Endocrine Journal 2011, **58** (3), 211-215

NOTE

Upregulation of circulating IL-15 by treadmill running in healthy individuals: Is IL-15 an endocrine mediator of the beneficial effects of endurance exercise?

Yoshiaki Tamura[1)], Keiichi Watanabe[2)], Tomomi Kantani[3)], Junichi Hayashi[4)], Nobuhiko Ishida[2)] and Masao Kaneki[1)]

[1)]*Department of Anesthesia, Critical Care, and Pain Medicine, Massachusetts General Hospital, Shriners Hospitals for Children, Harvard Medical School, Charlestown, MA 02129, USA*
[2)]*Wafukai Medical Corporation Tama Rehabilitation Academy, Ome 198-0004, Japan*
[3)]*Wafukai Tama Rehabilitation Hospital, Ome 198-0052, Japan*
[4)]*Department of General Medicine, Kyorin University School of Medicine, Mitaka 181-8611, Japan*

Abstract. The beneficial effects of endurance exercise include insulin-sensitization and reduction of fat mass. Limited knowledge is available about the mechanisms by which endurance exercise exerts the salutary effects. Myokines, cytokines secreted by skeletal muscle, have been recognized as a potential mediator. Recently, a role of skeletal muscle-derived interleukin-15 (IL-15) in improvement of fat-lean body mass composition and insulin sensitivity has been proposed. Yet, previous studies have reported that endurance training does not increase production or secretion of IL-15 in skeletal muscle. Here, we show that in opposition to previous findings, 30-min treadmill running at 70% of age-predicted maximum heart rate resulted in a significant increase in circulating IL-15 level in untrained healthy young men. These findings suggest that IL-15 might play a role in the systemic anti-obesogenic and insulin-sensitizing effects of endurance exercise, not only as a paracrine and autocrine but also as an endocrine factor.

Key words: IL-15, Myokine, Endurance exercise, Treadmill

OBESITY and metabolic syndrome are pandemic worldwide, which is related to sedentary life style in the modern society. Endurance (aerobic) exercise, such as running and cycling, and resistance (weight) training represent two major categories of exercise. The beneficial effects of endurance exercise have been well established in animal models and humans, which include reduction in adiposity, and insulin-sensitizing and anti-atherogenic actions. Hence, engagement in endurance exercise has emerged as a critical intervention in obesity and metabolic syndrome. Nonetheless, the molecular mechanisms responsible for the salutary effects of endurance exercise are poorly understood.

Recently, myokines, cytokines secreted by skeletal muscle, have been proposed as a mediator of systemic effects of exercise [1]. Endurance exercise upregulates the production of interleukin (IL)-6, IL-8, brain-derived nerve growth factor (BDNF) in skeletal muscle, which, in turn, leads to elevated circulating concentrations of these myokines following exercise. It remains unclarified, however, whether the insulin-sensitizing and anti-adipogenic effects of exercise are attributable to these myokines. IL-6 has been postulated as a mediator of insulin-sensitizing effects of exercise, but there is also controversy about the role of IL-6 in insulin sensitivity. Some studies have shown that IL-6 improves insulin sensitivity [2], while others have reported that IL-6 induces insulin resistance [3]. The role of IL-6 in adipogenesis is not clear. IL-8 does not seem a likely candidate mediator, since IL-8 functions as a pro-inflammatory cytokine and to the best of our knowledge no study has reported insulin-sensitizing action of IL-8. Little is known about the relation between muscle-derived BDNF and insulin sensitivity or adiposity [1].

IL-15 is produced by several tissues, including skeletal muscle, liver, placenta, epithelial cells, and activated

Received Dec. 20, 2010; Accepted Jan. 13, 2011 as K10E-400
Released online in J-STAGE as advance publication Feb. 5, 2011

Correspondence to: Masao Kaneki, Department of Anesthesia, Critical Care, and Pain Medicine, Massachusetts General Hospital, 149 Thirteenth Street, Rm. 6604, Charlestown, MA 02129, USA
E-mail: mkaneki@helix.mgh.harvard.edu

©The Japan Endocrine Society

macrophages, whereas little or no IL-15 is expressed in adipocytes [4]. IL-15 has an important role in muscle hypertrophy and prevention of muscle wasting as well as in immune function [5]. Recently, the functional role of IL-15 in the regulation of fat mass and energy expenditure has been highlighted [6-9]. IL-15 inhibits lipogenesis in white adipose tissues [8, 10, 11], while it enhances energy expenditure. Muscle-derived IL-15 is considered as a local factor which promotes muscle hypertrophy and inhibits lipogenesis. It is not known, however, whether muscle-derived IL-15 has a systemic effect as an endocrine factor, as well as an autocrine and paracrine.

These findings raise the possibility that IL-15 may be capable of serving as an important mediator of anti-adipogenic and insulin-sensitizing effects of endurance exercise. In previous studies, however, endurance exercise did not increase IL-15 mRNA content in skeletal muscle or circulating IL-15 concentration from 1 h up to 6 h after exercise [12-15]. One study showed that high intensity resistance training led to a transient increase in serum IL-15 concentration [16], while another study reported that plasma IL-15 concentration was not altered by heavy resistance training [17]. It should be noted that in opposition to endurance (aerobic) exercise, the beneficial effects of resistance (strength) exercise on insulin sensitivity and fat mass remain to be fully established. In aggregate, it remains an open question whether IL-15 may function as a mediator of the beneficial effects of endurance exercise. Here, we show that treadmill running induced a significant immediate upregulation of serum IL-15 level in untrained healthy adults.

Subjects and Methods

Subjects

Thirteen healthy untrained Japanese male subjects participated in the study. They were physically active but did not have habit of frequent periodical sports. None of them were taking any medication. The protocol was approved by the local Ethical Committee at Wafukai Hospital in accordance with the Declaration of Helsinki. Written informed consent was obtained from each individual.

Treadmill exercise

The subjects underwent 30-min treadmill running at 70% of age-predicted maximal heart rate (HR (max)) [1], after they refrained from exercise for 24 h.

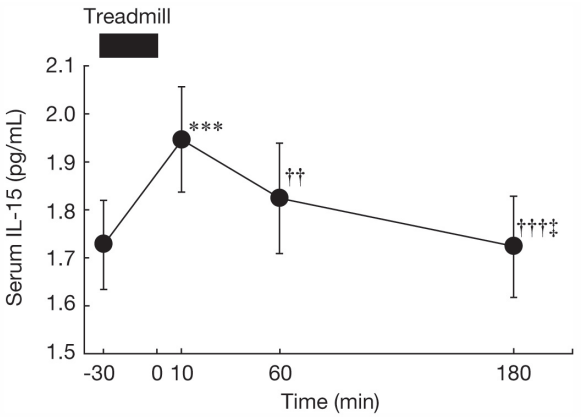


Fig. 1 Serum IL-15 concentration was increased after the treadmill running. Just before (-30 min) and 10 min, 60 min, and 180 min after the 30-min treadmill running, serum IL-15 concentrations were measured. *** $p<0.001$ *vs*. -30 min (pre-exercise), †† $p<0.01$, ††† $p<0.0001$ *vs*. 10 min, ‡ $p<0.05$ *vs*. 60 min.

Blood analysis

Blood samples were drawn from antecubital vein just before and 10, 60, 180 min after the exercise. Systolic blood pressure, diastolic blood pressure, and pulse rate were also measured at each time point. Blood samples were centrifuged immediately to isolate serum and sera were kept -80°C. Serum IL-15 concentrations were determined by enzyme-linked immunosorbent assay kits (HUMAN IL-15 QUANTIGLO ELISA KIT, R&D systems, Minneapolis, MN, USA).

Statistical analysis

Time dependent variability of each physiological and biochemical data was tested using a one-way ANOVA for repeated measures (Dr. SPSS II, IBM, Armonk, NY, USA). $p<0.05$ was considered statistically significant. All values are expressed as mean ± SEM.

Results

Serum IL-15 concentration was significantly increased at 10 min after the 30-min treadmill running as compared with that just before the inception of treadmill running (Fig. 1). At 3 h after the treadmill exercise, serum IL-15 concentrations were fully restored to the basal level. At 1 h post-exercise, serum IL-15 concentration was not significantly increased compared with pre-exercise, but it was significantly greater than

Table 1 The demographic information of the participants, and physiological and biochemical parameters during the exercise.

Age (yr)	28.1 ± 1.3	Body Height (cm)	169.8 ± 1.1
Body Mass (kg)	64.7 ± 2.4	Body Mass Index (kg/m^2)	22.4 ± 0.7

	Pre	10 min	1 h	3 h
WBC (/mm^3)	5922 ± 320	5725 ± 243	6365 ± 599	7079 ± 433**††
RBC (x10^4/mm^3)	476.2 ± 7.5	479.0 ± 8.5	479.5 ± 7.5	468.5 ± 9.7
Hb (g/dL)	14.7 ± 0.2	14.9 ± 0.2	14.9 ± 0.2	14.4 ± 0.3
Platelet (x10^4/mm^3)	24.4 ± 0.8	25.2 ± 0.9*	23.5 ± 0.9††	23.9 ± 0.9†
CK (IU/L)	154.7 ± 13.0	170.0 ± 12.7***	172.0 ± 12.4***	186.4 ± 12.6**
BUN (mg/dL)	14.3 ± 0.8	14.7 ± 0.9**	14.5 ± 0.9	14.1 ± 0.8†‡
Creatinine (mg/dL)	0.86 ± 0.01	0.92 ± 0.02**	0.92 ± 0.02**	0.89 ± 0.02††‡
Na (mmol/L)	141.2 ± 0.5	142.1 ± 0.4*	141.6 ± 0.4	141.3 ± 0.5†
K (mmol/L)	3.9 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	3.8 ± 0.1
Cl (mmol/L)	103.7 ± 0.4	103.8 ± 0.7	104.3 ± 0.6	103.8 ± 0.4
Lactate (mmol/L)	0.92 ± 0.11	1.35 ± 0.18*	0.80 ± 0.07†††	0.86 ± 0.06††
Systolic BP (mmHg)	124.3 ± 4.1	117.1 ± 4.1*	111.6 ± 3.2***†	117.7 ± 4.8*‡
Diastolic BP (mmHg)	69.4 ± 3.3	67.5 ± 3.0	63.8 ± 2.6	66.3 ± 3.3
Pulse Pressure (mmHg)	54.9 ± 3.3	49.6 ± 2.7	48.6 ± 3.0	51.4 ± 2.7
Pulse Rate (/min.)	68.9 ± 3.7	90.3 ± 3.6***	84.5 ± 2.6***	75.5 ± 3.2††‡‡

Data are means ± SEM. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ *vs.* -30 min (pre-exercise), $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$, $^{\dagger\dagger\dagger}p<0.001$ *vs.* 10 min, $^{\ddagger}p<0.05$, $^{\ddagger\ddagger}p<0.01$ *vs.* 1 h. Abbreviations: WBC, White blood cell; RBC, Red blood cell; CK, Creatine kinase; BUN, Blood urea nitrogen; BP, Blood pressure

that at 3 h post-exercise. The mean heart rate during the treadmill exercise was 143/min.

The demographic information of the participants, and physical and biochemical parameters during the exercise are summarized in Table 1. As expected, serum creatinine concentration increased after the treadmill running, presumably reflecting hypovolemic hemoconcentration during the exercise. Even after serum IL-15 concentrations were normalized to serum creatinine concentrations, circulating IL-15 levels were significantly greater at 10 min post-treadmill running compared to other time points ($p<0.05$). In contrast to increased serum IL-15 concentration, serum creatine kinase (CK) concentration at 10 min post-exercise did not significantly differ from pre-exercise level when normalized to serum creatinine, although serum CK concentration was significantly increased at 10 min post-exercise unless normalized to creatinine. On the other hand, serum CK concentrations were significantly greater at 1 h and 3 h after the exercise compared with pre-exercise, regardless of whether they were normalized to serum creatinine concentrations.

Discussion

Our data clearly demonstrate that the single-set of endurance exercise on a treadmill resulted in a significant increase in serum IL-15 concentration in untrained healthy young men. These findings suggest that exercise-stimulated secretion of IL-15 may have a role in mediation of the systemic as well as local beneficial effects of endurance exercise, which include insulin-sensitizing and anti-adipogenic effects in addition to anabolic actions in skeletal muscle. This also suggests that skeletal muscle-derived IL-15 might function not only as a paracrine and autocrine but also as an endocrine factor.

The patterns of increases in IL-15 and CK levels after the exercise are quite different. Serum IL-15 concentration was increased at 10 min after the exercise, and significant difference in IL-15 was not achieved at 1 h post-exercise. Then, serum IL-15 concentration was fully returned to the pre-exercise level at 3 h post-exercise. To the contrary, the maximum increase in serum CK concentration, a surrogate biomarker for muscle damage-related response, was observed at 3 h post-exercise. These findings support the notion that

the increase in circulating IL-15 may result from muscle contraction-induced active secretion rather than passive release associated with exercise-induced damage in muscle.

In contrast to our findings, previous studies have shown that endurance exercise has no or little effect on circulating IL-15 concentrations and IL-15 mRNA levels in skeletal muscle [2-5]. This discrepancy may be explained by differences in the study design, particularly the time points for circulating IL-15 measurements after exercise. In previous studies [2-5], plasma IL-15 was measured starting at 1 h post-exercise. We found a significant increase in circulating IL-15 concentration at 10 min after the exercise. It is important to reiterate that significant difference was no longer observed in IL-15 at 1 h and 3 h post-exercise compared to the pre-exercise level. These data at 1 h and 3 h post-exercise are consistent with the results in previous studies [2-5]. Taken together, one can speculate that IL-15 secretion may be stimulated immediately after or during endurance exercise, and that exercise-stimulated IL-15 secretion may not be sustained for hours.

Based on changes in parameters, including pulse rate, blood lactate, and serum CK, the intensity of the 30-min treadmill running at 70% HR (max) is considered within a range of moderate to submaximum intensity in untrained healthy adults. Our study suggest, therefore, that strenuous high intensity training may not be necessary to significantly increase circulating IL-15 level.

Acknowledgements

This work was supported by research grants to M.K. from the National Institutes of Health (R01DK05827) and American Diabetes Association (7-08-RA-77).

Appendix

The authors have no conflicts of interest.

References

1. Pedersen BK (2009) The diseasome of physical inactivity--and the role of myokines in muscle--fat cross talk. *J Physiol* 587: 5559-5568.
2. Wunderlich FT, Strohle P, Konner AC, Gruber S, Tovar S, Bronneke HS, Juntti-Berggren L, Li LS, van Rooijen N, Libert C, Berggren PO, Bruning JC (2010) Interleukin-6 signaling in liver-parenchymal cells suppresses hepatic inflammation and improves systemic insulin action. *Cell Metab* 12: 237-249.
3. Klover PJ, Clementi AH, Mooney RA (2005) Interleukin-6 depletion selectively improves hepatic insulin action in obesity. *Endocrinology* 146: 3417-3427.
4. Quinn LS, Strait-Bodey L, Anderson BG, Argiles JM, Havel PJ (2005) Interleukin-15 stimulates adiponectin secretion by 3T3-L1 adipocytes: evidence for a skeletal muscle-to-fat signaling pathway. *Cell Biol Int* 29: 449-457.
5. Inoue S, Unsinger J, Davis CG, Muenzer JT, Ferguson TA, Chang K, Osborne D, Clark AT, Coopersmith CM, McDunn JE, Hotchkiss RS (2010) IL-15 prevents apoptosis, reverses innate and adaptive immune dysfunction, and improves survival in sepsis. *J Immunol* 184: 1401-1409.
6. Barra NG, Reid S, MacKenzie R, Werstuck G, Trigatti BL, Richards C, Holloway AC, Ashkar AA (2010) Interleukin-15 contributes to the regulation of murine adipose tissue and human adipocytes. *Obesity (Silver Spring)* 18: 1601-1607.
7. Quinn LS (2008) Interleukin-15: a muscle-derived cytokine regulating fat-to-lean body composition. *J Anim Sci* 86: E75-83.
8. Quinn LS, Anderson BG, Strait-Bodey L, Stroud AM, Argiles JM (2009) Oversecretion of interleukin-15 from skeletal muscle reduces adiposity. *Am J Physiol Endocrinol Metab* 296: E191-202.
9. Nielsen AR, Hojman P, Erikstrup C, Fischer CP, Plomgaard P, Mounier R, Mortensen OH, Broholm C, Taudorf S, Krogh-Madsen R, Lindegaard B, Petersen AM, Gehl J, Pedersen BK (2008) Association between interleukin-15 and obesity: interleukin-15 as a potential regulator of fat mass. *J Clin Endocrinol Metab* 93: 4486-4493.
10. Ajuwon KM, Spurlock ME (2004) Direct regulation of lipolysis by interleukin-15 in primary pig adipocytes. *Am J Physiol Regul Integr Comp Physiol* 287: R608-611.
11. Carbo N, Lopez-Soriano J, Costelli P, Alvarez B, Busquets S, Baccino FM, Quinn LS, Lopez-Soriano FJ, Argiles JM (2001) Interleukin-15 mediates reciprocal regulation of adipose and muscle mass: a potential role in body weight control. *Biochim Biophys Acta* 1526: 17-24.
12. Chan MH, Carey AL, Watt MJ, Febbraio MA (2004) Cytokine gene expression in human skeletal muscle during concentric contraction: evidence that IL-8,

like IL-6, is influenced by glycogen availability. *Am J Physiol Regul Integr Comp Physiol* 287: R322-327.
13. Ostrowski K, Hermann C, Bangash A, Schjerling P, Nielsen JN, Pedersen BK (1998) A trauma-like elevation of plasma cytokines in humans in response to treadmill running. *J Physiol* 513 (Pt 3): 889-894.
14. Nieman DC, Davis JM, Brown VA, Henson DA, Dumke CL, Utter AC, Vinci DM, Downs MF, Smith JC, Carson J, Brown A, McAnulty SR, McAnulty LS (2004) Influence of carbohydrate ingestion on immune changes after 2 h of intensive resistance training. *J Appl Physiol* 96: 1292-1298.
15. Nieman DC, Davis JM, Henson DA, Walberg-Rankin J, Shute M, Dumke CL, Utter AC, Vinci DM, Carson JA, Brown A, Lee WJ, McAnulty SR, McAnulty LS (2003) Carbohydrate ingestion influences skeletal muscle cytokine mRNA and plasma cytokine levels after a 3-h run. *J Appl Physiol* 94: 1917-1925.
16. Riechman SE, Balasekaran G, Roth SM, Ferrell RE (2004) Association of interleukin-15 protein and interleukin-15 receptor genetic variation with resistance exercise training responses. *J Appl Physiol* 97: 2214-2219.
17. Nielsen AR, Mounier R, Plomgaard P, Mortensen OH, Penkowa M, Speerschneider T, Pilegaard H, Pedersen BK (2007) Expression of interleukin-15 in human skeletal muscle effect of exercise and muscle fibre type composition. *J Physiol* 584: 305-312.
18. McNeer JF, Margolis JR, Lee KL, Kisslo JA, Peter RH, Kong Y, Behar VS, Wallace AG, McCants CB, Rosati RA (1978) The role of the exercise test in the evaluation of patients for ischemic heart disease. *Circulation* 57: 64-70.