muscle (6,34). Although we hypothesized that muscle was "active" during passive leg movement, the present results did not show increments of the deoxy-Hb. According to the general principle, concentration changes in oxy- and deoxy-Hb are dependent on the dynamics of the equilibrium between tissue oxygen demand and supply (2,16). Therefore, a possible reason for our result is that oxygen delivery far exceeds the oxygen extraction in the acting muscle. The enhancement of HR during passive leg motion (Fig. 5) provides evidence to support this notion.

Changes in Hb concentration after exercise. After the cessation of the passive leg movement, the concentration of total Hb in the SCI group exceeded the preexercise level, whereas that in the normal group simply recovered to the preexercise level. Because the total Hb reflects the degree of muscle blood flow (6), these changes may suggest that enhancement of the muscle blood flow occurred in the SCI group, possibly resulting from the muscle contraction and oxygenation during the exercise period. It is likely that the excess total Hb following exercise resulted from the pooling of blood in the calf. Nevertheless, in this study, the subjects were kept in a standing posture on the apparatus before the initiation of the exercise period until the total Hb value reached a constant level; therefore, the above total Hb changes during the recovery stage cannot be explained solely by blood pooling in the calf. These total Hb changes may be due to postexercise hyperemia (35).

HR changes by imposing passive leg movement. As shown in the Figure 5, the HR increased after the onset of the passive leg movement in both SCI and normal subjects. These results provide evidence of the enhancement of central circulation by imposing passive leg motion even in the SCI patients. The simplest explanation is that increments of the venous return due to the muscle pump activity result in the central circulation (29). However, taken together with the results of differences in the EMG activity, there would be different mechanisms underlying the enhanced HR between two groups. In the case of the normal subjects, it is plausible that the enhancement of the HR is induced by the neuronal factor, which is an afferent neural signal from the mechanoreceptor by inducing muscle stretching (12). On the other hand, this neuronal factor is not a suitable explanation for SCI results because of the sensory paralysis. Rather, our results, the appearance of muscular activity and an alteration of the NIRS signals, imply that a metabolic change accompanied by muscle contraction seems to play a primary role in the enhancement of the central circulation. Because we do not still have any direct evidence, further investigations are needed to clarify this point.

Implications for rehabilitation. As mentioned at the beginning, chronic inactivity and hypocirculation of the paralyzed area are crucial factors in secondary impairment in SCI subjects (25). The present results provide indirect evidence that passive leg movement performed in a standing posture could alter the oxygenation level of the paralyzed muscle and has the potential to facilitate circulation of the paralyzed area. Given that the muscle contraction level during normal walking is about 15% MVC (21), it is considered that the muscle contraction level observed in this study is adequate to facilitate neural activity and circulation of the paralyzed area.

On a practical level, the subjects in the present study did not move their upper limbs and trunk voluntarily, because our aim was to examine whether the oxygenation level of the paralyzed muscle was altered by imposing passive leg movement. In a nonexperimental situation, however, patients would commonly operate the device themselves by manipulating the lever with their upper limbs. It is possible that the additional voluntary upper limb movement could enhance circulation not only in the voluntarily acting area, but also in the paralyzed area.

Although muscular activity in the paralyzed area can also be induced by applying electrical stimulation, as is the case in functional electrical stimulation (FES) (1,23,31), there are essential differences between our method and the FES technique. Previous investigations pointed out that one of major disadvantages of the FES technique is that it is difficult to generate FES-induced continuous muscle contractions without fatigue (for a review, see Stein et al. (32)). The muscle fatigue can be attributed to the fact that the fatigable motor unit is preferentially recruited by imposing electrical stimulation, in that large motor nerves are more easily activated than smaller ones. In contrast, in the case of the passive leg movement produced in the present study, the motor units are presumably recruited according to the size principle (15), because the afferent input was offered from proprioceptors by imposing muscle stretch and body load. Furthermore, passive leg movement is simpler and more practical than FES, and has a lower risk of misuse. Therefore, this type of passive leg movement might be a useful and efficient method for rehabilitation following SCI.

CONCLUSION

The present results demonstrate that passive leg movement can induce not only muscular activity, but also alteration of the muscle oxygenation level in the paralyzed lower limb. There may be increased oxygen consumption, but this could not be ascertained from the measurements in this study. Further study will be needed to clarify this issue.

The authors thank Dr. H. Ogata for comments on the manuscript. We also thank Dr. K. Mori for preparation of the experimental device. This work was supported by the Medical Frontier Project (ID: MF-15) of the Japanese Ministry of Health and Labor and the Japanese Society for Rehabilitation of Persons with Disabilities.

REFERENCES

1. Belanger, M., R.B. Stein, G.D. Wheeler, T. Gordon, and B. Leduc. Electrical stimulation: can it increase musclestrength and reverse osteopenia in spinal cord injured individuals? *Arch. Phys. Med. Rehabil.* 81:1090–1098, 2000.

2. Belardinelli, R., T. Barstow, J. Porszasz, and K. Wasserman. Skeletal muscle oxygenation during incremental exercise measured with near infrared spectroscopy. *Eur. J. Appl. Physiol.* 70:487–492, 1995.

3. BHAMBHANI, Y., C. TUCHAK, R. BURNHAM, J. JEON, and R. MAIKALA. Quadriceps muscle deoxygenation during functional electrical stimulation in adults with spinal cord injury. *Spinal Cord* 38:630–638, 2000.
4. BOOT, C. R. L., J. T. GROOTHUIS, H. VAN LANGEN, and M. T. E. HOPMAN. Shear stress levels in paralyzed legs of spinal cord-injured individuals with and without nerve degeneration. *J. Appl. Physiol.* 92:2335–2340, 2002.
5. BOUDAOUD, L., J. ROUSSI, S. LORTAT-JACOB, B. BUSSEL, O. DIZIEN, and L. DROUET. Endothelial fibrinolytic reactivity and the risk of deep venous thrombosis after spinal cord injury. *Spinal Cord* 35:151–157, 1997.
6. BOUSHEL, R., H. LANGBERG, J. OLESEN, J. GONZALES-ALONZO, J. BULOW, and M. KJAER. Monitoring tissue oxygen availability with near infrared spectroscopy (NIRS) in health and disease. *Scand. J. Med. Sci. Sports* 11:213–222, 2001.
7. CASTRO, M., R. APPLE, JR., D. STARON, G. CAMPOS, and G. A. DUDLEY. Influence of complete spinal cord injury on skeletal muscle within 6 mo of injury. *J. Appl. Physiol.* 86:350–358, 1999.
8. DIETZ, V., G. COLOMBO, L. JENSEN, and L. BAUMGARTNER. Locomotor capacity of spinal cord in paraplegic patients. *Ann. Neurol.* 37:574–582, 1995.
9. DIETZ, V., R. MULLER, and G. COLOMBO. Locomotor activity in spinal man: significance of afferent input from joint and load receptors. *Brain* 125:2626–2634, 2002.
10. DOBKIN, B. H., S. HARKEMA, P. REQUEJO, and R. EDGERTON. Modulation of locomotion-like EMG activity in subjects with complete and incomplete spinal cord injury. *J. Neurol. Rehabil.* 9:183–190, 1995.
11. FREY-RINDOVA, P., E. D. DE BRUIN, E. STUSSI, M. A. DAMBACHER, and V. DIETZ. Bone mineral density in upper and lower extremities during 12 months after spinal cord injury measured by peripheral quantitative computed tomography. *Spinal Cord* 38:26–32, 2000.
12. GLADWELL, V. F., and J. H. COOTE. Heart rate at the onset of muscle contraction and during passive muscle stretch in humans: a role for mechanoreceptors. *J. Physiol. (Lond).* 540:1095–1102, 2002.
13. GRIMBY, G., C. BROBERG, I. KROTKIEWSKA, and M. KROTKIEWSKI. Muscle fiber composition in patients with traumatic cord lesion. *Scand. J. Rehabil. Med.* 8:37–42, 1976.
14. HARKEMA, S. J. Neural plasticity after human spinal cord injury: Application of locomotor training to the rehabilitation of walking. *Neuroscientist* 7:455–468, 2001.
15. HENNEMAN, E., and L. M. MENDELL. Functional organization of motoneurone pool and its inputs. *In: Handbook of Physiology*, V.B. Brooks (Ed.). Bethesda: American Physiological Society, 1981, pp. 423–507.
16. HOMMA, S., H. EDA, H. OGASAWARA, and A. KAGAYA. Near-infrared estimation of O_2 supply and consumption in forearm muscles working at varying intensity. *J. Appl. Physiol.* 80:1279–1284, 1996.
17. HOPMAN, M. T. E., E. NOMMENSEN, W. N. J. VAN ASTEN, B. OESEBURG, and R. A. BINKHORST. Properties of the venous vascular system in the lower extremities of individuals with paraplegia. *Paraplegia* 32:810–816, 1994.
18. KAWASHIMA, N., D. NOZAKI, M. ABE, K. NAKAZAWA, and M. AKAI. Alternate leg movements contribute to amplify locomotion-like muscle activity in spinal cord injured patients. *J. Neurophysiol.* 93:777–785, 2005.
19. LOTTA, S., R. SCELSI, E. ALFONSI, et al. Morphometric and neurophysiological analysis of skeletal muscle in paraplegic patients with traumatic cord lesion. *Paraplegia* 29:247–252, 1991.
20. MARTIN, T., R. STEIN, P. HOEPPNERAND, and D. REID. Influence of electrical stimulation on the morphological and metabolic properties of paralyzed muscle. *J. Appl. Physiol.* 72:1393–1400, 1992.
21. MASUMOTO, K., S. TAKASUGI, N. HOTTA, K. FUJISHIMA, and Y. IWAMOTO. Electromyographic analysis of walking in water in healthy humans. *J. Physiol. Anthropol. Appl. Hum. Sci.* 23:119–27, 2004.
22. MAYNARD, JR., M. B., F. M. BRACKEN, G. CREASEY, et al. International standards for neurological and functional classification of spinal cord injury. *Spinal Cord* 35:266–274, 1997.
23. MUTTON, D. L., A. M. E. SCREMIN, B. J. BARSTOW, M. D. SCOTT, C. F. KUNKEL, and T. G. CAGLE. Physiologic responses during functional electrical stimulation leg cycling and hybrid exercise in spinal cord injured subjects. *Arch. Phys. Med. Rehabil.* 78:712–718, 1997.
24. NASH, M. S., M. S. BILSKER, H. M. KEARNEY, J. N. RAMIREZ, B. APPLEGATE, and B. A. GREEN. Effects of electrically-stimulated exercise and passive motion on echocardiographically-derived wall motion and cardiodynamic function in tetraplegic persons. *Paraplegia* 33:80–89, 1995.
25. NOREAU, L., P. PROULX, L. GAGNON, M. DROLET, and M. T. LARAMEE. Secondary impairments after spinal cord injury: a population-based study. *Am. J. Phys. Med. Rehabil.* 79:526–535, 2000.
26. OLIVE, J. L., G. A. DUDLEY, and K. K. MCCULLY, Vascular remodeling after spinal cord injury. *Med. Sci. Sports Exerc.* 35:901–907, 2003.
27. OLIVE, J. L., K. K. MCCULLY, and G. A. DUDLEY. Blood flow response in individuals with incomplete spinal cord injuries. *Spinal Cord* 40:639–645, 2002.
28. QUARESIMA, V., W. N. COLIER, M. VAN DER SLUIJS, and M. FERRARI. Nonuniform quadriceps O_2 consumption revealed by near infrared multipoint measurements. *Biochem. Biophys. Res. Commun.* 285:1034–1039, 2001.
29. ROWLAND, T. W. The circulatory response to exercise: role of the peripheral pump. *Int. J. Sports Med.* 22:558–565, 2001.
30. SAKO, T., T. HAMAOKA, H. HIGUCHI, Y. KUROSAWA, and T. KATSUMURA. Validity of NIR spectroscopy for quantitatively measuring muscle oxidative metabolic rate in exercise. *J. Appl. Physiol.* 90:338–344, 2001.
31. SAMPSON, E. E., R. S. BURNHAM, and B. J. ANDREWS. Functional electrical stimulation effect on orthostatic hypotension after spinal cord injury. *Arch. Phys. Med. Rehabil.* 81:139–143, 2000.
32. STEIN, R. B., S. L. CHONG, K. B. JAMES, et al. Electrical stimulation for therapy and mobility after spinal cord injury. *Prog. Brain Res.* 137:27–34, 2002.
33. SZOLLAR, S. M., E. M. MARTIN, D. J. SARTORIS, J. G. PARTHEMORE, and L. J. DEFTOS. Bone mineral density and indexes of bone metabolism in spinal cord injury. *Am. J. Phys. Med. Rehabil.* 77:28–35, 1998.
34. TAKAISHI, T., T. SUGIURA, K, KATAYAMA, et al. Changes in blood volume and oxygenation level in a working muscle during a crank cycle. *Med. Sci. Sports Exerc.* 34:520–528, 2002.
35. TAYLOR, J. A., P. B. CHASE, R. M. ENOKA, and D. R. SEALS. Cardiovascular adjustments to rhythmic handgrip exercise: relationship to electromyographic activity and post-exercise hyperemia. *Eur. J. Appl. Physiol. Occup. Physiol.* 58:32–38, 1988.
36. VAN BEEKVELT, M. C., W. N. J. C. VAN ASTEN, and M. T. E. HOPMAN. The effect of electrical stimulation on leg muscle pump activity in spinal cord-injured and able-bodied individuals. *Eur. J. Appl. Physiol.* 82:510–516, 2000.
37. VAN BEEKVELT, M. C., M. S. BORGHUIS, B. G. VAN ENGELEN, R. A. WEVERS, and W. N. COLIER. Adipose tissue thickness affects in vivo quantitative near-IR spectroscopy in human skeletal muscle. *Clin. Sci. (Lond).* 101:21–28, 2001.

歩行困難者への工学的支援

中澤　公孝　　国立身体障害者リハビリテーションセンター

ウォーキング研究　No. 10, 31 － 35
Walking Research

Walking Research
No.10, 2006

歩行困難者への工学的支援

Assistive technology for locomotor disabilities

キーワード：脊髄損傷、歩行、二次障害、歩行訓練機
Keywords ：Spinal cord injury, locomotion, secondary disorder, gait trainer

中澤 公孝[1)]
Kimitaka Nakazawa

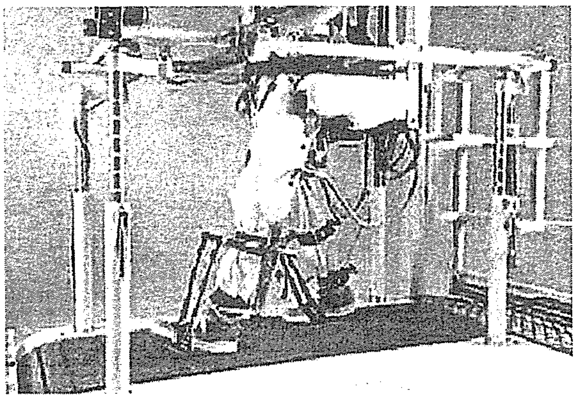
歩行困難者、中でも脊髄損傷者の歩行リハビリテーション理論は過去10年余りで大きく変貌した。それは脊髄の可塑的性質の発見に代表されるように、周辺の神経生理学的研究が急速に発展したことによるところが大きい。リハビリテーションの理論的枠組みの転換は、関連する機器開発にも反映され、従来とは異なったさまざまな装置が開発されるようになった。本稿では、近年筆者らのグループが開発した歩行訓練装置の基本コンセプトとそれが構築されるにいたった理論的背景を紹介しながら、この分野の新しい動向を解説したい。

筆者ら国立身体障害者リハビリテーションセンターと東京農工大、芝浦工大の研究グループは近年、動力付き歩行装具と免荷装置から成る移動型の歩行訓練装置を開発した。この装置は、現在のところ自立歩行が困難な後期高齢者や股関節術後患者を主たる対象としているが、近い将来、対麻痺者や片麻痺者の歩行リハビリテーションへとその用途を広げる予定である。以下ではこの装置の基本コンセプトの理論的背景、開発要件ならびに装置の概要について紹介する。

理論的背景

神経生理学的視点

本装置の理論的背景の第一は、1990年代中盤以降、主に脊髄損傷のニューロリハビリテーションとして考案された受動歩行トレーニング理論にある。受動歩行トレーニングでは、トレッドミル上で訓練者の下肢を理学療法士あるいは最近ではロボットがベルトスピードに合わせてステッピングさせる（図1）。それによって下肢の感覚受容器が刺激され、脊髄への感覚入力が喚起される。ステッピングにともなってパターン化した感覚入力が脊髄の歩行パターンジェネレーターおよび脊髄より上位の中枢神経を賦活する。それが繰り返し行われることで中枢神経系の再組織化が促進される[1)]。この時、不全脊髄損傷者のように上位中枢から脊髄運動ニューロンへの入力がわずかでも残っていると脊髄での伝達効率が改善され、自立歩行回復の可能性が高くなる。これが受動歩行トレーニングの理論である。



ロボット型歩行訓練機 Lokomat

図1

1）国立身体障害者リハビリテーションセンター Research Institute National Rehabilitation Center for Persons with Disabilities

我々は、受動歩行トレーニングのポイントを次の２点に集約し、新たな歩行訓練装置の要件とした。それは、①受動歩行あるいは半受動歩行による末梢感覚入力の喚起、および②脳からの下行性司令の確保、である。それぞれについて以下で詳しく説明する。

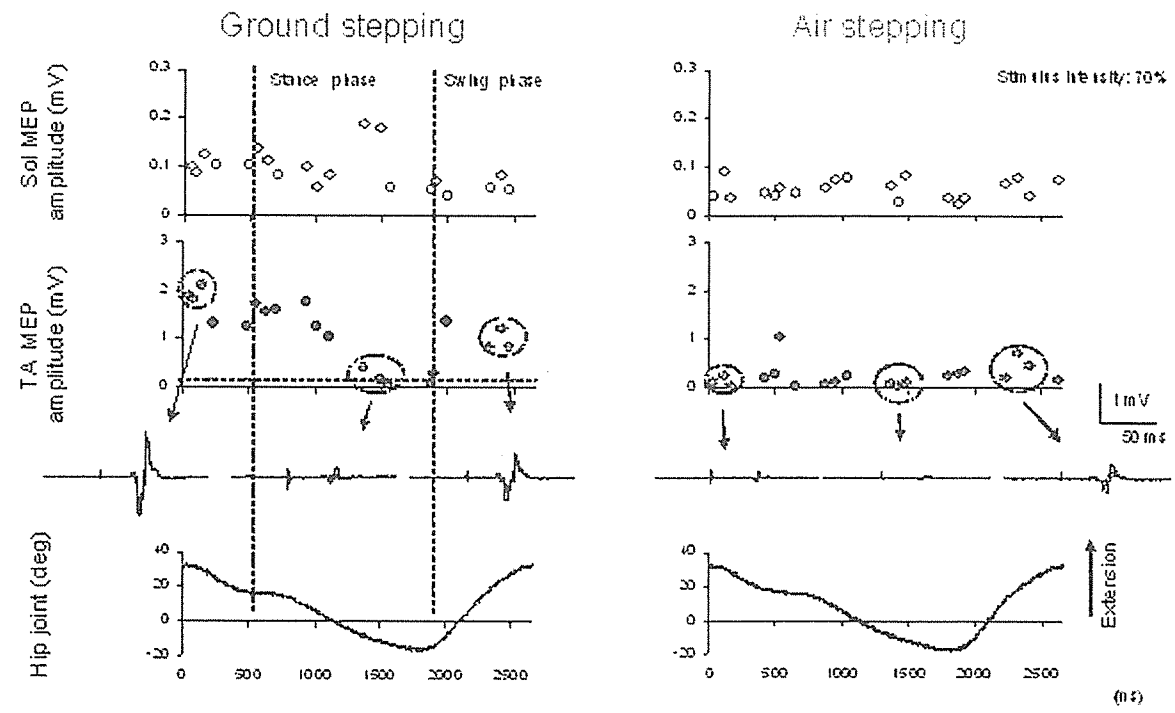
①　受動歩行の効果

対麻痺患者であっても下肢を受動的に動かしステッピングを行うと下肢の各筋群にステッピングと同調した筋活動が誘発される。これは下肢のステッピングに伴う感覚入力が脊髄の歩行パターンジェネレーターおよびその他の歩行に関連する神経回路を介し反射性出力を誘発した結果生じると考えられている。受動歩行トレーニングを続けると下肢の麻痺筋群に誘発されるそれらの筋放電が増大するとともに筋間の放電パターンも改善することが知られている。筆者らはさらにロボット型歩行訓練機を用いた実験によって、受動的ステッピングを行うと足関節屈筋である前脛骨筋の皮質脊髄路興奮性が増大することを見出した（図２）。この筋はそもそも皮質との結合が強く、足関節伸筋のヒラメ筋に比べて歩行中の皮質脊髄路興奮性が高いことが知られている[2,3]。今回の筆者らの結果は、随意歩行に似た下肢の動きを他動的に与えるだけで、皮質脊髄路の興奮性が増強することを示している。それはさらに他動的ステッピングを長期的に行うこと、あるいは下行性司令が加わることによってこの経路の再組織化が起こることも示唆する結果といえる。

以上を踏まえ、今回開発する機器には受動的ステッピング動作を実現する機構を組み込むこととした。

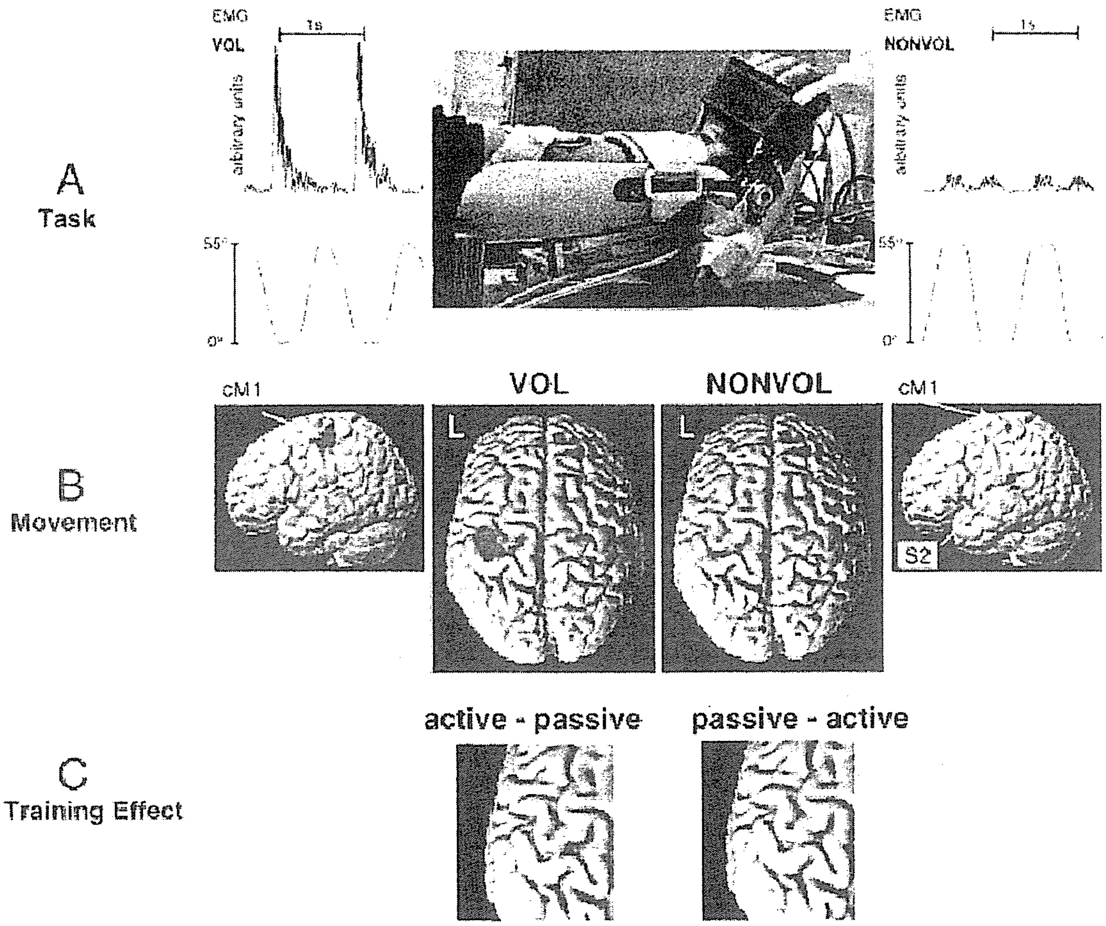
②　下行性司令の効果について

前述したように免荷式受動歩行トレーニングは不全脊髄損傷者の歩行能力回復に効果がある。逆に言えば脳と脊髄との結合が完全に遮断された完全脊髄損傷者では効果が見込めない。自立歩行の回復にはわずかであっても脳から脊髄への下行性入力の存在が必要なのである。最近のThomas and Gorasini（2005）[4] の報告によれば不全脊髄損傷者の皮質脊髄路機能は歩行トレーニングで改善する。またLotze ら（2003）[5] は受動運動トレーニングと随意司令を要する随意運動トレーニングを比較し、後者の方が大脳一次運動野の再組織化が著明であることを示した（図３）。この結果は、随意性の低い麻痺肢であっても、運動を起こそうとする意志が運動機能の回復



受動歩行中に経頭蓋磁気刺激によって誘発したヒラメ筋（SOL）と前脛骨筋（TA）の誘発電位（MEP）の変化。Ground stepping；トレッドミルベルト上でのステッピング、Air stepping；空中に吊られた状態でのステッピング。

図２



手関節の受動トレーニング（NONVOL）と能動トレーニング（VOL）による脳の再組織化の比較。A:動作課題特性の比較。VOLでは筋活動電位が発生するのに対し、NONVOLではほとんど生じていない。B:それぞれの運動課題で活性化する脳の領域の違い。C:トレーニング後に生じる活性領域の違い。文献5より引用。

図3

には重要であることを示唆している。すなわち、不全脊髄損傷者の受動歩行トレーニングにおいて、ただ他動的に下肢を動かされているのに比べて、自分の意志の下に動かそうとする随意司令がきわめて重要であることが示唆される。随意司令を引き出すためには、強い動機付けが必要である。この動機付けのため、今回の歩行訓練機は自分の意志の下で移動できることを重要な要件の一つとした。

体力医学的視点

上記したポイントは歩行回復にかかわる神経生理学的視点であった。もう一方の視点は体力医学的視点である。

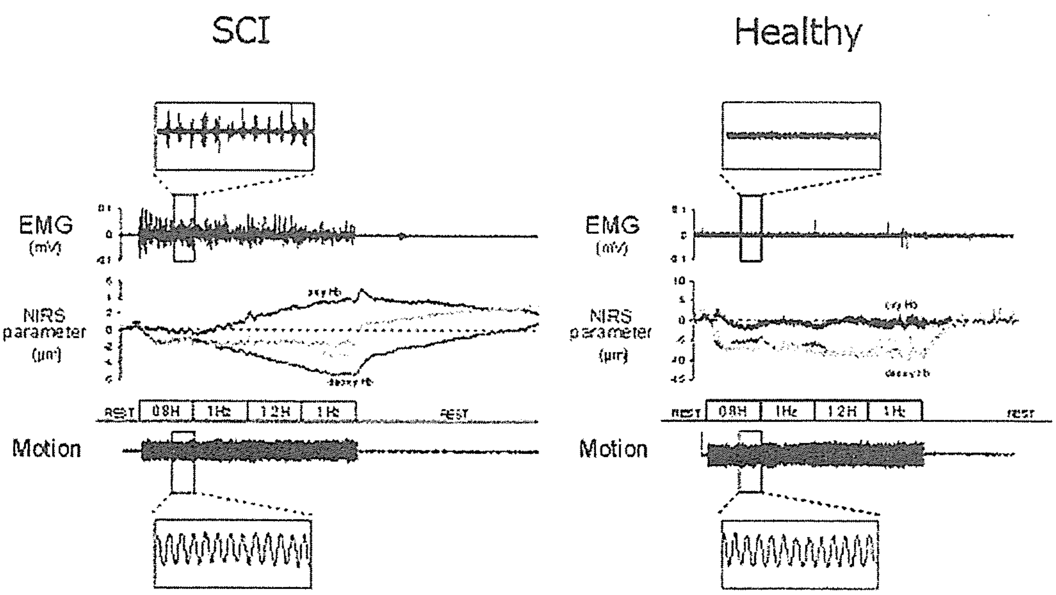
前述したように、上位中枢と脊髄との連絡が完全に遮断された完全損傷者では現状で理論的には自立歩行の改善は期待できない。そのため、完全損傷者にとって歩行機能回復を目指した受動歩行トレーニングを行うことの臨床的な意義は無い。しかし、立位での受動運動は麻痺領域の血液循環を促進するなど、神経系以外の運動に関連する諸器官の刺激効果にも優れる。筆者らは、歩行装具を用いた装具歩行トレーニング実験の結果から対麻痺者が立位で運動を行うことのいくつかの利点を知るに至った。例えば、装具歩行中の心拍数は150拍程度、酸素摂取量は16ml/kg/min程度となり[6]、これらの数値は座位で腕エルゴメーターをオールアウトまで行ったときの最大値で相対化するといずれも80%以上の数値となる。これは上肢の運動では心臓循環系を十分に働かせるに至っていないこと、すなわち最大心拍数と最大酸素摂

取量を過小評価していることを意味する。歩行装具を用いた立位歩行は体幹の筋など残余機能を十分に賦活させ、結果として心臓循環系、呼吸系へのトレーニングに適した負荷を与えることが容易になると考えられる。筆者らはまた、装具歩行や受動的ステッピングにおいて下肢の麻痺筋群に運動周期に一致した筋活動が誘発されることに着目し、それらの筋活動によって筋の末梢循環や代謝レベルが変化するのかどうかを調べた[7]。実験には立位歩行を模した受動的な下肢の左右交互性スウィング運動を用いた。この運動においても下肢麻痺領域に筋活動が誘発されることを確認した。実験では近赤外分光法を用い下腿三頭筋の酸素化レベルを定量化した。その結果、健常者の場合と異なり、対麻痺者では受動運動の結果、当該筋の酸素化レベルが大きく変化し、末梢循環が変調したことが示唆された（図４）。麻痺領域の末梢循環は普段の生活ではほとんど変化しないことが予想されることから、たとえ受動的運動であってもこれを促進することの意味は大きいと思われる。

脊髄損傷者では麻痺領域の不使用に伴う諸種の二次的障害が深刻な問題となる。その意味で、立位での受動運動はその予防に有効であるといえる。この点は完全損傷、不全損傷を問わない。したがって、脊髄完全損傷者にとっても立位運動を行うことは十分な臨床的意義があるといえる。本装置の理論的背景の第二のポイントがここにある。

以上の神経生理学的、体力医学的知見を踏まえ、本装置の要件を以下のように設定した。

1．下肢のステッピング運動を装置によって実現可能とする。対麻痺者など自力での歩行が困難な人に対して受動的ステッピング運動を実現し、歩行の再学習、神経の再組織化を促す。
2．移動型免荷装置によって訓練者の意志に基づく移動を可能にする。それによって訓練者の動機付け、トレーニング意欲を高める。上位中枢からの下行性指令を可能な限り高めることで脊髄への伝達効率を改善する。同時に残余機能を最大限動員することで呼吸循環系をはじめとする全身諸機関への刺激効果を高める。



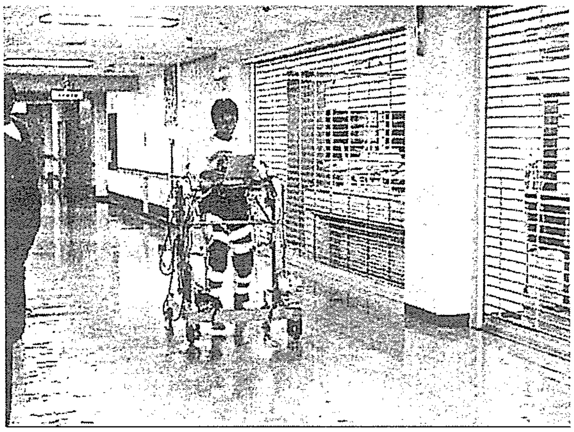
立位での受動的股関節屈曲伸展動作中の下腿三頭筋酸素化レベル。脊髄損傷者（SCI）と健常者（NORMAL）でのデータの比較。SCIでは動作中（Motion）に筋放電（EMG）が誘発され、近赤外分光法で計測した酸素化ヘモグロビンなどの変量（NIRS parameters）が変化していることがわかる。文献７より引用

図４

歩行訓練装置の概要

装置の基本構成はモーターで駆動される歩行装具部と身体を支え、あわせて免荷もおこなう免荷機構部から成る（図５)。歩行装具部は訓練者の状態、下肢のサイズに応じたステッピングを行う。免荷機構部は最大で約50kgの免荷をおこなうとともに、26 m/mの速度での移動を可能とする。

既に屋外での動作確認を行い、上記要件を満たす装置の試作機が完成した。今後さらに細部を改良し、臨床試験を行う予定である。



移動型歩行訓練装置を用いた試験歩行の様子

図５

参考文献

1) Raineteau O, Schwab ME. Plasticity of motor systems after incomplete spinal cord injury. Nature Rev 2: 263-273, 2001
2) Capaday C, Lavoie BA, Barbeau H, Schneider C, Bonnard M (1999) : Studies on the corticospinal control of human walking. I. Responses to focal transcranial magnetic stimulation of the motor cortex. J Neurophysiol 81: 129-139
3) Christensen LO, Andersen JB, Sinkiaer T, Nielsen J (2001) : Transcranical magnetic stimulation and stretch reflexes in the tibialis anterior muscle during human walking. J Physiol 531: 545-557
4) Thomas S and M A Gorassini: Increases in corticospinal tract function by treadmill training. J Neurophysiol 94:2844-2855, 2005
after incomplete spinal cord injury
5) Lotze M, Braun C, Birbaumer N, Anders S, Cohen LG: Motor learning elicited by voluntary drive. Brain 126: 866-872, 2003
6) Kawashima N, Sone Y, Nakazawa K, Akai M, Yano H: Energy expenditure during walking with weight-bearing control (WBC) orthosis in thoracic level of paraplegic patients. Spinal Cord 41, 506-510, 2003
7) Kawashima K, Nakazawa K, Akai M. Muscle oxygenation of the paralyzed lower limb in spinal cord injured persons. Med Sci Sport Exerc 37, 915-921, 2005.

Spinal Cord (2006) 44, 480–486

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Original Article

Cardiorespiratory responses during passive walking-like exercise in quadriplegics

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Study design: Cross-sectional and comparative investigation using quadriplegics (QP) and nondisabled subjects (ND).
Objective: To evaluate cardiorespiratory responses during passive walking-like exercise (PWE) in QP.
Setting: National Rehabilitation Center for Persons with Disabilities in Japan.
Method: The subjects were seven male QP with complete lesion (age: 27.0±5.4, injured level: C6–C7) and six male ND (age: 26.3±4.5). Cardiorespiratory responses were measured until voluntary fatigue during PWE, the rhythmical activity of paralyzed lower limbs synchronized with arm movements.
Results: There were no significant differences in oxygen consumption ($\dot{V}O_2$), pulmonary ventilation ($\dot{V}E$), heart rate (HR) and oxygen pulse (O_2 pulse) between QP and ND during PWE. ND showed increased ventilatory equivalent for oxygen ($\dot{V}E/\dot{V}O_2$ ratio) during exercise, while QP showed a significantly greater respiratory rate (RR) during exercise than ND ($P<0.05$).
Conclusion: PWE elicited an increase in $\dot{V}O_2$ with workload increment in QP similar to ND. However, higher RR suggested the intrinsic dysfunction of RR control during submaximal exercise in QP. From these results, it was thought that respiratory response would be the restriction factor of efficient oxygen transportation during PWE in QP.
Spinal Cord (2006) **44,** 480–486. doi:10.1038/sj.sc.3101875; published online 29 November 2005

Keywords: quadriplegics; passive walking-like exercise; cardiorespiratory response

Introduction

For individuals with spinal cord injury (ISCI), it is difficult to improve their cardiorespiratory function and to activate the oxygen supply function by exercise training such as wheelchair or arm swinging exercise because of the characteristics of the obstacles.[1,2] In general, the peak oxygen uptake (peak $\dot{V}O_2$) is dependent on the level of spinal cord injury (SCI), and quadriplegics (QP) and high lesion paraplegics (PP) show a lower peak $\dot{V}O_2$ than nondisabled (ND) or low lesion PP.[3–5] In addition, during submaximal exercise, QP and high lesion PP showed the reduced ventilation efficiency, stroke volume, venous return and sympathetic activity in comparison to ND or low lesion PP.[5,6]

For such ISCI with low physiological responses to exercise, the exercise posture is an important factor enhancing the effect of aerobic training. McLean *et al*[7] investigated the influence of body posture in training on aerobic capacity in ISCI and indicated that although improvements in aerobic capacity could be achieved by training in either a supine or a sitting posture, the supine posture had more effect on aerobic training than the sitting posture. One reason is that the supine posture is advantageous to ISCI circulation, because there is small amount of blood in the paralyzed leg compared to the sitting posture due to the lower effect of gravity.

In contrast, in a sitting posture during exercise, venous blood pools in the legs and abdomen, causing reduced filling pressure and diminished ventricular volume. Therefore, when QP and PP perform exercise in a sitting posture, blood is not efficiently redistributed

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to the working muscles.[8–11] To improve venous blood pooling during exercise in a sitting posture, some researchers have investigated the effect of passive leg exercise on circulation.[9,12] This exercise passively moves the paralyzed lower limbs, the blood stored in lower limbs returns to the heart, and stroke volume increases conjointly with the law of Frank-starling.[9,12]

It is reported that functional electrical stimulation (FES) is also a useful method to decrease venous blood pooling in the legs, because FES increases the activity of muscle pumping and vasoconstriction in the legs.[13–15] Bhambhani *et al*[16] measured the deoxygenation of the quadriceps muscle during FES using QP and PP. They indicated that the muscle deoxygenation of paralyzed muscles occurred more quickly with metabolic responses in the paralyzed muscles. Mutton *et al*[17] reported a significant increase in peak $\dot{V}O_2$ during hybrid exercise[18–20] that combined upper arm exercise with FES in QP and PP when compared with only FES. Moreover, Raymond *et al*[18] showed a higher oxygen intake and lower heart rate (HR) during hybrid exercise including arm-swinging exercise at a workload of 65% maximal oxygen uptake in PP. In addition, Hooker *et al*[21] compared the respiratory and circulatory responses during hybrid exercise with those during submaximal arm-swinging exercise and FES leg-cycle exercise in QP. They showed higher pulmonary ventilation ($\dot{V}E$), oxygen uptake ($\dot{V}O_2$) and carbon dioxide elimination during hybrid exercise than the other two exercises, but a higher stroke volume than only arm-swinging exercise. They concluded that hybrid exercise using whole-body exercise, including the paralyzed muscles, is effective in improving the cardiorespiratory function of ISCI.

More recently, it has been reported that standing gait exercise with orthoses has a good influence on cardiorespiratory function in ISCI.[22] Faghri *et al*[13,23] investigated the physiological reaction of a standing posture on ISCI with and without FES. They showed stable cardiac output, stroke volume and total peripheral resistance (TPR) during 30 min standing with FES in both QP and PP. In contrast, during passive standing without FES, QP demonstrated significantly higher TPR and significantly lower systolic blood pressure and mean arterial pressure than PP. Faghri *et al*[13,23] also indicated that standing without FES was disadvantageous to the regulation of hemodynamics during posture change in QP.

When ISCI passively walked on a treadmill using body weight support equipment, they showed a similar electromyographic pattern in the paralyzed muscles to ND.[24] Furthermore, Colombo *et al*[25] obtained the same result during passive stepping using driven gait orthosis for C3 (incomplete) and C5 (complete). It is naturally expected that passive walking with arm exercise increases energy expenditure and oxygen supply to the arm is elevated. However, as far as we know, there are no studies investigating the cardiorespiratory responses of QP during passive walking-like exercise (PWE) when standing.

The purpose of this study, therefore, was to clarify respiratory and circuratory responses during PWE by a stepwise incremental method and to compare the results of QP with those of ND.

Methods

Subjects

Seven male patients with complete chronic QP and six ND male subjects volunteered to participate in this study. Table 1 lists their physical characteristics. The lesion in SCI was located between C6 and C7. All subjects regularly performed wheelchair sports, such as twin basketball, quad rugby and distance running, for more than 60 min a day and more than twice a week. No subject had a history of cardiovascular, metabolic, or pulmonary disease. Informed consent was obtained from all subjects before their participation in this study. The subject refrained from food, caffeine and nicotine for at least 3 h before testing. The study was approved by the Ethical Research Committee in the National Rehabilitation Center for Persons with Disabilities.

Testing protocols

All subjects performed an incremental exercise test on an Easy Stand Glider 6000 (Altimate Medical Int.,

Table 1 Characteristics of the subjects

No	*Sex*	*Height (cm)*	*Weight (kg)*	*Age (years)*	*Level of injury*	*Zancolli*	*ASIA*	*Times of injury (month)*	*Sports*
a	M	163.0	57.3	34	C6	2B3	A	153	Twin basketball
b	M	168.0	55.7	23	C6	2B1	A	32	Twin basketball
c	M	174.0	55.1	29	C6	2B2	A	27	Twin basketball
d	M	166.0	52.3	29	C6	2B1	A	81	Distance running
e	M	160.0	41.3	20	C7	3A	A	25	Twin basketball
f	M	177.0	69.8	22	C7	3A	B	54	Quad rugby
g	M	172.0	66.6	32	C6	2B1	A	107	Quad rugby
h	M	168.0	59.7	25	ND				Track and field
i	M	180.0	62.2	26	ND				Soccer
j	M	172.0	62.5	35	ND				Track and field
k	M	175.0	68.4	23	ND				Track and field
l	M	168.0	65.0	26	ND				Baseball
m	M	170.0	54.0	23	ND				Skiing

Morton, MN, USA). The Easy Stand Glider 6000 is designed to strengthen both the upper and lower extremities while standing. It has a safety belt around the waist, a chest pad, hip guide and knee support. When the subject swings his arms back and forth, his legs simultaneously move passively just like walking. Tests were performed with the push and pull handle horizontal to the level of the shoulder joint and elbows slightly flexed at the point of maximal arm extension (Figure 1).

The subjects remained seated in a wheelchair or chair for at least 30 min. Baseline physiological measurements were recorded for the last 5 min during seating. They were subsequently guided with a metronome for reciprocal movement of the arms and legs while standing. Exercise commenced by swinging the arms back and forth at 20 times/min for 2 min. The swings were then increased to 10 times/min every 2 min until 50 times/min, and then 5 times/min every 2 min until exhaustion. The incremental exercise test was terminated when voluntary fatigue was attained. Voluntary fatigue was defined as the point at which the subject could no longer keep pace and his RPE was over 15. The experiment was carried out in a room with ambient temperature and relative humidity maintained at 22–25°C and 30–50%, respectively.

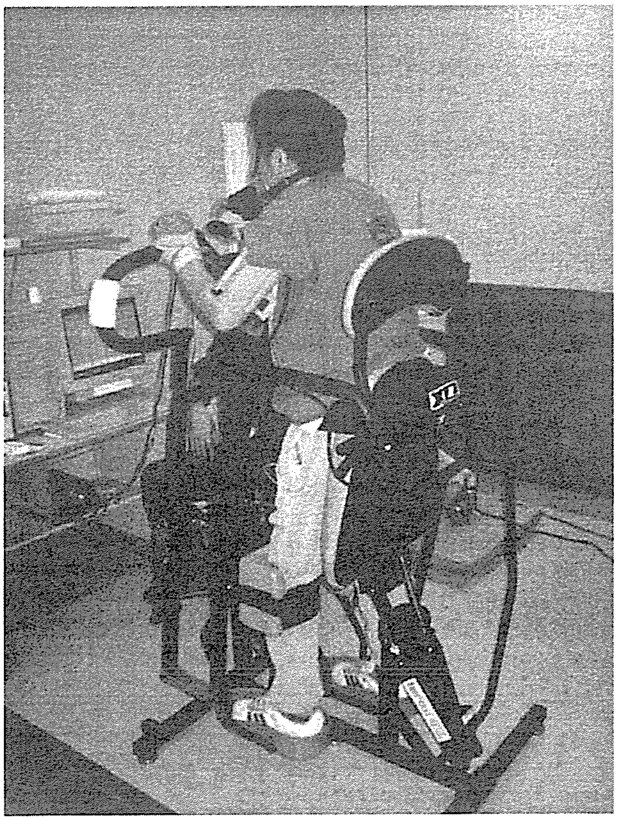

Figure 1 The arm swinging and passive walking machine. When a quadriplegic subject swings his arms back and forth, the paralyzed legs simultaneously move as if walking

Cardiorespiratory measurements

Cardiorespiratory measurements were continuously monitored during the test using the gas analyzer of the metabolic system (Model AT-3000, Anima, Tokyo, Japan). The gas analyzer was calibrated using standard gas concentrations (16.1% oxygen, 5.01% carbon dioxide). The volume transducer was calibrated using a syringe calibrated to 2 l. The gas analyzer was programmed to present the following results: absolute $\dot{V}O_2$ (l/min), relative $\dot{V}O_2$ (ml/kg/min), respiratory rate (RR, times/min) and $\dot{V}E$ (l/min). The following variables were calculated from the oxygen pulse (O_2 pulse, ml/beat) as the ratio between absolute $\dot{V}O_2$ and HR (beats/min), and the ventilatory equivalent for oxygen ($\dot{V}E/\dot{V}O_2$ ratio, l/ml) as the ratio between $\dot{V}E$ and absolute $\dot{V}O_2$.

HR was recorded during the last 10 s at each work stage using a wireless monitor (Life Scope 8/Two, Nihon Koden, Tokyo, Japan). Blood was sampled from the earlobe during rest and exercise and blood lactate accumulation (LA, mmol/ml) was measured using a simplified blood lactate test meter (Lactate Pro™ LT-1710, Arckly, Inc., Kyoto, Japan). Blood sampling was conducted immediately after rest and within 30 s after each workload. The sampling time was within 20 s.

Statistical analysis

All variables were expressed as the mean ± SD. Two-way repeated analysis of variance was used to compare the difference between groups. *P*-values <0.05 were considered significant.

Results

Figure 2a shows the relationship between $\dot{V}O_2$ and workload in QP and ND. $\dot{V}O_2$ within the workload of 60 times/min varied little and almost no rise was observed. At a workload of 65 times/min, $\dot{V}O_2$ started to increase rapidly. At any workload, there was no significant difference between the groups. Changes in $\dot{V}E$ and LA over time during exercise were similar to those in $\dot{V}O_2$. No significant differences were found in $\dot{V}E$ and LA between QP and ND.

HR increased with the workload increment in both groups (Figure 2b). When the workloads were between 40 and 70 times/min, there was little increase in HR for QP and ND. Although QP showed a higher HR than ND during rest and exercise, significant difference was only found at a workload of 30 times/min. Figure 2c indicates the O_2 pulse over the time course of exercise. In ND, the O_2 pulse remained unchanged from the beginning of exercise to a workload of 60 times/min. Subsequently, the O_2 pulse of QP increased rapidly. In contrast, QP showed a gradual increase in the O_2 pulse linearly with the workload. The O_2 pulse of QP was higher than that of ND at any workload and significant difference was only found at the beginning of exercise (20 times/min).

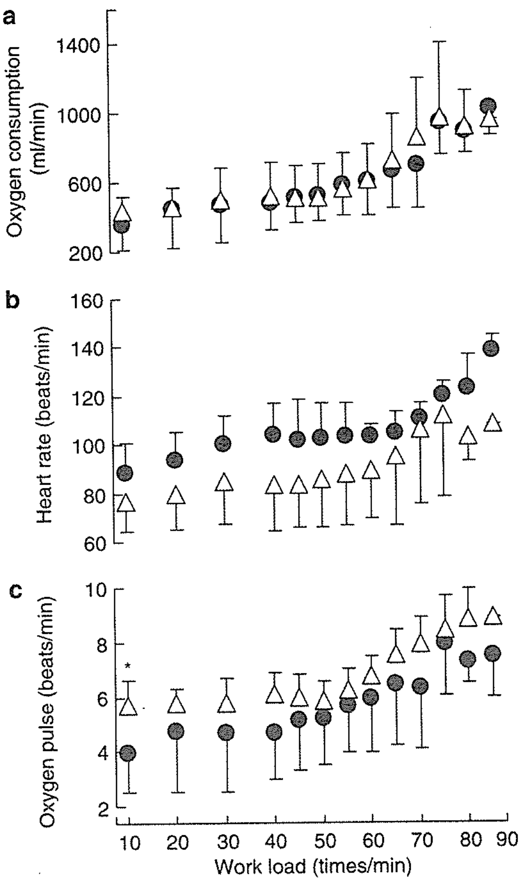

Figure 2 Change in oxygen consumption (**a**), heart rate (**b**), and oxygen pulse (**c**) during passive walking with arm swinging exercise in persons with Quadriplegics (●) and nondisabled (△). Although there was no difference of oxygen consumption between QP and ND, oxygen transportation of QP was inferior to ND during the exercise. $*P<0.05$: compared with nondisabled subjects

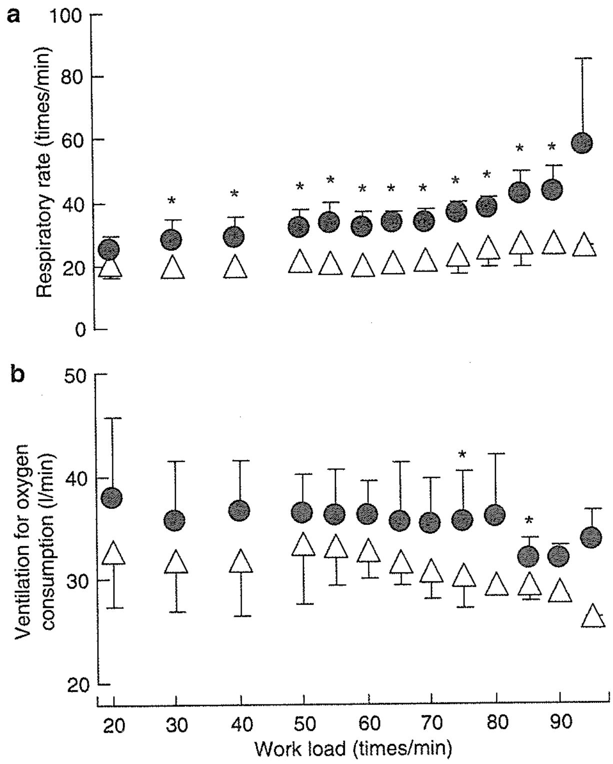

Figure 3 Change in respiratory rate (**a**) and ventilation for oxygen consumption (**b**) during passive walking with arm swinging exercise in persons with Quadriplegics (●) and nondisabled (△). Ventilation efficiency showed significant decrease in QP as compared with ND. $*P<0.05$, $^{\#}P<0.01$: compared with nondisabled subjects

Figure 4a illustrates the relationship of HR to $\dot{V}O_2$ in QP and ND. With increasing $\dot{V}O_2$, HR linearly and significantly increased in both groups. When $\dot{V}O_2$ was around 500 ml, HR of QP was apparently greater than ND.

There existed a great difference in RR between QP and ND during exercise (Figure 3a). ND had almost unchanged RR during exercise. In contrast to ND, QP showed increased RR over the time course of exercise. There were significant differences in RR between the groups at any workload except the lowest (20 times/min) and the highest (95 times/min). Figure 3b shows the $\dot{V}E/\dot{V}O_2$ ratio during the incremental exercise test in QP and ND. The $\dot{V}E/\dot{V}O_2$ ratio of QP was higher than that of ND at any workload and significant differences were found at higher workloads of 75 and 85 times/min.

The relationship of RR to $\dot{V}E$ is illustrated in Figure 4b with regression lines. The regression line of QP shifted to the upper side of ND, indicating that QP required more RR to achieve the same $\dot{V}E$ as ND.

Discussion

In this study, there were no significant differences in $\dot{V}O_2$ and $\dot{V}E$ during standing exercise between QP and ND (Figure 2a). Some investigators showed that the cardiorespiratory responses of QP during arm exercise were relatively lower than ND and PP,[4,5,26] suggesting that the cardiorespiratory responses of ISCI are largely dependent on the level of SCI.[3,6,27] These results were obtained from arm-swinging exercise or wheelchair ergometer exercise requiring mainly upper limb activity in a sitting posture. It may be considered that the exercise while sitting, using only the upper limbs, influences the $\dot{V}O_2$ of ISCI. Hopman *et al*[28] demonstrated that peak $\dot{V}O_2$ significantly increased in a supine posture during maximal arm-swinging exercise in comparison with a sitting posture. In addition, McLean *et al*[7] compared the power output (PO) of QP during an intermittent progressive peak exercise test in a sitting posture with that in a supine posture. As a result, they reported higher PO in a sitting than supine posture. These investigations suggest that cardiorespiratory responses during exercise are affected by exercise postures in QP.

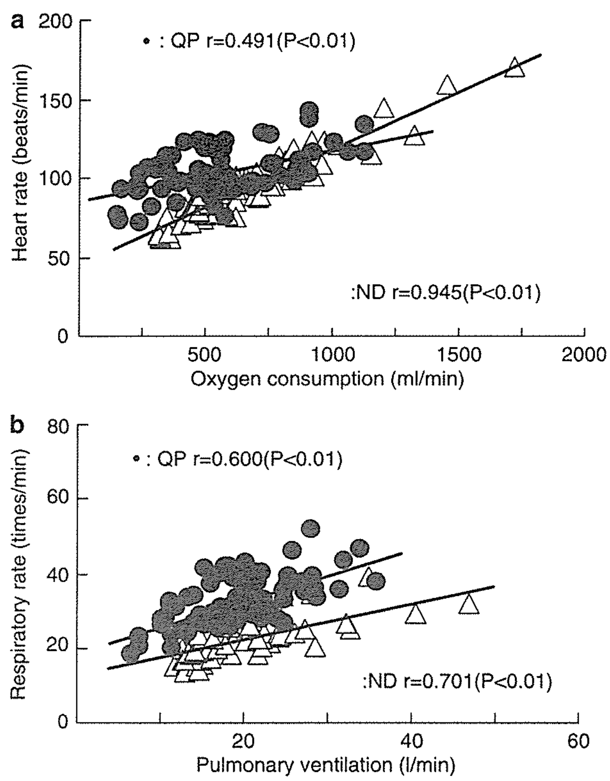

Figure 4 Relationship between oxygen consumption and heart rate (**a**), pulmonary ventilation and respiratory rate (**b**) during passive walking in persons with Quadriplegics (●) and nondisabled (△). QP showed inactive to HR and remarkable increase in RR as compared with ND

Cardiorespiratory responses during exercise in QP change with passive exercise by the paralyzed limbs in addition to the exercise posture. Pitetti *et al*[29] carried out arm-swinging exercise in ISCI and ND with lower body positive pressure (LBPP). They found a significant increase in $\dot{V}O_2$, $\dot{V}E$ and work rate during arm-swinging exercise with LBPP compared to without LBPP. Furthermore, there were no differences in $\dot{V}O_2$, $\dot{V}E$ and work rate between ISCI and ND during exercise with LBPP. From these results, Pitetti *et al*[29] suggested that for ISCI, LBPP augmented the exercise capacity by preventing the redistribution of blood to the lower extremities. Hopman *et al*[27,28] investigated the effects of exercise posture, wearing an antigravity suit (anti-G suit), elastic stocking and abdominal binder, and FES on blood redistribution and circulatory responses in QP and PP. They demonstrated that $\dot{V}O_2$ and HR decreased by wearing an anti-G suit and increased by FES, and increased by wearing elastic stockings and FES during submaximal exercise. In contrast, during maximal exercise, only FES increased $\dot{V}O_2$ and HR. From these results, Hopman *et al*[27,28] suggested that these methods of circulatory redistribution have different working mechanisms and the effects are dependent on the SCI level probably because of differences in active muscle mass, sympathetic impairment and blood pressure values. Furthermore, $\dot{V}O_2$ increased significantly during FES exercise of lower limbs in comparison with at rest[30] and $\dot{V}O_2$ during hybrid exercise was higher than that during arm-swinging exercise or leg cycle exercise by FES.[17,21,31]

The findings using the passive activity of paralyzed lower limbs and FES in addition to arm exercise clearly demonstrated good effects on cardiorespiratory responses and improving the efficiency of their oxygen utilization in ISCI. However, these studies were mostly performed in a sitting posture. If ISCI perform exercise in a standing posture, the cardiorespiratory responses may be different from those in a sitting posture. Nash *et al*[32] showed significant increases in $\dot{V}O_2$, $\dot{V}E$ and HR during PWE when standing by using lobotic-assisted locomotion in QP with a lesion level of C3–C4. In addition, Dietz *et al*[24] identified electromyographic activities of the musculus tibialis anterior and musculus soleus during passive walking on a treadmill in QP and PP. These investigations suggested that PWE in a standing posture with arm exercise in QP facilitated cardiorespiratory responses. Our study showed the same $\dot{V}O_2$ between QP and ND, indicating that the rhythmical activity of paralyzed limb increased $\dot{V}O_2$.

In the present study, QP showed a significantly higher RR from 30 to 90 times/min (Figure 3b), and QP increased RR to the equivalent $\dot{V}E$ of the ND (Figure 4b). Coutts *et al*[6] found lower respiratory parameters such as $\dot{V}E$ and ventilation equivalent in QP than in PP during submaximal arm-swinging exercise. The ventilation equivalent is generally considered to be a measure of breathing efficiency, and decreases during submaximal exercise are associated with increased tidal volume and relative decreases in dead space ventilation.[6] Bhambhani *et al*[16] demonstrated that the $\dot{V}E/\dot{V}O_2$ ratio in ISCI, including QP, is lower during FES cycle exercise than ND, indicating that the ventilatory efficiency of ISCI is inferior to that of ND. In good agreement with the data of Bhambhani *et al*,[16] we found a lower $\dot{V}E/\dot{V}O_2$ ratio of QP in comparison with ND. There was no difference in LA between QP (2.9 ± 1.1 mmol/l) and ND (3.0 ± 1.6 mmol/l) during peak exercise, indicating that the increase in RR was not related to metabolic factors, because LA stimulates the respiratory center and consequently increases the elimination of carbon dioxide. Furthermore, it has been reported that expiratory muscle contraction is influenced by sympathetic nerve activity more than muscle metaboreflex.[33]

In AB, the impulse from the motor area of the cerebral cortex via the center of breathing adjusts the ventilation equivalent to the exercise intensity during exercise.[34] In addition, it has been demonstrated that respiration is regulated by transmitting the afferent information from activity muscles to the center.[35] In QP, the afferent information from the agonist of the upper limbs was transmitted to the center during exercise; however, the nerve impulse of the ventilatory regulation corresponding to the workload is not sent to the respiratory muscles. That is, it is believed that the afferent information to the center increased excessively in our tests. Restrictive ventilatory impairment may disturb respiratory regulation during exercise in QP.

Green[36] found that excitations of the stretch receptors stimulated by the stretch reflex (the Hering–Breuer reflex) in the lung were relayed via the vagus nerve to the medullary respiratory center, leading to a reflex decrease in tidal volume. This reflex was not found in normal adults and in babies with undeveloped respiratory-related muscles and in some animals.[36]

We hypothesized that QP with restrictive ventilatory impairment might show a condition similar to that of the babies in Green's study. To compensate for the decrease in tidal volume by the Hering–Breuer reflex and to achieve the same ventilation as ND, QP increased RR from the commencement of exercise. Specifically, the increase in RR in QP could be a result of the increased afferent information from the agonist to the respiratory center and the increased reflex induced by restrictive ventilatory impairment.

Some investigators have shown a lower maximal HR below 110 beats/min in QP than that of PP and ND.[4,37] Bhambhani *et al*[16] found no significant difference of HR in ISCI including QP during FES exercise from that at rest. A lower HR during exercise is naturally expected in QP because of sympathetic activity dysfunction controlling the heart. In this study, however, during peak exercise, the HR of QP was higher than the data of Bhambhani *et al*,[16] and there were no significant differences of HR between QP and ND similar to $\dot{V}O_2$ (Figure 2b). Muraki *et al*[12] reported a significant increase in stroke volume and cardiac output without a rise of HR during passive leg cycle exercise in PP. They suggested the promotion of venous return related to the lengthening and shortening of the paralyzed muscle without tension in the lower limbs.

In this study, HR in QP increased during PWE. This is not consistent with the findings of Faghri *et al*,[23] who found no increase of HR in QP during FES when standing. On the other hand, they reported increased TPR, which could be a compensatory mechanism to control the significant drop in blood pressure occurring during standing in QP. Hooker *et al*[21] investigated cardiorespiratory responses during arm-swinging exercise, FES leg-cycle exercise and hybrid exercise in QP, and revealed that $\dot{V}O_2$, $\dot{V}E$ and HR were higher during hybrid exercise than the other two exercises and there was no significant difference in stroke volume between the hybrid exercise and FES leg-cycle exercise. Dela *et al*[14] reported that although HR increased immediately after the commencement of FES and attained a steady state in ND, QP showed a delay in the HR increment. HR responses in QP may be attributable to arterial baroreceptors that elevate HR in QP with the lower blood pressure developed during exercise.[14]

In this study, there was no significant difference in HR between QP and ND during peak passive walking. On the other hand, during submaximal exercise, a clear difference in HR was found between QP and ND. In ND, HR increased linearly with workload increment, while it increased in QP from the commencement of exercise to 40 times/min and HR increased gradually, showed a steady state between 50 and 75 times/min, increasing remarkably after 80 times/min (Figure 2b). It could be expected that HR in QP increased by the activation of arterial baroreceptors in compensation for deficient blood distribution from the beginning of exercise to 40 times/min, while between 50 and 75 times/min, HR showed a steady state because blood was distributed sufficiently to agonists (Figure 2c).

In conclusion, PWE, the rhythmical activity of paralyzed lower limbs synchronized with arm movements, elicited an increase in $\dot{V}O_2$ in QP similar to ND. However, higher RR suggested the intrinsic dysfunction of RR control during submaximal exercise in QP. From these results, it was thought that respiratory responses would restrict the efficiency of oxygen transportation during PWE in QP.

References

1 Hjeltnes N, Wallberg-Henriksson H. Improved work capacity but unchanged peak oxygen uptake during primary rehabilitation in tetraplegic patients. *Spinal Cord* 1998; **36:** 691–698.
2 Birk TJ, Nieshoff E, Gray G, Steeby J, Jablonski K. Metabolic and cardiopulmonary responses to acute progressive resistive exercise in a person with C4 spinal cord injury. *Spinal Cord* 2001; **39:** 336–339.
3 Bernard PL, Mercier J, Varry A, Prefaut C. Influence of lesion level on the cardioventilatory adaptations in paraplegic wheelchair athletes during muscular exercise. *Spinal Cord* 2000; **38:** 16–25.
4 Coutts KD, Rhodes EC, McKenzie DC. Maximal exercise responses of tetraplegics and paraplegics. *J Appl Physiol* 1983; **55:** 479–482.
5 Schimid A *et al*. Catecholamines, heart rate, and oxygen uptake during exercise in persons with spinal cord injury. *J Appl Physiol* 1998; **85:** 635–641.
6 Coutts KD, Rhodes EC, McKenzie DC. Submaximal exercise responses of tetraplegics and pareplegics. *J Appl Physiol* 1985; **59:** 237–241.
7 McLean KP, Skinner JS. Effect of body training position on outcomes of an aerobic training study on individuals with quadriplegia. *Arch Phys Med Rehabil* 1995; **76:** 139–150.
8 Davis GM. Exercise capacity of individuals with paraplegia. *Med Sci Sports Exerc* 1993; **25:** 423–432.
9 Hopman MT. Circulatory responses during arm exercise in individuals with paraplegia. *Int J Sports Med* 1994; **15:** 126–131.
10 Mathias CJ, Frankel HL. Cardiovascular control in spinal man. *Ann Rev Physiol* 1988; **50:** 577–592.
11 Olive JL, Mccully KK, Dudley GA. Blood flow response in individuals with incomplete spinal cord injuries. *Spinal Cord* 2002; **40:** 639–645.
12 Muraki S, Yamasaki M, Ehara Y, Kikuchi K, Seki K. Cardiovascular and respiratory responses to passive leg cycle exercise in people with spinal cord injuries. *Eur J Appl Physiol* 1996; **74:** 23–28.
13 Faghri PD, Yount J. Electrically induced and voluntary activation of physiologic muscle pump: a comparison

between spinal cord-injured and able-bodied individuals. *Clin Rehabil* 2002; **16:** 878–885.
14 Dela F *et al.* Cardiovascular control during exercise insights from spinal cord-injury humans. *Circulation* 2003; **107:** 2127–2133.
15 Kjaer M, Pott F, Mohr T, Linkis P, Tornoe P, Secher NH. Heart rate during exercise with leg vascular occlusion in spinal cord-injured humans. *J Appl Physiol* 1999; **86:** 806–811.
16 Bhambhani Y, Tuchak C, Burnham R, Jeon J, Maikala R. Quadriceps muscle deoxygenation during functional electrical stimulation in adults with spinal cord injury. *Spinal Cord* 2000; **38:** 630–638.
17 Mutton DL, Scremin AME, Barstow TJ, Scott MD, Kunkel CF, Cable TG. Physiologic responses during functional electrical stimulation leg cycle and hybrid exercise in spinal cord injured subjects. *Arch Phys Med Rehabil* 1997; **78:** 712–718.
18 Raymond J, Davis GM, Fahey A, Climstein M, Sutton JR. Oxygen uptake and heart rate responses during arm versus combined arm + ES-induced leg exercise in people with paraplegia. *Spinal Cord* 1997; **35:** 680–685.
19 Laskin JJ *et al.* Electrical stimulation-assisted rowing exercise in spinal cord injured people. A pilot study. *Paraplegia* 1993; **31:** 534–541.
20 Wheeler GD *et al.* Functional electric stimulation-assisted rowing: increasing cardiovascular fitness through functional electric stimulation rowing training in persons with spinal cord injury. *Arch Phys Med Rehabil* 2002; **83:** 1093–1099.
21 Hooker SP *et al.* Metabolic and hemodynamic responses to concurrent voluntary arm crank and electrical stimulation leg cycle exercise in quadriplegics. *J Rehabil Res Dev* 1992; **29:** 1–11.
22 Kawashima N, Sone Y, Nakazawa K, Akai M, Yano H. Energy expenditure during walking with weight-bearing control (WBC) orthosis in thoracic level of paraplegic patients. *Spinal Cord* 2003; **41:** 506–510.
23 Faghri PD, Yount JP, Pesce WJ, Seetharama S, Votto JJ. Circulatory hypokinesis and functional electric stimulation during standing in persons with spinal cord injury. *Arch Phys Med Rehabil.* 2001; **82:** 1587–1595.
24 Dietz V, Nakazawa K, Wirz M, Erni T. Level of spinal cord lesion determines locomotor activity in spinal man. *Exp Brain Res* 1999; **128:** 405–409.
25 Colombo G, Wirz M, Dietz V. Driven gait orthosis for improvement of locomotor training in paraplegic patients. *Spinal Cord* 2001; **39:** 252–255.
26 Irizawa M, Yamasaki M, Muraki S, Komura T, Seki K, Kikuchi K. Relationship between heart rate and oxygen uptake during submaximal arm cranking in paraplegics and quadriplegics. *Ann Physiol Anthropol* 1994; **13:** 275–280.
27 Hopman MT, Monroe M, Dueck C, Phillips WT, Skinner JS. Limits to maximal performance in individuals with spinal cord injury. *Int J Sports Med* 1998; **19:** 98–103.
28 Hopman MT, Monroe M, Dueck C, Phillips WT, Skinner JS. Blood redistribution and circulatory responses to submaximal arm exercise in persons with spinal cord injury. *Scand J Rehabil Med* 1998; **30:** 167–174.
29 Pitetti KH, Barrett PJ, Campbell KD, Malzahn DE. The effect of lower body positive pressure on the exercise capacity of individuals with spinal cord injury. *Med Sci Sports Exerc* 1994; **24:** 463–468.
30 Figoni SF *et al.* Physiologic responses of paraplegics and quadriplegics to passive and active leg cycle ergometry. *J Am Paraplegia Soc* 1990; **13:** 33–39.
31 Raymond J, Davis GM, Climstein M, Sutton JR. Cardiorespiratory responses to arm cranking and electrical stimulation leg cycling in people with paraplegia. *Med Sci Sports Exerc* 1999; **31:** 822–828.
32 Nash MS, Jacobs PL, Johnson BM, Field-Fote' E. Metabolic and cardiac responses to robotic-assisted locomotion in motor-complete tetraplegia: a case report. *J Spinal Med* 2004; **27:** 78–82.
33 Derchak PA, Shell AW, Morgan BJ, Dempsey JA. Effects of expiratory muscle work on muscle sympathetic nerve activity. *J Appl Physiol* 2002; **92:** 1539–1552.
34 Krough A, Lindhard J. The reguration of respiration and circulation during the initial stages of muscular work. *J Physiol* 1913; **47:** 112–136.
35 Morikawa T *et al.* Afferent and cardiodynamic drives in the early phase of exercise hyperpnea in humans. *J Appl Physiol* 1989; **67:** 2006–2013.
36 Green JH. *The Autonomic Nervous System and Exercise.* Chapman & Hall: London 1990, pp 126–128.
37 Van Loan MD, McCluer S, Loftin JM, Boileau RA. Comparison of physiological responses to maximal arm exercise among able-bodied, paraplegics and quadriplegics. *Paraplegia* 1987; **25:** 397–405.

Spinal Cord (2006), 1–8

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Original Article

Effect of lesion level on the orthotic gait performance in individuals with complete paraplegia

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Study design: Cross-sectional, experimental research.
Objectives: To clarify the effect of lesion level on cardio-respiratory responses and biomechanical characteristics of walking with a reciprocating gait orthosis in complete paraplegia with spinal cord injury (SCI).
Setting: National Rehabilitation Center for Persons with Disabilities, Japan.
Methods: Ten SCI individuals (age: 20–34 years, injured level: Th5–12) who experienced orthotic gait training at least for 10 weeks participated in two experiments: (1) measurement of the cardiorespiratory responses during 20 min of orthotic gait exercise; and (2) three-dimensional motion analysis and ground reaction force measurement using the VICON system. We calculated the following parameters: pulmonary ventilation, oxygen consumption ($\dot{V}O_2$), heart rate (HR), gait speed, cadence, stride length, crutch force (CF), hip range of motion (ROM), and hip angular velocity (VEL). Further, energy consumption and energy cost were calculated using the steady-state value of $\dot{V}O_2$ and gait speed.
Results: The steady-state value of the $\dot{V}O_2$ (18.2 ± 3.80 ml/kg) and HR (133.0 ± 21.63 b/min) tended to be larger in higher thoracic SCI subjects. There were strong positive correlations between the lesion level and walking speed ($r = 0.74$), energy cost ($r = 0.85$), and hip ROM ($r = 0.78$). On the other hand, negative correlation between the lesion level and peak CF ($r = -0.78$) was clarified.
Conclusions: The physiological intensity of the orthotic gait strongly depended on the level of lesion. It seems likely that a limited hip range of motion and excess upper limb load result in the low energy cost of orthotic gait for the higher thoracic level of paraplegic patients.
Spinal Cord advance online publication, 21 March 2006; doi:10.1038/sj.sc.3101916

Keywords: spinal cord injury; orthotic gait; energy consumption; motion analysis; cardio-respiratory response

Introduction

Orthotic gait exercise is usually prescribed for patients with spinal cord injury (SCI) in their therapeutic phase to promote their general health. Although the effectiveness of orthotic gait exercise is well recognized, there are several obstacles to achieve walking for complete paraplegic persons, in particular the high energy cost of the orthotic gait.[1–5] SCI persons inevitably require larger energy expenditure for orthotic gait because they need to produce complementary upper limb and trunk motion in order to swing their paralyzed lower limb.[6–9] Further, it can be pointed out that the neurological level of paralysis considerably influences the achievement of orthotic gait motion and energy expenditure.

We have evaluated the energy expenditure during orthotic gait of thoracic level of SCI paraplegics, and suggest that, even with the higher level of lesion, the physiological intensity required was in a feasible range for cardiorespiratory function.[10] We have also found that subjects who had higher levels of lesion demonstrated relatively slower gait speed and a relatively higher physiological intensity. These findings confirm the clinical impression that higher thoracic SCI subjects have some difficulties in performing orthotic walking. Although physicians and therapists already know this because of their clinical experience, it is not clear to what extent the motor paralysis influences orthotic gait performance, and what is the primary reason for limited

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gait performance for higher level SCI subjects. In the present study, we aimed to clarify the effect of injured level on the physiological intensity and biomechanical characteristics of orthotic gait on the complete paraplegiac persons.

Methods

Subjects
Ten subjects with the thoracic level of SCI participated in this study. All subjects had complete motor paralysis in the lower limb muscles (ASIA classification; grade A or B[11]) and no history of cardiorespiratory disease. Criteria for participation of this study were (1) judged to be better general health condition and have adequate exercise tolerance at the health check, (2) no cardiovascular disease, and (3) had past at least a half year after injury. The characteristics of the subjects in detail are summarized in Table 1. All subjects had participated in the basic rehabilitation process, and had undergone at least 10 weeks of orthotic gait training using the advanced reciprocating gait orthosis® (ARGO). Each subject gave written informed consent for the experimental procedure, which was approved by the local biological ethics committee of the National Rehabilitation Center for Persons with Disabilities (NRCD).

Table 1 Characteristics of the SCI subjects

	Sex	*Age (years)*	*Weight (kg)*	*Lesion level*	*Grade of ASIA*	*Duration of paraplegia (months)*
A	M	30	67	Th12	A	32
B	M	25	79	Th12	A	28
C	M	26	80	Th12	A	16
D	M	29	72	Th11	B	12
E	F	27	45	Th10	A	18
F	M	32	74	Th10	A	10
G	M	30	74	Th8	A	22
H	M	22	65	Th7	A	30
I	M	34	54	Th6	A	36
J	M	20	53	Th5	B	28

Orthotic gait
Sequential pictures of walking with the ARGO are shown in Figure 1a. The ARGO has a single cable which connects both sides of the leg frame. With this device, a torque exerted by the right (left) hip joint is mechanically transmitted to the left (right) hip joint, resulting in the torque to the opposite direction exerted by the left (right) hip joint. Although there is individual variation, in many cases, paraplegic patients with injury to a lower thoracic level could walk after 10 weeks of gait training independently, while patients with injury at a higher thoracic level needed additional practice. After the training period, each subject could perform the orthotic gait (subjects F, H, and J still required light support to avoid falling) independently, and were able to walk continuously for at least 20 min.

All subjects participated in two experiments on the separate day; one was the measurement of cardiorespiratory responses during 20 min of orthotic gait exercise, and another was the three-dimensional motion analysis with the use of the VICON system.

Measurement of the cardiorespiratory responses
Subjects were asked to abstain from alcohol and caffeine for at least 12 h before the experiment. The temperature and humidity during the experiment were 23.5 ± 4.2°C and 58.3 ± 3.3%, respectively. The experimental procedure was as follows: 3 min of rest in the standing position followed by 20 min of continuous walking at the most comfortable speed. The cardiorespiratory responses at rest and during walking were measured continuously with a telemetric device (K4-RQ Cosmed

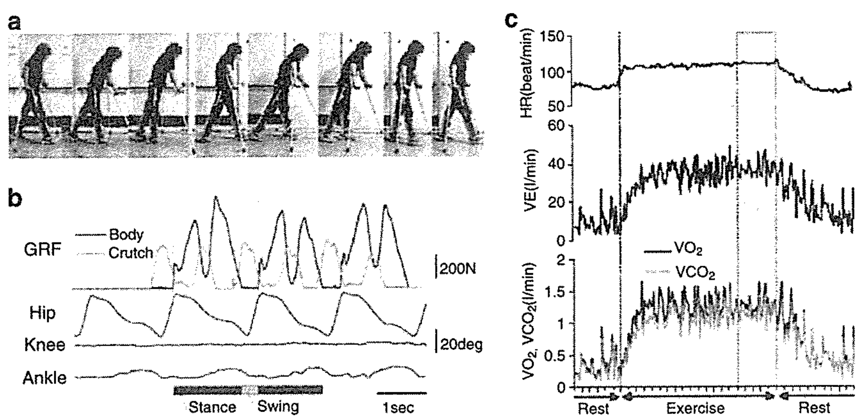

Figure 1 (**a**) Sequential picture of walking with the ARGO in a SCI subject (injured at Th12). (**b**) Time series data of the GRF (body and stick) and joint angle motion (Hip, Knee, and Ankle) obtained by the VICON system. (**c**) Typical example of the changes in the cardiorespiratory parameters during orthotic gait exercise

s.r.l., Rome, Italy) and were analyzed in real time. The telemetric device consisted of a transmitting unit, a facemask for sampling the expired gas, a heart rate (HR) chest strap, a battery, and a receiving unit. The following cardiorespiratory parameters were obtained: pulmonary ventilation (VE), oxygen uptake (VO_2), and HR. Typical example of the changes in the cardiorespiratory parameters during orthotic gait exercise was shown in the Figure 1b. The amount of time required to walk 10 m was recorded during the exercise period, and gait speed was calculated after the experiment. After the experiments, the energy consumption and energy cost were calculated. The terms adopted were those of Nene and Patrick,[12] and calculations were performed according to their protocol:

$$\text{Energy consumption (J/kg/s)} = \frac{\text{Ambulatory min } VO_2 \text{ (ml/ min)}}{\text{Weight (kg)} \times 60} \times K$$

$$\text{Energy cost (J/kg/m)} = \frac{\text{Ambulatory min } VO_2 \text{ (ml/ min)}}{\text{Speed (m/min)} \times 60} \times K$$

where $K = 20.19$ J/ml, since 1 ml $O_2 = 4.825$ cal and 1 cal $= 4.184$ J.

Motion analysis
Subjects performed orthotic walking along a 10-m walkway in the laboratory at least five times at a comfortable (self-determined) speed. In order to obtain the kinematics and kinetics variables of the orthotic gait, the gait motion was measured with a three-dimensional motion-analysis system (VICON 370, Vicon Motion Systems Ltd, Oxford, UK). The motion-analysis system consisted of a conventional video-analysis system with seven cameras and force plates (Kistler, Switzerland). The force plates, 160×450 cm in size, consisted of two 80×200 cm plates and four 40×250 cm plates. These separate force plates enabled us to measure ground reaction forces (GRF) under the feet and canes on both sides, separately. A total of 17 markers were attached to the orthosis and to the body of the subject on the skin overlying the following marks: the vertex, both sides of the acromium (SHO), the lateral aspects of the hip (HIP), knee (KNE), and ankle (AKL) joints of the orthosis, the top of the great toe (TOE), the protrusion of the ulna at the elbow and wrist joint, and the tip of the crutch. We defined the hip angle as the angle formed by the SHO, HIP, and KNE, and the ankle angle as that formed by the KNE, AKL, and TOE, respectively. Typical time series data of the GRF (body and stick) and joint angle motion (Hip, Knee, and Ankle) obtained by the VICON system was shown in the Figure 1c.

We sampled 10-step cycles for the analysis. The following kinematic and kinetic variables were evaluated on the basis of the motion analysis: cadence, stride length, hip joint range of motion (ROM), hip angular velocity (VEL), and crutch force (CF) (peak crutch force (PCF) and mean crutch force (MCF)). Cadence was calculated as the time required between heel contacts detected by the body GRF. Stride length was calculated as the distance of the toe marker between two consecutive gait cycles.

Statistics
Values were given as means$\pm$SEM. Pearson's product moment correlation coefficient was used to examine the relationship between the level of lesion and the kinematic and kinetic variables. Moreover, since the injured level is not strictly regarded as a parametric variable, we also examined this relationship using the Spearman rank-correlation coefficient. For this analysis, the parameters used were the average value for each subject. Significance was accepted at $P<0.01$ and $P<0.05$.

Results

Figure 1a shows a sequential picture of walking with the ARGO , the typical waveform of the GRF of each body and of the opposite side of the crutch, joint motion (Hip, Knee, and Ankle) (Figure 1b), and the typical data of the cardiorespiratory responses (Figure 1c). As shown in this figure, dynamic hip joint motion and periodic load application appeared during orthotic gait. In the orthotic gait, in contrast with normal walking, the knee joint was held in an extended position throughout the locomotion cycle. Although the ankle was held by the plastic socket, angle changes were observed to some extent in the stance phase.

Gait speed
The average walking speeds calculated during field walking (during cardiorespiratory measurement) and laboratory walking (during motion analysis) were 19.8 ± 6.16 and 21.3 ± 5.82 m/min, respectively. There was a strong relationship between these variables ($r = 0.87$, $P<0.01$). Therefore, in this study, the former value was used as the gait speed. Eight of 10 subjects were able to walk continuously for 20 min without a long break. Subjects H and J, who had relatively higher levels of injury, required rest intervals due to arm fatigue and a pressure on the heels of hands.

Cardiorespiratory responses during orthotic gait
Table 2 shows the cardiorespiratory responses at rest and the steady-state value during orthotic walking. In the subjects H and J, the resting value of the HR was much higher than in other subjects. This is presumably caused by a disorder of the autonomic nervous functions. As clearly shown in Figure 1c, cardiorespiratory parameters rapidly increased after the beginning of the walking exercise. HR reached a plateau level in the first few minutes, whereas some subjects with a higher level of lesion (subjects I and H) showed further

increases of HR during prolongation of the exercise. The steady-state value of HR ranged from 99.2 to 166.4 b/min (average value: 130.0±21.63 b/min). As with HR, VO_2 reached a plateau level about 3–4 min after the beginning of exercise. The steady-state value of VO_2 ranged from 14.91 to 24.83 ml/kg (18.17±3.80 ml/kg), and this value is approximately 3–4 times the resting level.

The energy consumption and energy cost during walking were 6.11±1.28 J/kg/s and 20.12±7.35 J/kg/m, respectively (Table 3). Figure 2a shows the relationship between energy consumption (y-axis) and energy cost (x-axis) in each subject. As the walking speed was determined by dividing the energy consumption by the energy cost, the slope of the line from zero to each point plotted reflects the walking speed of each subject. It was found that the plots of persons with higher level injuries tended to shift to the right side, which signifies a relatively slower gait speed and a higher energy cost.

Table 2 VE, VO_2, and HR at rest and during orthotic gait exercise

	VE (ml/kg)		*VO₂ (ml/kg)*		*HR (beat/min)*	
	Rest	*Exercise*	*Rest*	*Exercise*	*Rest*	*Exercise*
A	194.0	437.3	6.70	16.01	60.1	99.2
B	171.5	525.1	4.48	17.63	70.4	114.3
C	139.2	574.2	4.29	14.91	87.6	140.2
D	172.9	664.9	4.66	15.62	92.1	129.5
E	185.8	624.2	6.78	24.20	62.1	132.5
F	175.3	592.9	6.62	15.41	78.3	131.5
G	155.0	634.4	4.43	16.75	77.9	110.1
H	221.8	560.6	5.80	15.19	107.8	163.0
I	277.9	660.5	9.75	24.83	81.0	143.7
J	265.1	687.7	8.29	21.14	144.1	166.4
Mean	195.8	596.2	6.18	18.17	86.13	133.0
SD	45.61	75.67	1.83	3.80	24.78	21.63

In order to confirm the data reproducibility, we compared the energy consumption and energy cost evaluated at 2 and 3 months after the beginning of the training in six of 10 subjects (Figure 2b). Although these data include the training effect (improvement of the energy cost), data reproducibility can be well recognized from this figure.

Kinematics and kinetics
The parameters obtained from motion analysis are summarized in Table 3. Cadence and stride length were 44.0±6.27 step/min and 97.4±16.36 cm, respectively. Hip ROM was 44.1±5.20 deg. The angular velocities of the flexion and extension phases were 124.3±11.56 and 21.7±7.12 deg/s, respectively. Figure 3 shows the stick picture (top) and ensemble averaged waveform of the hip angle and GRF for both body and crutch during orthotic gait obtained from subjects A (injured at Th12) and J (Th5). As clearly shown in this figure, there are remarkable differences in the gait motion between the SCI subjects injured at a lower and higher level. This figure also shows good reproducibility of both hip motion and GRF during orthotic gait.

Relationship between injury level and each parameter
Table 4 summarizes the Pearson's product moment correlation coefficient (r_p) and the Spearman rank-correlation coefficient (r_s) between the injured level and each parameter obtained in this study. Figure 4 also shows the correlation diagram between the level of injury and with gait speed, energy consumption, energy cost, hip ROM, and mean GRF of the body and crutch. The parameters that showed strong relevance to the injury level were gait speed (r_p = 0.74; P<0.01, r_s = 0.89; P<0.01), energy cost (r_p = −0.88; P<0.01, r_s = −0.92; P<0.01), stride length (r_p = 0.79; P<0.01, r_s = 0.78; P<0.01), peak CF (r_p = −0.78; P<0.01, r_s = 0.80; P<0.01), and hip ROM (r_p = 0.78; P<0.01, r_s = 0.77; P<0.01). By evaluating the Spearman rank-correlation

Table 3 All parameters calculated in this study

	Gait speed (m/min)	*E consmp. (J/kg/s)*	*E cost (J/kg/m)*	*Cadence (step/min)*	*Stride length (cm)*	*Peak CF (N/kg)*	*Mean CF (N/kg/s)*	*Hip ROM (deg)*	*Hip fVEL (deg/s)*	*Hip eVEL (deg/s)*
A	20.06	5.39	16.12	40.86	106.6	1.81	0.32	45.44	110.2	24.35
B	32.58	5.93	10.93	56.92	122.5	2.81	0.34	51.04	142.6	38.90
C	27.22	5.02	11.06	47.62	107.7	2.62	0.33	47.95	126.0	23.91
D	21.55	5.26	14.63	40.59	118.8	3.93	0.46	49.52	127.6	24.17
E	19.99	8.14	24.44	36.34	79.9	3.73	0.49	40.91	119.0	13.36
F	18.35	5.19	16.95	47.10	102.9	3.37	0.60	46.35	131.0	22.20
G	11.64	5.64	29.05	36.81	83.8	4.31	0.47	43.08	110.0	15.78
H	15.58	5.11	19.67	42.25	89.8	4.39	0.50	42.23	132.9	18.00
I	17.09	8.35	29.33	48.98	77.5	5.02	0.45	38.83	133.9	18.75
J	14.69	7.11	29.06	42.01	84.8	3.92	0.55	41.92	109.7	17.79
Mean	19.88	6.11	20.12	43.95	97.4	3.59	0.45	44.73	124.3	21.72
SD	6.16	1.28	7.35	6.27	16.36	0.96	0.09	3.98	11.56	7.12

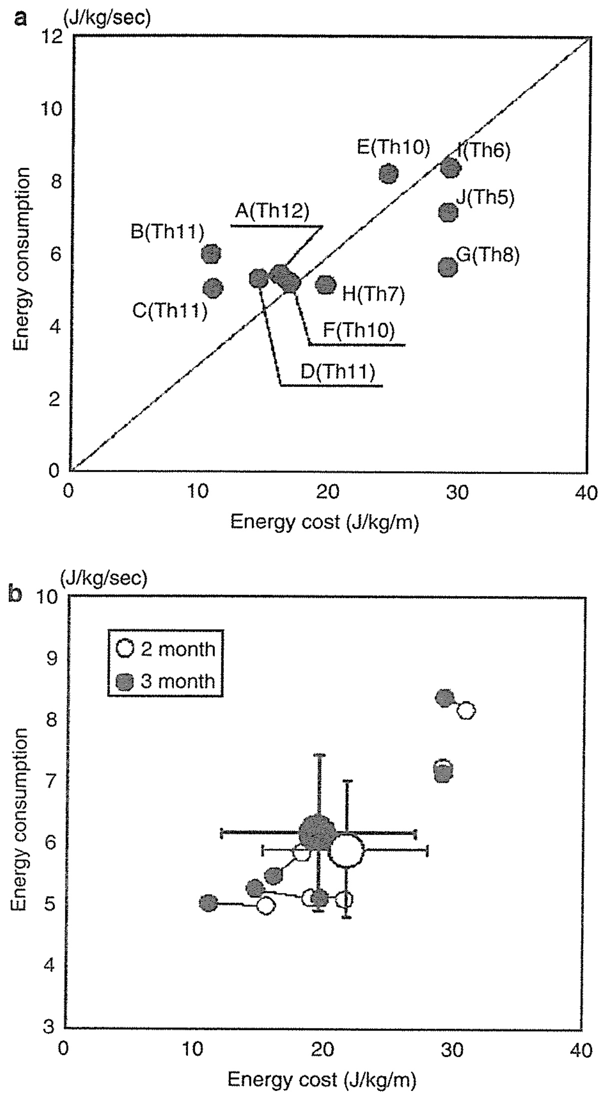

Figure 2 (**a**) The relationship between energy consumption and cost in each investigation. The slope of each line from zero to each plot reflects the walking speed. The subjects represented by points plotted on the upper and left side of the figure can be considered to have adequate aerobic conditioning. (**b**) Differences of the energy consumption and cost between 2 and 3 months after the beginning of the orthotic gait training. Gray and black markers indicate each subject's data and averaged data, respectively. Error bar indicates standard deviation

coefficient, mean CF ($r_s = -0.67$; $P < 0.05$) and hip extension VEL ($r_s = 0.74$; $P < 0.05$) also showed a strong relevance to the injury level.

Discussion

The present results clearly show the significant relationship between the level of neurological lesion and orthotic gait performance (Figure 4 and Table 4). The injured segment of the spinal cord in our subjects ranged from Th5 to 12. This included the anatomical levels of those innervating muscles that move the trunk, for example, the volitional control of the abdominal and iliopsoas muscle. This was intact in those injured at Th12, but not in those injured at Th5. It is therefore likely that these results are attributable to the degree of residual motor function around the trunk.

Physiological intensity of orthotic gait exercise

In the present study, the steady-state values of the $\dot{V}O_2$ and HR during orthotic gait were 18.2±3.80 ml/kg and

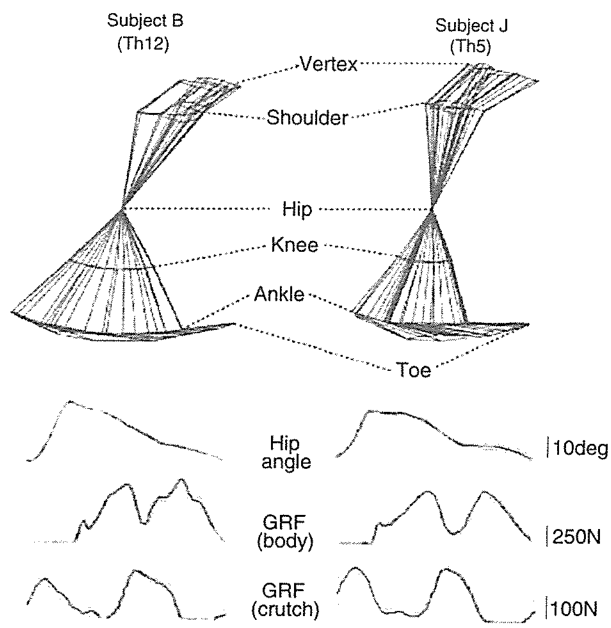

Figure 3 Differences in the gait motion between SCI subjects injured at a lower (left; Th12) and higher level (right; Th5). The stick pictures represented were standardized by the marker on the GTR

Table 4 Pearson's product moment correlation coefficient and Spearman rank-correlation coefficient between injured level and each parameter

Level versus	*Gait speed*	*E consmp.*	*E cost*	*Cadence*	*Stride length*	*Peak CF*	*Mean CF*	*Hip ROM*	*Hip fVEL*	*Hip eVEL*
Pearson	0.74**	−0.54	−0.88**	0.22	0.79**	−0.78**	−0.60	0.78**	0.23	0.60
Spearman	0.89**	−0.44	−0.92**	0.14	0.78**	−0.80**	−0.67*	0.77**	0.20	0.74*

*$P<0.05$, **$P<0.01$