptake ($\dot{V}O_2$) and the rate of carbon dioxide elimination ($\dot{V}CO_2$) were analyzed by online respirometry (ARCO-2000, ARCO SYSTEM, Chiba, Japan), with the RQ calculated as the ratio between both measures. These values were averaged over the last 5 minutes of each of the three experimental periods, i.e. thermoneutrality, cold stress and recovery.

Measures to provide an indication of autonomic completeness included catecholamine concentrations, whilst sympathetic skin responses (SSR) and a sit-up tilt test were performed in 5 out of 9 participants with SCI_C for additional confirmation [19]. To test SSR, electrodes were placed on the dorsal and ventral sides of the left hand and foot of the supine participants and the signals were collected by a data acquisition unit (PowerLab 26T (LTS), AD Instruments, Colorado Springs,

CO, USA). Electrical stimulation was applied to the left median, the left posterior tibial, and the left supra orbital nerves, in series, intermitted with 30-40 seconds. Five stimuli were applied at each nerve, with the pulse amplitude set to a threshold where a motor response could be elicited (12-20 mA, 0.2 ms, single pulse). The presence of a response was recorded at each nerve (maximal score was 5). For the sit-up tilt test, after 5 min supine rest, participants were passively sat up to a seated position for 15 minutes, while they breathed at a rate of 15 breaths/min. Automated oscillometric blood pressure (STBP-780, Colin, Komaki, Japan) and HR detected from electrocardiogram were recorded every minute, or until participant demonstrated signs of syncope, then averaged for each stage. Orthostatic hypotension was defined as a drop in SBP of ≥ 20 mmHg, or a drop in DBP of ≥ 10 mmHg.

Blood samples

Twelve milliliters of blood were withdrawn at the end of thermoneutrality, immediately after cold stress, as well as 60 and 120 min after cold stress (post-CS60 and post-CS120, respectively). One milliliter of the whole blood sample was transferred to tubes containing heparin for measurements of hemoglobin concentration and hematocrit using an automatic blood cell counter (MEK-6400, Nihon Koden, Tokyo). The remaining 9 milliliters of aliquots were placed in a tube containing EDTA2Na^{+}, which was centrifuged at 4 °C at 3000 rpm for 30 minutes. Serum and plasma samples were stored at -80 °C until the analysis performed by a specialist company (SRL, Hachioji, Japan). Serum samples were used to determine $[FFA]_s$, $[tKB]_s$, acetoacetic acid, 3-hydroxybutyric acid, and $[Ins]_s$. Plasma concentrations of adrenaline and noradrenaline ($[Ad]_p$ and $[NA]_p$, respectively), and glucagon ($[Gcg]_p$) and $[Glc]_p$ were also analyzed. The insulin/glucagon ratio was calculated. The homeostatic model assessment (HOMA) was used to quantify insulin resistance (HOMA-IR) and β-cell function (HOMA-β) and then calculated according to the following equations [20]:

HOMA-IR = fasting $[Glc]_p$ (mg/dl) × fasting $[Ins]_s$ (μU/ml) / 405

HOMA-β = 360 × fasting $[Ins]_s$ (μU/ml) / (fasting $[Glc]_p$ (mg/dl) - 63)

Statistical analysis

Data are expressed as mean (SD). Prior to inferential analyses, normality was checked using the Shapiro-Wilk test. Differences in participants` characteristics between both groups were tested by the Student's t-test. Two-way ANOVAs with repeated measures were applied for comparisons of all other parameters. Main effects of comparisons between the groups and between each time point and Group x Time interactions were evaluated. Subsequent post-hoc Tukey-Kramer tests were performed when significance was detected. The null hypothesis was rejected at the level of $P<0.05$.

RESULTS

Physiological parameters

The physiological responses to cold stress in the SCI_C and AB groups are shown in Table 2. The mild cold stress decreased T_{sk} by 2.3 (0.5) °C in both groups and did not return to baseline during the recovery period; T_{oes} dropped by 0.2 (0.4) °C at recovery in both groups. HR was lower by ~10 bts·min^{-1} in SCI_C when compared to AB throughout the experiment (p=0.008). MAP increased at recovery in SCI_C (p<0.0001, interaction of Group x Time p=0.002), yet there was no notable change in MAP in AB. $\dot{V}O_2$ and $\dot{V}CO_2$ did not change during cold stress in either group (p=0.210 and p=0.392, respectively. RQ decreased at recovery in both groups (p=0.016), without a Group x Time interaction (p=0.772).

Catecholamines

Figure 2 shows that $[Ad]_p$ was lower in SCI_C compared with AB throughout the trial (p=0.0002), and did not change in either group following cold stress (p=0.238). There was a Group x Time interaction for $[NA]_p$ (p=0.006). While $[NA]_p$ significantly increased in AB at post-CS60

and post-CS120 compared with thermoneutrality (P<0.0001), this was unchanged in SCI_C (p=0.778).

Lipids and ketone bodies

$[FFA]_s$ was elevated immediately after cold stress and decreased at post-CS60 and CS120 in both groups (p=0.003) as shown in Figure 3. There were no differences between both groups (p=0.654) and no Time x Group interaction effect (p=0.696). $[tKB]_s$ was increased at both post-CS60 and post-CS120 compared with thermoneutrality in SCI_C (p<0.0001), while it was elevated at post-CS120 in AB (p< 0.0001). There were no differences between groups (p=0.538) and no Group x Time interaction (p=0.773).

Glycemic parameters

There was a Time x Group interaction for $[Glc]_p$ (p=0.003). While $[Glc]_p$ was higher in SCI_C compared with AB at thermoneutrality (p=0.013), $[Glc]_p$ was reduced in SCI_C after cold stress (p=0.010) but remained unchanged in AB throughout the trial (p=0.177). $[Ins]_s$ was higher in SCI_C than AB throughout the trial (p=0.022), while there was also a Group x Time interaction (p=0.012). $[Ins]_s$ in SCI_C decreased following cold stress, but remained unchanged in AB (p=0.343). In keeping with $[Ins]_s$, $[Gcg]_p$ decreased immediately after cold stress in SCI_C only (p=0.026; Table 3). HOMA-IR (p=0.032), but not HOMA-β (p=0.052), was higher in SCI_C compared with AB.

Degree of autonomic dysfunction

The outcomes of the SSR and sit-up tilt test were in line with the lower catecholamine concentrations found in SCI_c. Four out of the five participants experienced orthostatic hypotension during the sit-up tilt test. During the SSR test, no nerve responses were observed in two participants, responses in the radial nerve in one participant, and responses in the radial and orbital nerve in the remaining two participants.

DISCUSSION

The main finding of this study is that the elevation in $[FFA]_s$ and $[tKB]_s$ after mild cold stress was similar between SCI_C and AB, despite sympathetic dysfunction and lack of an increase in plasma catecholamine concentration in SCI_C.

Catabolic processes (e.g., glycolysis, gluconeogenesis and lipolysis) are activated by catecholamines. In persons with autonomic complete SCI_C, the sympathetic nervous pathway in the spinal cord is dissected and central sympathetic activation, including catecholamine production, upon physiological stress impaired [4]. The mild cold stress applied in the present study elevated noradrenaline concentration in AB but not in SCI_C. This is in corroboration with Mizushima et al. [21], who found an attenuated noradrenaline response in persons with SCIs after a cold pressor test. Investigating AB individuals, Koska et al. [22] showed that the increase in lipolysis after cold stress is associated with noradrenaline concentration but not with other candidate markers such as adrenaline, glucagon and growth hormone concentration. Therefore, the increase in lipid metabolism in AB in the present study may have been the result of the increase in noradrenaline concentration. However, the similar increase in lipid metabolism in SCI_C suggests that sympathetic activation is not *essential* for lipid mobilization in response to physiological stress in humans.

In keeping with the FFA response, there was no difference in the acute increase in ketone body concentration between SCI_C and AB. This is in line with Hoekstra et al. [23], who found a similar increase in total ketone body concentration in persons with SCI_C and AB individuals following dopamine infusion. The lack of difference between both groups in the present study may be partly explained by the similar increase in FFA concentration, as FFA act as a substrate for ketone bodies [15]. It should be noted that ketone body metabolism in response to other physiological stressors than cold stress, such as exercise, may be altered in persons with SCI_C [24]. Exercise increases ketone body uptake by skeletal muscle [24]. Considering the reduced muscle

mass and the deconditioned state of skeletal muscle in persons with SCI_C [16], the rate of ketone body removal from the circulation is possibly impaired. Indeed, unpublished data show an elevated ketone body concentration after a wheelchair marathon in people with SCI_C compared with those with SCI, while FFA concentration was comparable (Nishiyama et al., in preparation); suggesting that the ketone body removal from the circulation also needs to be considered when studying circulating concentrations. In the present study, ketone body uptake is likely to have been limited because the passive nature of the experiment may have limited the need for ketone body oxidation.

The similar response in lipid and ketone body metabolism after cold stress in SCI_C and AB corresponds with previous studies investigating other stressors [13,14]. Karlsson et al. [13] found a similar lipolytic rate in adipose tissue at rest between SCI_C and AB individuals, and between adipose tissue above and below the lesion level. Moreover, no difference in lipolysis between SCI_C and AB individuals was observed in response to mental stress and handgrip exercise [14]. We speculate that, as insulin inhibits lipolysis, the reduction in insulin concentration during cold stress has stimulated FFA mobilization in SCI_C. Additionally, or alternatively, other mechanisms to compensate for the reduced sympathetic activity develops in persons with SCI_C. For instance, people with SCI_C show an exacerbated blood pressure response to cold stress and catecholamine infusion, suggested to be partly related to hypersensitivity of adrenergic receptors [21,25]. The catecholamine concentrations confirm that participants of the present study had an autonomic complete SCI. While no direct evidence exists for this supposition with regards to lipid metabolism, peripheral sympathetic activation of tissue below the lesion cannot be excluded. For instance, peripheral stimulation can trigger local sympathetic activity in persons with SCI_C, as indicated by noradrenaline spillover at the nerve terminal [26]. Finally, there is evidence that circulating factors other than catecholamines can stimulate lipid metabolism [27]. Even when blocking β-adrenergic receptors using propranolol, atrial natriuretic peptide stimulates lipid metabolism in AB individuals

[27]. Future studies could include a wider array of plasma markers to assess the significance of additional parameters for lipid metabolism.

While the primary focus of this study was to provide insight into the role of sympathetic activation in the metabolic response to cold stress, the findings also have practical implications for the population with SCI. Since the discovery of brown adipose tissue in humans [28], cold acclimation has received increased attention as an intervention to improve cardiometabolic health [10]. The majority of chronic studies investigating this suggestion are conducted in animals [29], but more recent work shows that 10-day cold acclimation improves insulin sensitivity in individuals with type II diabetes mellitus [30]. Interestingly, although no test of glucose tolerance was employed in the present study, glucose and insulin concentration were reduced after cold stress in SCI_C. The potential use of passive cold exposure to improve cardiometabolic health is particularly appealing for populations restricted from engaging in exercise, such as persons with SCI_C. Of note, no adverse events (e.g. autonomic dysreflexia) were reported in any of the trials. Therefore, the use of cold stress as a health promoting intervention for persons with SCI_C may be an interesting avenue for future research.

Several limitations of this study deserve further discussion. First, two members of the SCI_C group showed signs of diabetes mellitus and/ or insulin resistance. Although we confirmed by an interview that they were not diabetic, they had high fasting blood glucose ($[Glc]_p$ at TN > 126 mg/dl) and 4 SCIc with high serum insulin ($[Ins]_s$ at TN > 19.6 µIU ml^{-1}). Relative insulin deficiency might decrease glycolysis and glucose oxidation, resulting in increasing gluconeogenesis and FFA oxidation; therefore potentially impacting the findings of the present study. Second, we could perform the SSR and the sit-up tilt test in 5 out of 9 participants with SCI_C only. However, the lower catecholamine concentrations in SCI_c throughout the trial in combination with the SSR and

283 sit-up tilt test results in this subgroup support the presence of impaired sympathetic nerve activity in
284 our sample.

285

286 In conclusion, the findings suggest that mild cold stress elevates lipid and ketone body
287 metabolism in persons with SCI_C, despite the presence of sympathetic dysfunction. This indicates
288 that an increase in circulating catecholamines is not essential to stimulate lipid metabolism in
289 response to mild cold stress.

DATA ARCHIVING

There were no data to deposit.

ACKNOWLEDGMENTS

We thank Mr. Shinji Kawasaki for the recruitment of study participants. We thank Mrs. Daisuke Kojima, Makoto Kawanishi, Takashi Moriki, Yasuhisa Fujita, and Ryosuke Miyake for their support with data collection.

ETHICS DECLARATIONS

Conflict of Interest

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

Statement of Ethics

The study protocol and the methods applied in this study conformed to the guidelines of the Declaration of Helsinki and were approved by the Review Board on Human Experiments, Wakayama Medical University.

Informed consent

Informed consent for inclusion in this study was obtained from the participants.

AUTHOR CONTRIBUTIONS

Kazunari Nishiyama (K.N.), Yoshi-ichiro Kamijo (Y-i.K.), V.L.G-T., Yukihide Nishimura (Y.N.), and F.T. conceived and designed research; K.N., Y-i.K., Jan W. van der Scheer (J.W.v.d.S.), Tokio Kinoshita (T.Ki.) and Takashi Kawasaki performed the experiments; K.N and Y-i.K. analyzed the data; K.N., Y-i.K., J.W.v.d.S., T.Ki., V.L.G-T., Y.N., Sven Hoekstra (S.H.) and F.T. interpreted the

313 results; K.N. prepared the figures; K.N. and Y-i.K. drafted the manuscript; all authors edited and
314 revised the manuscript and approved the final version of the manuscript.

315

316 **FUNDING**

317 This study was supported by grants from the Joint Usage/Research Center of Sport for Persons with
318 Impairments, authorized by the Ministry of Education, Culture, Sports, Science and Technology in
319 Japan, to Victoria L. Goosey-Tolfrey (V.L.G-T.) in 2015 and Nachikatsuura Research Foundation
320 (L1221) to Fumihiro Tajima (F.T.). Jan W. van der Scheer (J.W.v.d.S.) is based in The Healthcare
321 Improvement Studies Institute (THIS Institute), University of Cambridge. THIS Institute is
322 supported by the Health Foundation, an independent charity committed to bringing about better
323 health and healthcare for people in the UK.

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409

410 **FIGURE LEGENDS**

411 Figure 1: A schematic overview of experiment. TN, thermoneutrality; CS, mild cold stress; Rec,
412 recovery. Syringe symbols indicate blood draws.

413

414 Figure 2: Plasma concentrations of noradrenaline ($[NA]_p$) and adrenaline ($[Ad]_p$) at
415 thermoneutrality (TN), immediately after mild cold stress (CS), 60 and 120 minutes after CS
416 (post-CS60 and post-CS120, respectively) in 9 individuals with cervical spinal cord injury (SCI_C; ●)
417 and 11 able-bodied (AB; ○). Data are means (SD). *, compared between groups; †, from TN; ‡,
418 from CS ($P<0.05$).

419

420 Figure 3: Serum concentrations of free fatty acids ($[FFA]_s$) and total ketone bodies ($[tKB]_s$) at
421 thermoneutrality (TN), immediately after mild cold stress (CS), 60 and 120 minutes after CS
422 (post-CS60 and post-CS120, respectively) in 9 individuals with cervical spinal cord injury (SCI_C; ●)
423 and 11 able-body persons (AB; ○). Data are means (SD). †, compared from TN; ‡, from CS; #, from
424 post-CS60 ($P<0.05$).

Table 1. Characteristics of 11 AB and 9 subjects with SCIc.

	SCIc (n=9)	AB (n=11)	P values
Age, years	37 (10)	37 (13)	0.929
Height, cm	176 (8)	173 (4)	0.431
Weight, kg	61.2 (13.1)	67.2 (8.7)	0.237
Time since injury, years	19 (11)		
Injury level	C5 - C7		
AIS	A		

Data are means (SD) of 9 persons with cervical spinal cord injuries (SCIc) and 11 able-bodied (AB). AIS; ASIA Impairment Scale. Grade “A” indicates complete injury clinically, in other words motor and sensory function are compromised in the sacral segments S4-S5.

Table 2. Body temperatures, cardiovascular responses, and metabolic rates before and after mild cold stress.

		SCIc (n=9)			AB (n=11)			P values		
		TN	CS	Rec	TN	CS	Rec	Group	Time	GxT
T_{sk}, ºC		34.9 (0.5)	32.5 (0.7) †	34.6 (0.4) †‡	35.1 (0.4)	32.9 (0.7)†	34.8 (0.3)†‡	0.138	<0.0001	0.291
T_{oes}, ºC		36.8 (0.5)	36.6 (0.4)	36.5 (0.4) †	36.9 (0.3)	36.9 (0.3)	36.7 (0.3)†‡	0.202	0.051	0.427
HR, beats min^{-1}		56 (7)*	54 (7)*	54 (9)*	64 (6)	62 (7)	64 (7)	0.008	0.347	0.661
SBP, mmHg		111 (13)	115 (11)	129 (13)†‡	124 (12)	127 (16)	127 (18)	0.204	0.0004	0.007
DBP, mmHg		67 (8)	72 (9)	81 (9)†‡	74 (4)	75 (7)	76 (8)	0.511	<0.0001	0.003
MAP, mmHg		82 (9)	86 (9)	97 (9)†‡	91 (5)	92 (6)	93 (9)	0.232	<0.0001	0.002
PP, mmHg		44 (8)	44 (7)	48 (9)	50 (12)	52 (19)	51 (17)	0.331	0.396	0.338
$\dot{V}O_2$	ml min^{-1}	191 (40)*	184 (41)*	190 (41)*	225 (34)	214 (33)	225 (28)	0.036	0.210	0.919
	ml kg^{-1} min^{-1}	3.2 (0.5)	3.0 (0.6)	3.1 (0.5)	3.4 (0.5)	3.2 (0.5)	3.4 (0.5)	0.345	0.269	0.917
$\dot{V}CO_2$	ml min^{-1}	155 (34)*	151 (37)*	149 (34)*	185 (32)	175 (33)	178 (29)	0.050	0.392	0.866
	ml kg^{-1} min^{-1}	2.6 (0.4)	2.5 (0.5)	2.5 (0.4)	2.8 (0.5)	2.6 (0.5)	2.7 (0.5)	0.329	0.396	0.906
RQ		0.81 (0.03)	0.82 (0.03)	0.78 (0.05)‡	0.82 (0.04)	0.81 (0.05)	0.79 (0.06) †‡	0.872	0.016	0.772

Data are means (SD) of 9 persons with cervical spinal cord injuries (SCIc) and 11 able-bodied (AB). TN, thermoneutral condition; CS, immediately after cold stress; Rec, 30 minutes after the end of CS; T_{sk} and T_{oes}, skin and oesophageal temperatures; HR, heart rate; SBP and DBP, systolic and diastolic blood pressures; MAP, mean arterial pressure; PP, pulse pressure; $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, cabon dioxide emission; RQ, respiratory quotient. *, compared between groups; †, from TN; ‡, from CS ($P<0.05$).

Table 3. Glucose metabolism in response to mild cold stress.

	SCIc (n=9)				AB (n=11)				P values		
	TN	CS	post-CS 60	post-CS 120	TN	CS	post-CS 60	post-CS 120	Group	Time	G×T
$[Glc]_p$ mg dl^{-1}	106 (24)*	106 (19)*	98 (15)*	89 (8)†‡	88 (7)	84 (9)	85 (6)	88 (6)	0.013	0.010	0.003
$[Ins]_s$ μIU ml^{-1}	17.7 (16.5)*	16.2 (17.5)*	11.9 (10.1)*	5.6 (3.4)†‡	5.1 (3.2)	4.4 (2.1)	4.6 (0.9)	4.1 (1.1)	0.022	0.003	0.012
$[Gcg]_p$ pg ml^{-1}	13.5 (8.2)	8.1 (5.9)†	10.1 (5.9)	11.7 (3.9)	15.1 (6.0)	12.2 (6.0)	16.0 (8.7)	15.7 (7.9)	0.134	0.026	0.519
I/G ratio	3.5 (1.6)*	3.6 (1.7)*	1.9 (0.9)	0.5 (0.1)	0.4 (0.3)	0.4 (0.3)	0.4 (0.3)	0.4 (0.4)	0.049	0.009	0.011

Data are means (SD) of 9 subjects with cervical spinal cord injury (SCIc) and 11 able-bodied (AB). TN, thermoneutral condition; CS, immediately after cold stress; post-CS60 and post-CS120, 60 and 120 minutes after the end of CS; $[Glc]_p$, plasma concentration of glucose; $[Ins]_s$, serum concentration of insulin; $[Gcg]_p$, plasma concentration of glucagon; I/G ratio, ratio of $[Ins]_s$ and $[Gcg]_p$. *, compared between groups; †, from TN; ‡, from CS; #, from post-CS60 ($P<0.05$).

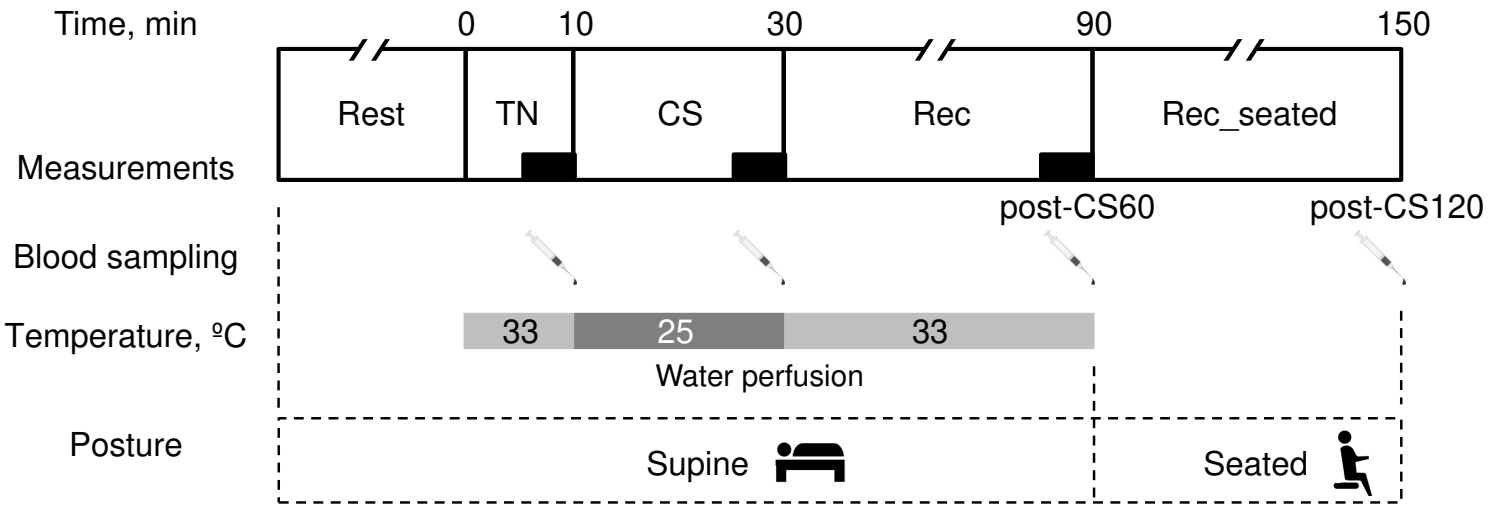
Time, min
0
10
30
90
150
Rest
TN
CS
Rec
Rec_seated
Measurements
post-CS60
post-CS120
Blood sampling
Temperature, ºC
33
25
33
Water perfusion
Posture
Supine
Seated

Figure 1

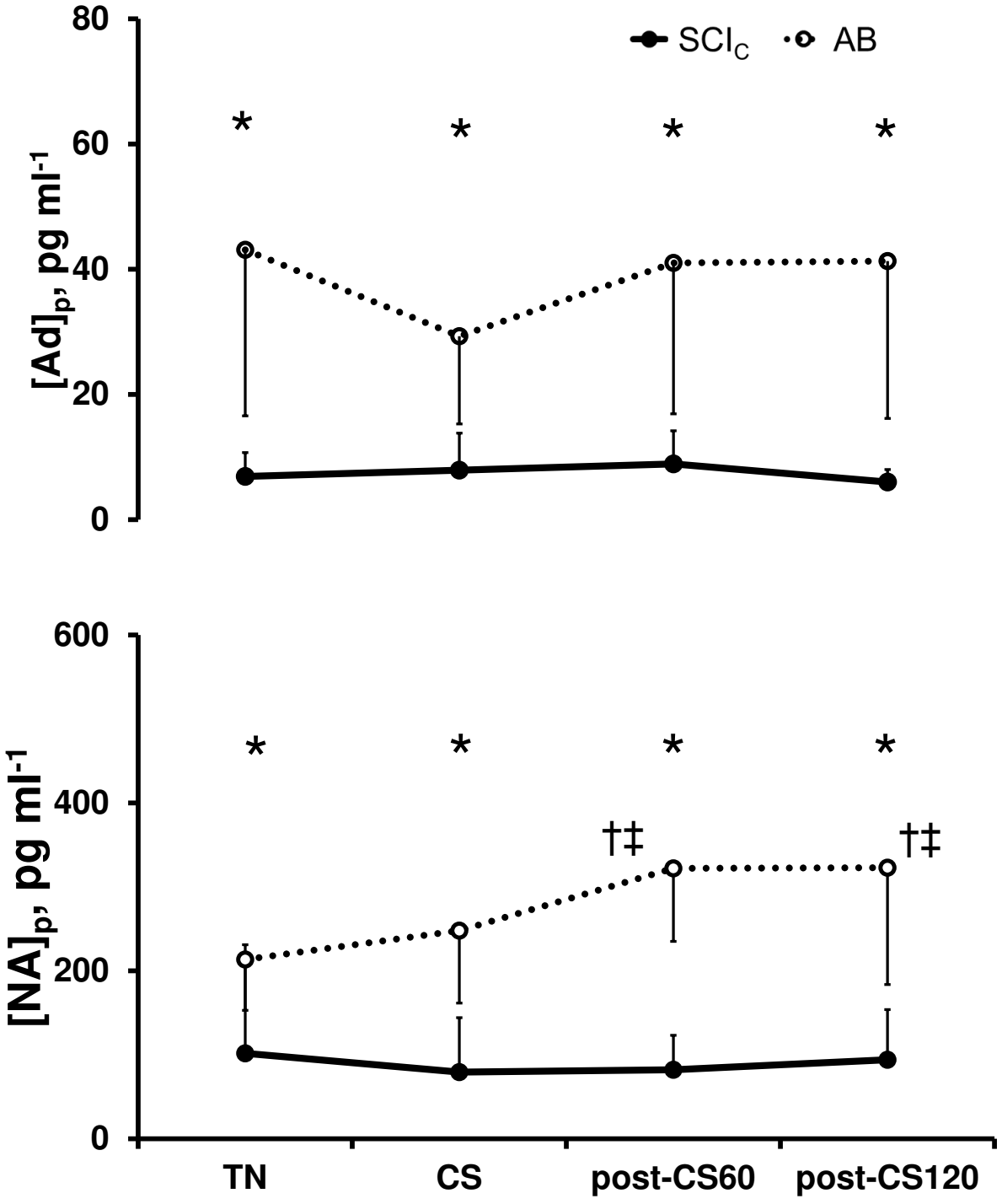
SCI$_C$
AB
[Ad]$_p$, pg ml^{-1}
[NA]$_p$, pg ml^{-1}
0
20
40
60
80
200
400
600
*
†‡
TN
CS
post-CS60
post-CS120

Figure 2

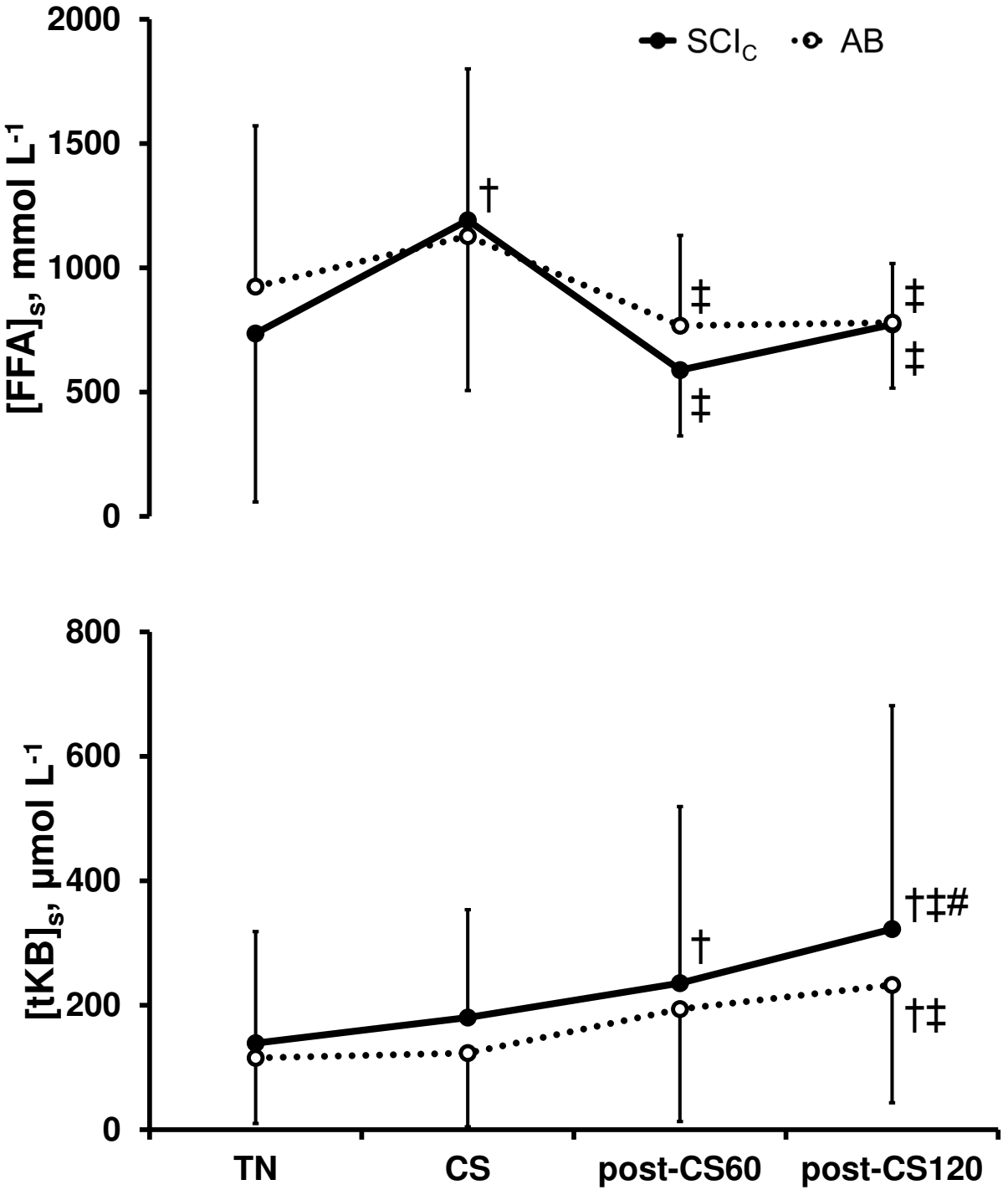
2000
1500
1000
500
0
[FFA]s, mmol L-1
SCIC
AB
†
‡
‡
‡
‡
800
600
400
200
0
[tKB]s, µmol L-1
†
†‡#
†‡
TN
CS
post-CS60
post-CS120

Figure 3