

Serum concentration of cartilage oligomeric matrix protein (COMP) is sensitive to physiological cyclic loading in healthy adults¹

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Summary

Objective: To test the hypothesis that physiological cyclic loading during a 30-min walking exercise causes an increase in serum cartilage oligomeric matrix protein (COMP) concentration in a healthy population.

Methods: Blood samples (5 ml) were drawn from 10 physically active adults immediately before and after, and 0.5 h, 1.5 h, 3.5 h and 5.5 h after a 30-min walking exercise on a level outdoor walking track at self-selected normal speed. On a separate day, blood samples were drawn from the same 10 subjects during 6 h while they were resting in a chair. Serum COMP concentrations were determined using a commercial enzyme-linked immunosorbent assay (COMP[®] ELISA). An activity monitor was used to record basic time–distance measurements of gait. Serum COMP concentrations within the exercise protocol and within the resting protocol were compared using separate repeated measures analyses of variance ($\alpha = 0.05$).

Results: In the exercise protocol, a first increase (9.7%; $P = 0.003$) occurred immediately after the walking exercise. A second increase in serum COMP concentration (7.0%; $P = 0.024$) occurred 5.5 h after the walking exercise. In the resting protocol, the concentration at baseline was significantly higher than at all subsequent time points (8.2%; $P < 0.050$). Serum COMP concentration decreased from the 3.5-h to the 5.5-h sample (–4.8%; $P = 0.012$).

Conclusions: Even a moderate walking activity can significantly influence serum COMP concentration. The immediate response points to a diffusion time of COMP fragments from cartilage to the blood of 30 min or less. The response at 5.5 h indicates a metabolic delay for COMP in the range of 5 h to 6 h.

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Key words: COMP, Cartilage, Walking, Exercise.

Introduction

Cartilage oligomeric matrix protein (COMP) is a prominent constituent of articular cartilage¹. Increased serum concentrations of COMP fragments have been reported for patients with knee osteoarthritis (OA)^{2–5} and early rheumatoid arthritis (RA)^{6,7}. It has thus been suggested⁸ that patients with high levels of COMP in serum might represent individuals with increased degradation of their articular cartilage. Indeed, patients with greater serum COMP concentration experience a faster progression of their disease^{6–10}.

It has been proposed that COMP molecules are important for maintaining the properties and integrity of the collagen network¹¹ and contribute to the material properties of biological tissue¹². Increases in blood serum

concentrations of COMP have been reported¹³ in response to strenuous running exercise in healthy adults, and COMP levels remain elevated for up to 1 h after completion of the exercise. The loss of COMP fragments from the cartilage (indicated by increased serum levels) following exercise is important since COMP molecules may also transfer forces from the cartilage matrix to the cell, and thus are involved in the regulation of tissue synthesis and degradation¹⁴. Hence, COMP may be especially important for linking biological or pathogenic processes to mechanical loading of articular cartilage. However, the diffusion time of COMP fragments from cartilage to the blood and the metabolic delay for COMP in a healthy population have not been specified.

While increased mechanical loads at the knee during walking have been related to the progression of knee OA over a 6-year period¹⁵, it is not known whether everyday activities such as walking for a short period will cause a change in serum COMP concentration. In a healthy population, such activities may cause an increase in serum COMP concentration that may indicate normal adaptation to altered functional demand. Thus, the purpose of this study was to test the hypothesis that a 30-min walking exercise causes an increase in serum COMP concentration.

¹ Support: AnaMar Medical AB (Lund, Sweden) provided COMP ELISA kits. Dynastream Innovations Inc. (Cochrane, Canada) provided an activity monitor.

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Received 17 April 2004; revision accepted 24 September 2004.

Methods

POPULATION

Ten adults (five male, five female; age: 31.9 ± 5.9 years; height: 1.74 ± 0.10 m; mass: 70.7 ± 15.9 kg; body mass index (BMI): 23.1 ± 2.8 kg/m²; Table I) participated in this study after giving informed consent in accordance with the Institutional Review Board at Stanford University. All subjects were physically active, had no history of injuries to the lower extremities, and were free of pain for at least 6 months prior to the experiment.

PROCEDURE

Subjects were asked to limit their physical activity 36 h prior to the experiment. On the day of the experiment, subjects consumed breakfast within 1 h of waking, and the experiment started within 3–4 h of waking. Subjects were seated in a chair for 15 min immediately before the experiment. Five-milliliter blood samples were drawn by a certified research nurse from the same antecubital vein immediately before, immediately after, and 0.5 h, 1.5 h, 3.5 h, and 5.5 h after a 30-min walking exercise. Subjects consumed lunch immediately following the 1.5-h blood draw. During the walking exercise of the exercise protocol, subjects walked at their self-selected normal speed on a level paved outdoor walking track. An accelerometer based activity monitor (AMP331; Dynastream Innovations Inc., Cochrane, Canada) was attached to the subjects' right ankles to record basic time–distance measurements of gait including total step count, distance and average walking speed, cadence and stride length. Subjects performed office tasks in a seated posture after the 30-min walking exercise and were asked to perform a minimal amount of physical activity after completing the 30-min walking exercise.

On a separate day, data were collected for a resting protocol. Similarly to the exercise protocol, subjects self-limited their physical activity 36 h prior to the experiment, consumed breakfast within 1 h of waking, and the experiment started within 3–4 h of waking. Subjects were seated in a chair for 15 min immediately before the experiment. Blood samples were drawn at the same time points as in the exercise protocol over the course of 6 h while they were resting in a chair. An activity monitor was used to quantify the number of steps taken during the 6 h of the resting protocol.

ENZYME-LINKED IMMUNOSORBENT ASSAY

Blood was collected and allowed to clot for 30 min. Sera were separated and frozen to -20°C within 1 h of collection and then transferred for storage at -80°C until assayed. Serum COMP concentrations were determined using a commercial enzyme-linked immunosorbent assay (COMP[®] ELISA; AnaMar Medical AB, Lund, Sweden).

Briefly, 25 μl of six standards, diluted human control sera, as well as the diluted subject sera were added to an anti-COMP coated plate (mouse monoclonal antibodies) and incubated with 100 μl enzyme conjugate peroxidase–anti-COMP (mouse monoclonal antibodies, approximately 10 $\mu\text{g}/\text{ml}$) for 2 h at room temperature. After rinsing, the bound antibody was incubated with an enzyme substrate (tetramethylbenzidine) for 15 min at room temperature followed by incubation with stop solution (0.5 M H₂SO₄). The light absorbance of the reaction was read at 450 nm in a plate reader (Bio-Rad 550; Bio-Rad Laboratories, Hercules, CA).

An in-house cubic spline regression algorithm written in Mathematica version 4.1 (Wolfram Research Inc., Champaign, IL) was used to define the standard curve of light absorbance vs COMP concentration and to interpolate serum COMP concentrations of the samples. Investigators were blinded to the samples, which were analyzed in duplicate and in random order. The detection limit of the assay was <0.1 Units/liter (U/l), and the intra-assay and inter-assay coefficients of variation were $<1.9\%$ and $<2.7\%$, respectively (10 U/l convert to approximately 1 $\mu\text{g}/\text{ml}$)¹⁶. Differences due to inter-assay variation were eliminated by comparing concentrations within subjects and by testing all samples of any subject on the same plate.

STATISTICAL ANALYSIS

All statistical computations were performed using SPSS 11.5 software (SPSS Inc., Champaign, IL). Descriptive statistics, sample means, and standard deviation for serum COMP concentrations and basic time–distance measures of gait were calculated. Serum COMP concentrations within the exercise protocol and within the resting protocol were compared using separate repeated measures analyses of variance (ANOVAs). Repeated measures Student's *t* tests were used for *post hoc* comparisons. The significance level α for all statistical tests was set at 0.05.

Results

At baseline of the exercise protocol (i.e., immediately before the 30-min walking exercise), the mean serum COMP concentration was 9.98 ± 3.38 U/l. While female subjects had significantly lower serum COMP concentrations (6.72 ± 1.55 U/l) than male subjects (11.59 ± 2.35 U/l; $P = 0.005$), all individual serum COMP concentrations at baseline were within the previously reported normal distribution¹⁶ (Fig. 1). Serum COMP concentrations immediately after the 30-min walking exercise were elevated, on average, by 9.7% (Figs. 1 and 2; $P = 0.003$) and returned to the baseline concentration 30 min later. A second increase in serum COMP concentration was observed 5.5 h after the 30-min exercise [Fig. 2(a); $P = 0.024$].

Table I

Mean (± 1 SD) of demographic data and basic time–distance measurements during the 30-min walking exercise for all subjects by gender

Gender	Age (years)	Height (m)	Mass (kg)	BMI [†] (kg/m ²)	Speed (m/s)	Distance (m)	Number of steps	Cadence (steps/min)	Stride length (m)
Male, $N = 5$	31.4 (4.5)	1.82 (0.06)	83.4 (11.4)	25.2 (1.8)	1.47 (0.12)	2685.2 (217.0)	3369.2 (231.5)	110.8 (8.6)	0.80 (0.04)
Female, $N = 5$	32.4 (7.6)	1.66* (0.06)	57.9* (6.0)	20.9* (1.8)	1.52 (0.11)	2812.6 (171.2)	3644.8* (69.1)	117.8 (2.3)	0.77 (0.06)

*Significantly different from male subjects ($P < 0.050$) based on two-tailed Student's *t* tests using SPSS 11.5 software (SPSS Inc., Champaign, IL).

[†]BMI = mass (kg)/height (m)².

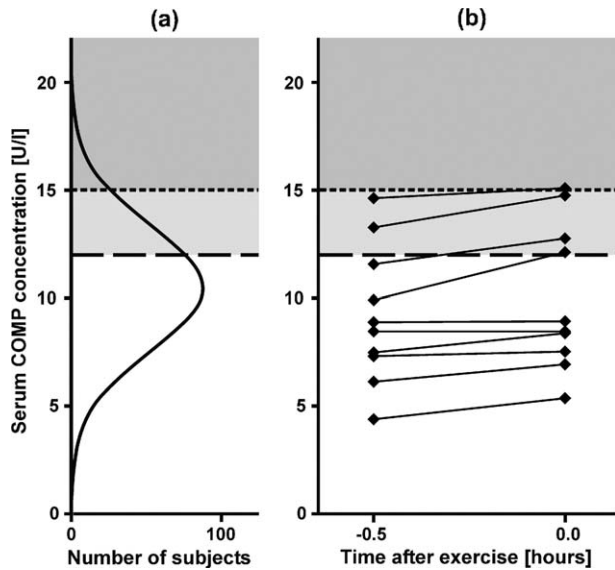


Fig. 1. Comparison of serum COMP concentrations at the first two time points of the exercise protocol in the present study with the normal distribution data from the manufacturer: (a) normal distribution ($N = 336$) of serum COMP concentration in blood donors provided by the manufacturer¹⁶ and risk of joint destruction in RA (<12 U/l: low; $12\text{--}15$ U/l: increasing; >15 U/l: high)⁹; (b) individual serum COMP concentration immediately before and immediately after a 30-min walking exercise measured in the present study ($N = 10$). Note that serum COMP concentration of all subjects increased and that three subjects would be classified into higher risk categories after the 30-min walking exercise.

During the 30-min walking exercise, subjects took, on average, 3507 ± 217 steps to travel an average distance of 2748.9 ± 196.1 m (Table I). Average walking speed, cadence and stride length for all subjects during the 30-min walking exercise were 1.49 ± 0.11 m/s, 114.3 ± 7.0 steps/min and 0.78 ± 0.05 m, respectively. While the female subjects were significantly lighter and shorter and took more steps ($P = 0.002$, $P = 0.004$, and $P = 0.034$, respectively) than male subjects, these two groups did not differ in age, self-selected walking speed, distance traveled, cadence, or stride length ($P > 0.050$; Table I). The magnitude of the increases in COMP concentrations immediately after and 5.5 h after the 30-min walking exercise was not related to gender, age, height, mass, BMI, or any of the basic time–distance gait measures ($P > 0.050$; linear regression analysis).

At baseline of the resting protocol the mean serum COMP concentration was 8.89 ± 2.75 U/l. This concentration was not significantly different from the baseline concentration of the exercise protocol ($P = 0.620$). In the resting protocol, the concentration at baseline was significantly higher than at all subsequent time points [8.2%; $P < 0.050$; Fig. 2(b)]. Interestingly, serum COMP concentration further decreased from the 3.5-h to the 5.5-h sample (−4.8%; $P = 0.012$). During the resting protocol (duration: 6 h), subjects took on average 505 steps and traveled an average distance of 210.0 m.

Discussion

The results of this study showed that an activity as simple as walking for 30 min can cause elevated levels of COMP in

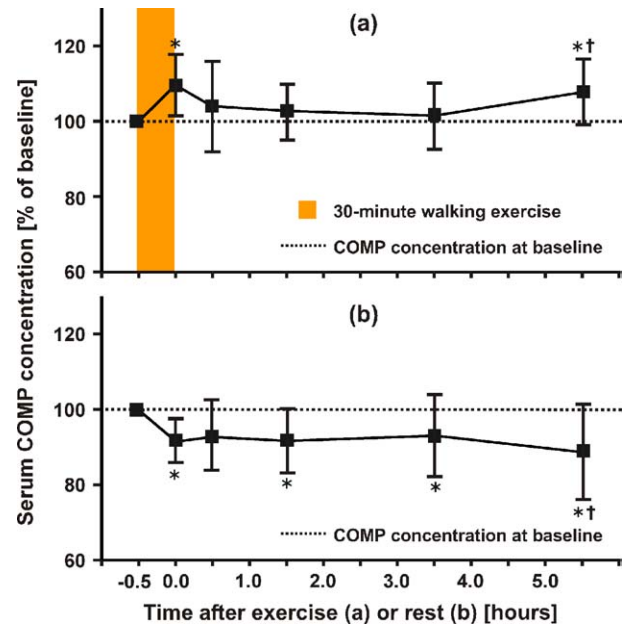


Fig. 2. (a) Mean (SD) serum COMP concentration before (−0.5 h) and up to 5.5 h after 30-min walking exercise ($N = 10$ subjects); (b) mean (SD) serum COMP concentration during 6 h of resting in a chair for the same 10 subjects. *Significantly different from baseline; †significantly different from preceding time point ($\alpha = 0.05$). Serum COMP concentrations after exercise were normalized to serum COMP concentration at baseline (before exercise) for each subject for visual presentation. Comparisons of serum COMP concentrations before and after exercise and between resting time points were performed with repeated measures ANOVA ($\alpha = 0.05$) using SPSS 11.5 software (SPSS Inc., Champaign, IL).

serum. The number of steps taken during the 30-min exercise in this study corresponds to approximately 30% of the average number of steps taken per day in a healthy population¹⁷. In comparison, simply traveling to the clinic for blood testing may represent a similar portion of daily walking activity in a mostly sedentary population of patients with knee pain. Thus, uncontrolled activity such as walking to or within the clinic prior to a diagnostic blood test may alter serum COMP concentrations.

Average serum COMP concentrations in the current study were lower than values reported for patients with OA^{18,19}, patients with knee OA²⁰ and patients with early RA⁷ using the same assays. While all baseline values were within the normal distribution of a large sample of blood donors¹⁶ [Fig. 1(a)], three of our subjects would be classified into higher categories of risk for joint destruction for RA patients⁹ after the 30-min walking exercise [Fig. 1(b)]. Similarly, it is possible that the effect of an OA or RA patient walking to the clinic could also bias the result of the diagnostic test.

To our knowledge, the diffusion time of COMP fragments from cartilage to the blood and the metabolic delay for COMP in a healthy population are unknown. Consequently, repeated blood sampling over the course of 6 h was chosen because the time delay between physiological cyclic loading of the joints of the lower extremity during a walking exercise and the consecutive increase in serum COMP concentration was not known. Neidhart *et al.*¹³ investigated the kinetics of COMP concentration in serum before, during, immediately after and 2 h after a marathon run. Serum

COMP concentrations increased throughout the run and decreased within 2 h after the run. Neidhart *et al.*¹³ did not take additional blood samples more than 2 h after the run on the same day. Nevertheless, our observations are consistent with these results. We suggest that the immediate elevation of serum COMP concentration after 30 min of mild exercise and during a marathon run reflects enhanced diffusion of COMP fragments from the cartilage to the blood due to the physiological cyclic joint loading corresponding to a diffusion time of COMP of 30 min or less. Moreover, the immediate increases in serum COMP concentration after physiological cyclic loading indicate that free COMP antigens are indeed also present in the living tissue of healthy adults.

In our study, we observed a second increase in serum COMP concentration 5.5 h after the 30-min exercise. It has previously been shown^{14,21} that COMP expression is enhanced following cyclic confined and unconfined compression (10% amplitude, 0.5 Hz) of bovine articular cartilage explants *in situ* for 18 h and 45 h. Thus, although load was applied over a much shorter period (30 min) in the current study, the increase in serum COMP concentration following physiological cyclic loading *in vivo* such as the 30-min walking exercise of healthy adults (females and males) may in fact reflect increased tissue metabolism. While COMP is a proposed marker for cartilage degradation⁴, and increased serum COMP concentrations after exercise thus likely reflect increased COMP catabolism; the anabolic activity of the tissue is unknown. However, everyday activities such as a 30-min walking exercise will most likely stimulate cartilage turnover rather than initiate cartilage degradation in a healthy population. Further studies are necessary to quantify anabolic activity of cartilage following a walking exercise.

While serum COMP concentrations increased immediately and 5.5 h after the 30-min walking exercise, in the resting protocol serum COMP concentrations decreased from the -0.5-h (baseline) value to the 0-h value and further decreased to the 5.5-h value. Thus, the increases observed in the exercise protocol are clearly due to the 30-min walking exercise and suggest a metabolic delay in the order of 5–6 h. The observed increases in the exercise protocol might have been even greater had the subjects not been required to travel to the blood clinic. In the resting protocol, the concentration at baseline was significantly higher than at all subsequent time points suggesting an increase in serum COMP concentration following walking activity required to travel to a blood clinic followed by a decrease after resting. A previous study²² reported decreased serum COMP concentrations during bed rest at night reaching the lowest levels around 5 am. Hence, we recommend that future biomarker studies draw subjects' blood in the morning and after the subject was in a seated posture for at least 30 min.

Wong *et al.*¹⁴ suggested that cyclic loading has an important regulatory function in cartilage matrix remodeling. Our study is a first attempt to link serum COMP concentration to physiological cyclic loading *in vivo*. This is the first study to report diffusion time of COMP fragments from cartilage to the blood, metabolic delay for COMP, and the effect of controlled walking exercise on serum COMP concentration in a healthy population. The results of this study showed that controlling physical activity prior to blood sampling for biomarker tests is critical and suggest a diffusion time of COMP fragments to the blood of 30 min or less and a metabolic delay for COMP in the range of 5 h to 6 h in the exercise protocol used in this study.

Acknowledgements

We thank Louise McManus from the Clinical Studies Unit at the Palo Alto VA Health Care System, Palo Alto, CA for taking the blood samples; AnaMar Medical AB for providing the ELISA kits; and Dynastream Innovations Inc. for providing the activity monitor.

References

- Hedborn E, Antonsson P, Hjerpe A, Aeschlimann D, Paulsson M, Rosa-Pimentel E, *et al.* Cartilage matrix proteins. An acidic oligomeric protein (COMP) detected only in cartilage. *J Biol Chem* 1992;267: 6132–6.
- Clark AG, Jordan JM, Vilím V, Renner JB, Dragomir AD, Luta G, *et al.* Serum cartilage oligomeric matrix protein reflects osteoarthritis presence and severity: the Johnston County Osteoarthritis Project. *Arthritis Rheum* 1999;42:2356–64.
- Jordan JM, Luta G, Stabler T, Renner JB, Dragomir AD, Vilím V, *et al.* Ethnic and sex differences in serum levels of cartilage oligomeric matrix protein: the Johnston County Osteoarthritis Project. *Arthritis Rheum* 2003;48:675–81.
- Neidhart M, Hauser N, Paulsson M, DiCesare PE, Michel BA, Häuselmann HJ. Small fragments of cartilage oligomeric matrix protein in synovial fluid and serum as markers for cartilage degradation. *Br J Rheumatol* 1997;36:1151–60.
- Pavelka K, Forejtová Š, Olejárová M, Gatterová J, Šenolt L, Špaček P, *et al.* Hyaluronic acid levels may have predictive value for the progression of knee osteoarthritis. *Osteoarthritis Cartilage* 2004;12: 277–83.
- Forslind K, Eberhardt K, Jonsson A, Saxne T. Increased serum concentrations of cartilage oligomeric matrix protein. A prognostic marker in early rheumatoid arthritis. *Br J Rheumatol* 1992;31:593–8.
- Skoumal M, Kolarz G, Klingler A. Serum levels of cartilage oligomeric matrix protein. A predicting factor and a valuable parameter for disease management in rheumatoid arthritis. *Scand J Rheumatol* 2003;32: 156–61.
- Vilím V, Olejárová M, Macháček S, Gatterová J, Kraus VB, Pavelka K. Serum levels of cartilage oligomeric matrix protein (COMP) correlate with radiographic progression of knee osteoarthritis. *Osteoarthritis Cartilage* 2002;10:707–13.
- Lindqvist EK, Eberhardt K, Heinegård D, Saxne T. Serum COMP for risk assessment of joint destruction in early rheumatoid arthritis (Abstract). In: Proceedings of EULAR 2002, June, Stockholm, Sweden 2002;60.
- Sharif M, Kirwan JR, Elson CJ, Granell R, Clarke S. Suggestion of nonlinear or phasic progression of knee osteoarthritis based on measurements of serum cartilage oligomeric matrix protein levels over five years. *Arthritis Rheum* 2004;50:2479–88.
- Rosenberg K, Olsson H, Mörgelin M, Heinegård D. Cartilage oligomeric matrix protein shows high affinity zinc-dependent interaction with triple helical collagen. *J Biol Chem* 1998;273:20397–403.
- Smith RK, Gerard M, Dowling B, Dart AJ, Birch HL, Goodship AE. Correlation of cartilage oligomeric matrix protein (COMP) levels in equine tendon with mechanical properties: a proposed role for COMP in

- determining function-specific mechanical characteristics of locomotor tendons. *Equine Vet J Suppl* 2002; 34:241–4.
13. Neidhart M, Müller-Ladner U, Frey W, Bosserhoff AK, Colombani PC, Frey-Rindova P, *et al.* Increased serum levels of non-collagenous matrix proteins (cartilage oligomeric matrix protein and melanoma inhibitory activity) in marathon runners. *Osteoarthritis Cartilage* 2000;8:222–9.
 14. Wong M, Siegrist M, Cao X. Cyclic compression of articular cartilage explants is associated with progressive consolidation and altered expression pattern of extracellular matrix proteins. *Matrix Biol* 1999;18: 391–9.
 15. Miyazaki T, Wada M, Kawahara H, Sato M, Baba H, Shimada S. Dynamic load at baseline can predict radiographic disease progression in medial compartment knee osteoarthritis. *Ann Rheum Dis* 2002;61: 617–22.
 16. AnaMar Medical AB. COMP® ELISA Enzyme Immunoassay: Directions for use. 3–11.
 17. Sequeira MM, Rickenbach M, Wietlisbach V, Tullen B, Schutz Y. Physical activity assessment using a pedometer and its comparison with a questionnaire in a large population survey. *Am J Epidemiol* 1995;142: 989–99.
 18. Petersson IF, Boegård T, Svensson B, Heinegård D, Saxne T. Changes in cartilage and bone metabolism identified by serum markers in early osteoarthritis of the knee joint. *Br J Rheumatol* 1998;37:46–50.
 19. Petersson IF, Boegård T, Dahlström J, Svensson B, Heinegård D, Saxne T. Bone scan and serum markers of bone and cartilage in patients with knee pain and osteoarthritis. *Osteoarthritis Cartilage* 1998;6:33–9.
 20. King KB, Lindsey CT, Dunn TC, Ries MD, Steinbach LS, Majumdar S. A study of the relationship between molecular biomarkers of joint degeneration and the magnetic resonance-measured characteristics of cartilage in 16 symptomatic knees. *Magn Reson Imaging* 2004;22:1117–23.
 21. Giannoni P, Siegrist M, Hunziker EB, Wong M. The mechanosensitivity of cartilage oligomeric matrix protein (COMP). *Biorheology* 2003;40:101–9.
 22. Andersson M, Jpnsson N, Petersson IF, Heinegård D, Saxne T. Diurnal variation of serum-COMP in individuals with knee pain and osteoarthritis (Abstract). In: *Proceedings of the EULAR 2002, June, Stockholm, Sweden* 2002;48.
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