

ARTICLE



Serum concentrations of ketones increase after hand-ergometer exercise in persons with cervical spinal cord injuries: a preliminary prospective study

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STUDY DESIGN: Experimental study.

OBJECTIVES: To compare lipid profiles during moderate-intensity exercise between persons with cervical spinal cord injuries (SCI_C) and able-bodied controls (AB).

SETTING: Wakayama Medical University, Japan.

METHODS: Six participants with SCI_C and six AB performed 30-min arm-crank exercise at 50% VO_{2peak}. Blood samples were collected before (PRE), immediately (POST), and 60 min after exercise (REC). Concentrations of serum free fatty acids ([FFA]_s), total ketone bodies ([tKB]_s), acetoacetic acid ([AcAc]_s), insulin ([Ins]_s), and plasma catecholamines and glucose ([Glc]_p) were assessed.

RESULTS: Catecholamine concentrations in SCI_C were lower than AB throughout the experiment ($P < 0.001$) and remained unchanged, while increased at POST in AB ($P < 0.01$). [FFA]_s remained unchanged in both groups with no differences between groups. [tKB]_s in SCI_C tended to increase at REC from PRE ($P = 0.043$), while remaining unchanged in AB ($P > 0.42$). [AcAc]_s in SCI_C increased at REC from PRE and POST ($P < 0.01$) while remaining unchanged in AB (interactions of Group \times Time $P = 0.014$). [Glc]_p and [Ins]_s were comparable between the groups throughout the study.

CONCLUSION: Serum ketone bodies in SCI_C increased after exercise while remaining unchanged in AB, suggesting that suppressed uptakes of serum ketone bodies from blood to the muscles in SCI_C would partially contribute the increased serum ketones.

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INTRODUCTION

Daily physical activity in persons with spinal cord injuries (SCI) tends to be restricted due to locomotor deficits, likely increasing the prevalence of diabetes mellitus, dyslipidemia, and metabolic syndrome more than in the able-bodied (AB) population [1]. As reported from Sasakawa Peace Foundation, participation in sport and recreation should be encouraged and promoted, but rates of activity participation in individuals with impairments was still less than 20% in Japan [2]. Accordingly, the identification and development of ideal physical environments to encourage physical activity participation safely in persons with impairments are strongly desired.

Persons with cervical spinal cord injuries (SCI_C) face multiple challenges to exercise performance due to both somatic and autonomic dysfunction associated with higher-level SCI [3]. Working muscles are smaller in SCI_C athletes and skeletal muscle glycogen stores are likely depleted faster during exercise [4]. In addition, after SCI, there is a shift to type II muscle fibers while the existing type I fibers selectively atrophy [5], further contributing to

altered exercise metabolism in this population. Because lipid profiles in persons with SCI are characterized primarily by lower high-density lipoprotein cholesterol (HDL-C) and higher triglyceride/HDL-C ratio than those in AB [6], lipid metabolism is possibly different from AB controls during prolonged exercise. However, there is limited information about fat metabolism that would inform nutritional guidance for exercise performance in SCI.

Free fatty acid (FFA) usage can exceed that of glucose during mild to moderate exercise, leading to increase production of ketone bodies in the liver. Ketone bodies are crucial to metabolic pathways such as β -oxidation, the tricarboxylic acid (TCA) cycle, gluconeogenesis, and lipogenesis [7]. Serum concentrations of ketone bodies are determined by the difference between production in the liver and uptake in peripheral tissues such as skeletal muscles and brain. Sympathetic dysfunction possibly attenuates the production and release of ketone bodies in SCI_C, while the smaller muscle mass and decreased oxidative capacity in SCI_C can reduce the removal of ketone bodies from the bloodstream [7]. The assessment of serum concentrations of FFA

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([FFA]_s), respiratory exchange ratio (RER), and serum ketone bodies would deepen our understanding of lipid metabolism in persons with SCI_C.

The purpose of our study was to examine serum ketone bodies and [FFA]_s before, during, and after exercise in SCI_C and compare the responses to normative changes in AB persons. The hypothesis was that serum ketone bodies would be elevated more in SCI_C than in AB after exercise.

METHODS

Participants

Participants were recruited from the community surrounding Wakayama Medical University. Six males with SCI_C volunteered for the study and six AB males were recruited as sex-matched controls. Participants were excluded if they had comorbid other neurological/medical conditions or were taking medications that primarily affected cardiac function. Participants were provided the details of the study protocol and possible risks, and they provided informed written consent form before voluntary participation in this study. The procedures in this study conformed to the guidelines in the *Declaration of Helsinki* and were approved by the Review Board on Human Experiments, Wakayama Medical University (#2076).

Study protocol

Participants were reported to come to the laboratory for two separate visits for a baseline testing session, as well as an acute exercise trial. Prior to each visit, all participants were asked to refrain from alcohol, caffeine and heavy exercise for 24 h preceding the experiments, as well as fast for at least 4 h before measurements. In the first visit, participants performed an arm-crank graded exercise test to exhaustion for determining their peak rate of oxygen consumption (VO_{2peak}). After arrival at the laboratory, participants emptied their bladders and body mass was taken on a wheelchair scale (PW-630, TANITA corporation, Tokyo); in participants with SCI_C, the mass of their wheelchair was subtracted from the total to calculate body mass. Participants then entered an environmental chamber controlled at 25 °C ambient temperature and 50% relative humidity. Participants rested quietly for 30 min prior to measurements while they were outfitted with equipment. After measurements at rest for 3 min, participants started arm-crank exercise at 50 revolutions/min without loading for 3 min. Since the optimal workload increment for eliciting VO_{2peak} of SCI_C using an arm-crank ergometer is 2–6 W min^{-1} [8], the exercise intensity was increased by 5 W every 3 min until it reached 10 W, and above this intensity, 5 W every minute for persons with SCI_C and 10 W for AB every minute until they could not maintain the rhythm due to exhaustion [9]. We assessed minute oxygen consumption (VO_2), carbon dioxide elimination (VCO_2), and ventilation (VE) every 15 s (MetaMax 3B, CORTEX, Leipzig, Germany) and monitored electrocardiogram continuously during the graded exercise. We determined VO_{2peak} by averaging the three largest consecutive values at the end of exercise [10].

At the second visit, participants emptied their bladders, were weighed, entered the same environmental chamber at the same condition as the first visit, and then rested for 60 min on a wheelchair or a chair for SCI_C or AB, respectively, while they were outfitted with measurement equipment. Previously, a hand-ergometer exercise at 60% of VO_{2peak} for 20 min was used as the exercise load for trained SCI_C [11], but it was difficult for untrained SCI_C in present study to do 20-min exercise at the same load as the above. The participants performed the exercise in two separate sessions because total VO_2 and energy expenditure during intermittent aerobic exercise are comparable to continuous exercise with the same intensity and total duration and are greater after exercise [12]. After 10-min resting measurements, participants performed 2 sets of arm-crank exercise for 15 min intermediated by 2 min. They were able to exercise for 30 min, the minimum daily exercise time recommended for SCI to improve their cardiometabolic health [13]. Work intensity was set as 50% VO_{2peak} calculated by the Karvonen formula [14] at 50 revolutions/min, 7 (2) [mean (SD)] and 43 (6) W for SCI_C and AB, respectively. After exercise, participants rested in a sitting position for 60 min during de-instrumentation and recovery measurements.

Measurements

Heart rate. A single-lead electrocardiogram (BSM-2401; Nihon Koden, Tokyo) was used to measure heart rate (HR) by averaging R-R intervals every minute during rest and throughout exercise.

Blood samples. Eight milliliters of blood was collected from the antecubital vein via a butterfly needle each at rest (PRE), immediately after the exercise (POST), and 60 min after the exercise (REC). One milliliter of blood was put into sodium-fluoride-containing tubes to assess plasma concentration of glucose ([Glc]_p). Three milliliters of blood were placed into EDTA-2Na⁺-containing tubes to assess plasma concentrations of adrenaline ([Ad]_p) and noradrenaline ([NA]_p). Four milliliters of blood was transferred to serum-separating medium tubes to assess serum concentrations of insulin ([Ins]_s), ketone bodies, acetoacetic acid ([AcAc]_s), β-hydroxybutyrate ([βOHB]_s) and total ketone bodies ([tKB]_s), as well as [FFA]_s. All tubes, except those containing heparin, were centrifuged at 4 °C at 3000 rpm for 20 min. Following centrifugation, serum or plasma components were transferred into sterilized tubes. These samples were stored in a –80 °C freezer for later analysis.

Blood properties. [Ad]_p and [NA]_p were measured by HPLC (model HPLC-725CA; Toso, Tokyo). [AcAc]_s, [βOHB]_s, [tKB]_s and [FFA]_s were assessed by enzyme method. [Ins]_s was determined by chemiluminescent enzyme immunoassay. [Glc]_p was measured using an ultraviolet absorption spectrophotometry.

Data analysis

Cardiorespiratory responses. HR was averaged for 5 min at rest, during the 2-min rest interval, and during the first and last 5 min of each exercise bout.

Group characteristics were compared using independent Student's *t*-tests. A 2 × 3 (group × time) repeated-measures ANOVA was used to evaluate all outcome responses. Subsequent post hoc evaluations were performed using a Tukey–Kramer test. All values are presented as means (SD), unless otherwise indicated. The null hypothesis was rejected at the level of $P < 0.05$. The standard least squares method was used to perform regression analyses between a change (Δ) in [Ad]_p versus Δ lipid profiles from PRE. All statistical evaluations were performed using SPSS 25.0 (IBM, Chicago, IL). Based on the previous data of [tKB]_s and [AcAc]_s in SCI_C and AB during mild cold stress [15] and assumed that alpha error probability and power (1- β err prob) were 0.05 and 0.85, respectively, the effect size were 0.454 and 0.851 and the total sample sizes were 10 and 6, respectively, using “ANOVA Repeated measures, within factors” of statistical test. Sample sizes in SCI interventional studies tend to be small [16] due to their severe disabilities. Type II errors are more likely to occur when sample sizes are too small. However, statistical power (1- β err prob) for [tKB]_s and [AcAc]_s in the present study calculated by using the same test as above were both >0.999 and the effect sizes were 2.255 and 3.133, respectively, assuming a error probability was 0.05.

RESULTS

There were no significant differences in any physical characteristics between SCI_C and AB, including age [40 (11) vs. 37 (12) years, $P = 0.639$], self-reported body length [173 (7) vs. 170 (5) cm; $P = 0.513$] and body mass [58.8 (14.4) vs. 64.8 (12.2) kg; $P = 0.459$]. SCI_C participants' level of injury ranged from C5 to C7, and the duration of injury was 23 (12) years (range 7–39 years). Four SCI_C participants were clinically classified as grade A of the American Spinal Injury Association Impairment Scale (AIS A) and the rest were graded as AIS B, all having motor-complete injuries. Maximal power output was 7 (1) W in SCI_C, which was lower than that in AB of 43 (3) W ($P < 0.0001$). Power output during continuous exercise was 7 (2) and 43 (6) W in SCI_C and AB, respectively.

Cardiorespiratory responses

HR increased from 61 (8) to 76 (7) bpm in SCI_C, and from 69 (7) to 138 (18) in AB (both $P < 0.0001$; Table 1).

Catecholamines

At all-time points, SCI_C had significantly lower [Ad]_p and [NA]_p than AB ($P < 0.007$), which remained unchanged during exercise in SCI_C ($P > 0.6$) but was increased at POST in AB compared to PRE ($P = 0.037$) and REC ($P = 0.020$; Fig. 1).

Table 1. Heart rate [beats/min] response during rest and exercise.

	ID	Rest	1st set		Interval	2nd set	
			5 min	End		5 min	End
SCI _C	1	73	86	79	80	87	80
	2	64	72	72	68	72	73
	3	50	71	70	63	69	71
	4	59	65	61	57	60	73
	5	58	73	72	64	71	71
	6	61	80	92	89	93	90
Means		61	75* [†]	74* [†]	70* [†]	76* [†]	76* [†]
SD		8	8	10	12	12	7
AB	21	68	123	139	105	138	147
	22	59	125	136	104	136	146
	23	72	93	165	162	163	166
	24	67	107	117	87	115	121
	25	79	112	115	95	114	123
	26	68	117	118	95	116	123
Means		69	113 [†]	131 [†]	108 [†]	130 [†]	138 [†]
SD		7	12	19	27	19	18

Values, means and SD for 6 participants with cervical spinal cord injuries (SCI_C) and 6 with able-bodies (AB).

*Significant differences between the groups.

[†]Significant differences from Rest at the level of $P < 0.05$.

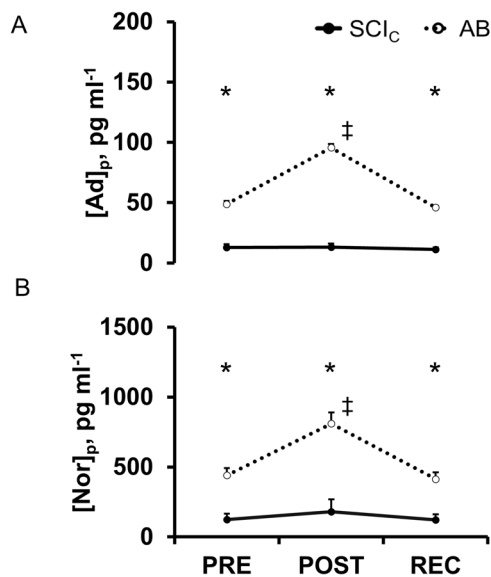


Fig. 1 Plasma concentrations of adrenaline and noradrenaline. Plasma concentrations of adrenaline (A) and noradrenaline (B) before and after exercise in individuals with cervical spinal cord injury and able-bodied. Blood samples were collected before (PRE), immediately after exercise (POST) and 60 min after the cessation of exercise (REC) in 6 individuals with cervical spinal cord injury (●; SCI_C) and 6 able-bodied (○; AB). [Ad]_p, plasma concentrations of adrenaline; [NA]_p, plasma concentrations of noradrenaline. Data are means (SD). *, compared between groups; ‡, from PRE and REC ($P < 0.05$).

Metabolic markers

Figure 2 shows lipid profiles in both groups. [FFA]_s remained unchanged throughout the experiment in both groups (time effect $P = 0.212$; Fig. 2A) and no significant differences between the groups were observed (group effect $P = 0.288$). [tKB]_s in SCI_C

tended to increase at REC from PRE ($P = 0.082$; Fig. 2B), while remaining unchanged in AB ($P > 0.42$). [AcAc]_s in SCI_C increased at REC from PRE and POST ($P = 0.006$ and 0.020 , respectively; Fig. 2C), while remaining unchanged in AB ($P > 0.42$). [βOHB]_s remained unchanged in both groups over time (time effect $P = 0.083$; Fig. 2D) with no differences between groups ($P = 0.309$) and no Group × Time interaction ($P = 0.181$).

[Glc]_p at each time point was similar between the two groups (group effect $P = 0.216$) and remained unchanged throughout the study in both groups (time effect $P = 0.103$; Fig. 3A). [Ins]_s was comparable between the groups ($P = 0.811$) and there was no Group × Time interaction ($P = 0.839$; Fig. 3B), however, it tended to decrease at REC compared with PRE in SCI_C ($P = 0.068$; time effect of ANOVA for $P = 0.011$), while remaining unchanged in AB ($P > 0.170$).

Relationships between Δ[Ad]_p and lipid profiles

There were no significant correlation between Δ[Ad]_p vs. Δ[FFA]_s, Δ[AcAc]_s, Δ[βOHB]_s, and Δ[tKB]_s (all $P > 0.694$).

DISCUSSION

The new finding from this study was that serum indicators of lipolysis, [tKB]_s and [AcAc]_s, tended to be elevated one hour after exercise in SCI_C, while [FFA]_s and [Glc]_p remained unchanged during exercise. The results were not associated with differences in plasma catecholamine concentrations between the groups. Together, these results suggest that the increased [tKB]_s after exercise is likely explained by reduced uptake of ketone bodies from the skeletal muscles.

[tKB]_s increased after exercise only in SCI_C

Endurance activities are associated with increased fat utilization. Triglycerides, stored in adipose tissue and within muscle fibers, are the main source of FFA during exercise [17] and during the first few hours of recovery [18]. The release of FFA into the blood is primarily due to sympathetic nervous activity directed towards the adipocytes [17]. After β-oxidation of FFA in the mitochondria,

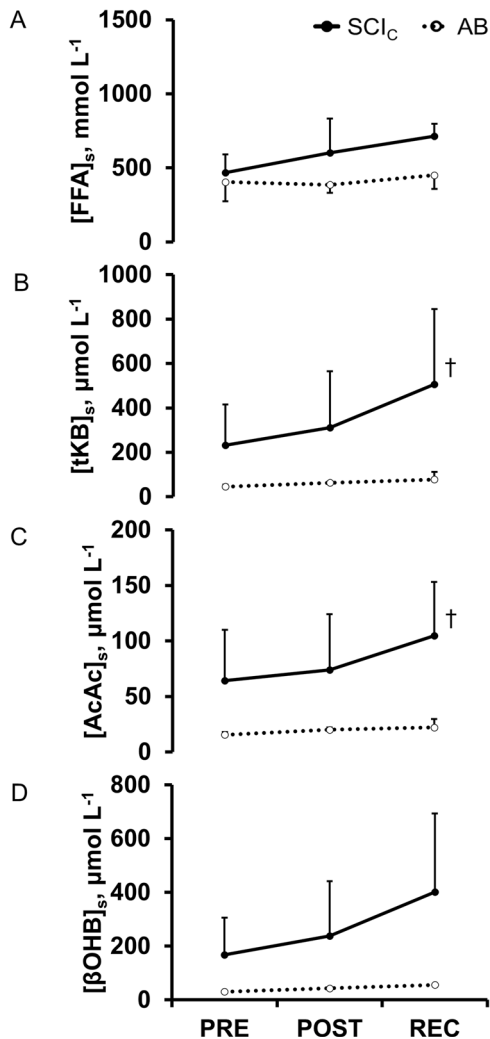


Fig. 2 Serum concentrations. Serum concentrations of free fatty acids (A), total ketone bodies (B), acetoacetic acid (C) and 3-hydroxybutyric acid (D) before and after exercise in individuals with cervical spinal cord injury and able-bodied. Blood samples were collected before (PRE), immediately after exercise (POST) and 60 min after the cessation of exercise (REC) in 6 individuals with cervical spinal cord injury (●; SCI_C) and 6 able-bodied (○; AB). [FFA]_s, serum concentrations of free fatty acids; [tKB]_s, serum concentrations of total ketone bodies; [AcAc]_s, serum concentrations of acetoacetic acid; [βOHB]_s, serum concentrations of 3-hydroxybutyric acid. Data are means (SD). †, compared from PRE ($P < 0.05$).

acetyl-coenzyme A (acetyl-CoA) is provided to the TCA cycle as substrate. Ketone bodies are synthesized in the liver by the condensation of 2 acetyl-CoA molecules, which are then transported to the extrahepatic tissues and used as an energy source in the brain and muscles [17]. [tKB]_s is determined by the balance between ketone supply from the liver into the blood and disappearance from blood into peripheral tissues.

Persons with SCI_C have muscular atrophy and increased storage of intramuscular fat [19]. Furthermore, accumulation of lipids could interfere with glucose metabolism in skeletal muscles due to reduced sensitivity of insulin receptors [20], resulting in enhanced fat utilization. With respect to the observed increase in serum ketone bodies, higher production of ketones bodies could be considered. However, similar [FFA]_s, [Glc]_p, and [Ins]_s between groups suggest ketone supply was not substantially altered. Thus, it is more likely that there was reduced uptake of ketones bodies from the bloodstream to the muscles in SCI_C during and after

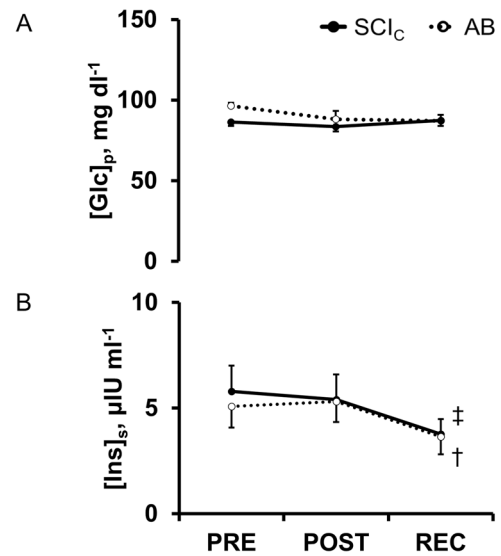


Fig. 3 Plasma concentrations of glucose and serum concentrations of insulin. Plasma concentrations of glucose (A) and serum concentrations of insulin (B) before and after exercise in individuals with cervical spinal cord injury and able-bodied. Blood samples were collected before (PRE), immediately after exercise (POST) and 60 min after the cessation of exercise (REC) in 6 individuals with cervical spinal cord injury (●; SCI_C) and 6 able-bodied (○; AB). [Glc]_p, plasma concentrations of glucose; [Ins]_s, serum concentrations of insulin. Data are means (SD). †, compared from PRE in AB; ‡, from PRE and POST in SCI ($P < 0.05$).

prolonged exercise, not enhanced production of ketones bodies. If RER is lower in SCI_C than in AB, this idea would be supported (see Supplementary Appendix for metabolic responses). Additional studies are needed to study the time course of changes immediately post-exercise, and whether similar results would be expected under different exercise conditions; for example, under long-duration exercise with glycogen depletion or with different nutritional strategies before and after exercise in SCI_C.

Ketones are critical energy sources in the peripheral tissues, and act as the substrates of lipolysis during non-glycolytic aerobic metabolism. MCT1 and MCT2 transporters control the uptake of ketones and are expressed in the skeletal muscles, heart, brain, muscle, and kidney [7]. Skeletal muscle tissues are a primary location for ketone uptake in AB persons, but individuals with SCI_C have drastically less muscle mass in which to uptake ketone bodies from the blood [21]. Muscle atrophy and selective transformation from type I to type II fibers in paralyzed areas [5] would further disadvantage ketone use as an energy source, as ketone usage is related with mitochondrial volume [22], which is larger in non-atrophied type I muscle fibers. Although it is possible the production of ketones was enhanced in SCI_C compared with AB, the actual usage of ketone bodies in the peripheral tissues may not be augmented, leading to our observation of [tKB]_s in the blood.

Insulin inhibits lipolysis, gluconeogenesis, and glycogenolysis in the liver. In the present study, the decrease in [Ins]_s following exercise in both groups suggests that lipolysis would be enhanced after exercise, although this would not solely account for the increase in serum ketones in SCI_C. Previous studies have suggested that insulin sensitivity itself decreased in SCI_C compared with paraplegia [23] and AB persons [24]. As a result, decreased insulin sensitivity could lead to secondary increases in lipid metabolism to overcome an energy deficit during exercise in SCI_C [25]. While this is an attractive theory, the change in plasma glucose was also similar between groups in the present study, suggesting trivial differences in insulin sensitivity.

Finally, one other potential mechanism for increased post-exercise $[tKB]_s$ is negative energy balance secondary to SCI_C . In a negative energy balance state, the central nervous system and red blood cells have priority use of glucose because they almost entirely dependent on glucose metabolism. However, as other tissues can rely on fat metabolism, the liver increases synthesis of ketone bodies to supply fuel to tissues that can use substrate other than glucose, such as skeletal muscle [26]. Nutritional health after SCI_C varies widely, with previous reports indicating 40–50% of SCI_C patients admitted to hospitals were at risk of nutritional deficiency, including up to 12% who were severely malnourished. At the opposite end of the spectrum, there are also individuals who suffer from obesity due to high energy intake, which likely exceeds energy expenditure [27]. In the present study, body weight in persons with SCI_C was comparable to AB. The influences of the negative energy balance would be minor in the study.

Increasing $[FFA]_s$ after exercise in SCI_C

In general, catabolic processes (i.e., glycolysis, gluconeogenesis and lipolysis) are activated by catecholamines. Venous infusion of catecholamines has been shown to increase $[Glc]_p$ and $[FFA]_s$ in healthy subjects [28]. However, catecholamines do not seem to influence glucose and lipid profiles during and after endurance exercise in SCI_C , given the lack of correlation in the present study. A previous study using the microdialysis technique in healthy participants revealed that lipolysis in the abdominal subcutaneous adipose tissue was stimulated by phentolamine (an α -adrenergic inhibitor) during 120-min rest, while in contrast, lipolysis was attenuated by propranolol (a β -adrenergic blocker) during 30-min exercise [29]. In the present study, energy expenditure in exercising SCI_C was lower than in AB, because the low absolute workload was ~ 5 W. However, the increase in $[FFA]_s$ after exercise was comparable to AB. Mechanisms of lipolysis in SCI_C would not be related with sympathoexcitation, which does not occur in the SCI_C group.

Limitations

Finally, we have to mention some limitations of the study. First, although the statistical powers of the ketones were relatively high enough to guarantee minimum effects of type II error, the sample size was small. So, the preliminary results of the present study may help the future research plans. Even the exercise load performed in this study wearing the measurement devices was a hurdle for SCI_C who do not have an exercise routine, because of their multiple disabilities. Unfortunately, metabolic responses could only be assessed for just 3 persons with SCI_C and there may be low power to detect differences in some biomarkers. These data were presented as supplementary files. Direct metabolic markers (outside serum and plasma) immediately after exercise were not measured. For instance, lactate is a metabolic by-product of glycolysis and is also produced by glycogenolysis. We did not evaluate intramuscular glycogen content. Muscle biopsies or non-invasive magnetic resonance spectroscopy are established procedures for measuring metabolic activity inside the muscle, but we are unable to conduct these procedures in our laboratory. Although there was no *a priori* hypothesis for a sex difference, only men participated in this study. This is reflective of the sex distribution of our rehabilitation program. It has been reported that there are sex differences in lipid metabolism in able-bodied individuals [30], and the results of this study may not apply to women with SCI_C .

CONCLUSION

Serum concentrations of ketone bodies increased after exercise only in SCI_C , with unchanged $[FFA]_s$ and $[Glc]_p$. Reduced utilization of ketones as an energy substrate during endurance exercise in SCI_C may be due to less active skeletal muscle mass. The present

results will provide constructive information to establish a strategy of nutritional promotion before and after exercise in SCI_C , for example, matching glucose intake for exercise intensity during and after exercise in persons with SCI_C . Future work is warranted to better understand fat metabolism during prolonged exercise in persons with SCI_C , as well as best methods to incorporate this knowledge into nutritional recommendations to improve exercise performance.

DATA AVAILABILITY

The dataset generated and analyzed during the current study is available from the corresponding author upon reasonable request.

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AUTHOR CONTRIBUTIONS

KN, Y-iK, YN, and FT conceived and designed research; KN, Y-iK, JSA, TM, YM, YU, KK, and TO performed the experiments; KN and Y-iK analyzed the data; KN, Y-iK, JSA, YN, and FT interpreted the results; KN prepared the figures; KN and Y-iK drafted the manuscript; all authors edited and revised the manuscript; all authors approved the final version of the manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

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.

ADDITIONAL INFORMATION

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