

REVIEW ARTICLE

REPETITIVE PHYSICAL ACTIVITY AND THE INCIDENCE OF ARTHRITIC CHANGES IN THE LOWER LIMBSG. MANN^{1,2}, N. WEEG³, I. HETSRONI^{1,2}, O. MEI-DAN¹, M. NYSKA¹ and L. CANATA^{4,5}¹Meir Medical Centre, Kfar Saba; ²The Wingate Institute, Netanya, Israel; ³Mayday Hospital, London, United Kingdom; ⁴Koelliker Hospital, Torino; ⁵SUISM, University of Torino, Italy*Received May 25, 2009- Accepted July 30, 2009*

In order to evaluate the impact of physical activity, especially continuous activity such as walking, cycling or running, on the lower limb joints, we reviewed nearly one hundred articles reporting the various aspects and different research angles on this topic. The sources were divided into five categories: those claiming damage, those which are not conclusive, those claiming no damage, reviews as well as animal studies. Overall the material shows only borderline evidence that High Impact Sports Activity will damage a normal joint which has not previously suffered injury or an anatomic variation and which does not present a genetic fault. In cases presenting with osteoarthritis, single or recurrent injuries, which have gone unobserved, have often occurred previously. The evidence that continuous Non-High Impact Physical Activity like walking, cycling or running could damage a joint with no prior anatomical damage and which does not suffer a biomechanical or genetic fault, is weak and not convincing, while literature supporting no damaging effect or even improvement of the joint structure is far more persuasive and abundant. This evidence observed in human subjects and in animal models, is still stronger in animal studies than in research performed with human subjects. The safest activity, it seems, is gradual and graded activity with no exploding force, not extreme and which is not irregular for the individual trainee. The advantages of physical activity to general health as well as to the musculoskeletal system seem to strongly outdo its potential risks.

In the 2000 World Congress on Orthopaedic Sports and Trauma held in Queensland, Australia, and in the 2003 ISAKOS Meeting (International Society of Arthroscopy, Knee Surgery and Orthopaedic Sports Medicine) held in Auckland, New Zealand, presentations were given discussing the arthritic changes that occur in the lower limb joints as a result of running (1). These were later published in "Orthopaedics Today" as they were believed to be of interest to the general public. The subject was reviewed again in 2007 at the Sports

Medicine Meeting of the University of Turin, Italy (2). For many years this subject, understandably, has worried recreational sportsmen, including those who practice a sport for fun and those who practice in order to keep fit, as well as causing concern to general practitioners, sports physicians, physical education (PE) teachers, coaches, and other healthcare professionals when recommending physical activity to the general population.

It seems that, invariably, the presence of a congenital anatomical abnormality or an injury

Key Words: Osteoarthritis, Physical Activity, overuse, running

Mailing address: Dr. Gideon Mann,
P.O.Box 34400,
Givat Shaul,
91342 Jerusalem, Israel
Tel: ++972 52 251 4608
Fax: ++972 2 6528231
email: drmann@regin-med.co.il

that has changed the anatomical or biomechanical configuration of a joint, will lead to arthritic changes in a relatively high proportion of injured subjects (3-12), a large proportion of whom have suffered a torn meniscus (13-15) or anterior cruciate ligament (ACL) disruption (7,14-17).

In the absence of a clinical history for joint injury, it seems that high impact activities, such as some ball games, may be a risk factor for the development of degenerative (i.e. wear and tear) changes in the knee and hip (9,11,18-21), although some authors argue that in the absence of anatomical or neuromuscular damage, extreme high impact physical activity and repeated rotation, the activity will lead to radiological changes (such as osteophytes) but not to true arthritic changes (22), or in the absence of defined injury, might not bring to any locatable arthritic changes at all (23). Melzer et al in 2001 showed a higher rate of degenerative changes in the contralateral knee of traumatic amputees (24) while no significant difference was shown when comparing volleyball player amputees to amputees who led a sedentary life style. This observation though was statistically not significant.

Nonetheless, some papers tend to support the view that high intensity sport may be a contributing factor in the development of arthritic changes in the lower limb joints. In a review of sports fields, without differentiating individual sports, Vingard et al in 1993 (25) demonstrated a 4.5 fold rise in hip osteoarthritis in a population previously active in high intensity sports. The rate rose to 8.5 fold when work exposure was added. Track and field and racket sports were specified as higher risk sports. Without specifying the type of sport, Vingard et al further reported in 1998 (26) an occurrence of degenerative changes in active women 1.5 to 2.3 times higher than in the normal population, depending on their exposure to sport. L'Hemette et al showed a rise of degenerative changes in the hip in past hand ball players (18). Several publications have discussed the issue of high rates of arthritic changes in the knee and hip joints of footballers; where the highest incidence is seen in professional players, while some authorities have claimed that the hip joints are not affected more in amateur footballers than in the general population, the knees show high rates of arthritic changes in both professional and amateur

players (27-35). Certain degenerative changes in the hip have been demonstrated by other authors (36-37) in connection to general sports activities. Lane, in 1999, reviewed 5,818 women, average age 72 yrs (38), and showed an increased risk of osteoarthritis (OA) of the hip in women who reported significant physical activity in their past, without documenting running as a separate issue. In 2000, Sandmark (39) detected high rate arthritic changes and injuries in the knees of 571 Physical Education (PE) graduates, on the basis of personal reports alone, without the use of X ray findings. Cooper, in 2000, (40) performed a radiological review of a physically active population average age 76 years and detected an excess risk for OA of the knees, without progression compared to X rays taken five years previously. Lahr in 1996 (12) noted no increased risk of osteoarthritis nor acceleration of existing osteoarthritis in individuals involved in moderate running and who did not suffer anatomical variances.

Hunter and Eckstein in their recent 2009 review (41) similarly observed no negative effect of vigorous low impact activity on joints and positive benefits of exercise on joint tissues. Accordingly they suggested that the increased rate of osteoarthritis observed in elite sports by some investigators may well be dependant on injury occurrence and not on the sports as such.

In the next few lines we intend to address repetitive physical activity (i.e. walking, cycling and, mainly, running) as a possible contributing factor to the wear and tear (i.e. OA) of the joints of the lower limbs. When preparing this review, we used material that was previously presented at the World Congress on Orthopaedic Sports Trauma, Queensland, Australia, in 2000 (1); material used in our lectures to doctors during the post-graduate program for Sports Medicine at the Tel Aviv University, Israel; material from literature, reviews published in the past; and material from an updated literature search.

The subject will be discussed according to the following topics:

1. Publications supporting a link between repetitive continuous physical activity and arthritic changes in the joints of the lower limbs;
2. Controversial or "problem" publications;
3. Publications which found no relationship

between running or continuous low impact sport and osteoarthritic changes in the joints of the lower limbs;

4. Literature reviews;
5. *In vivo* studies (animal studies).

1. Publications supporting a link between repetitive physical activity and arthritic changes in the joints of the lower limbs

Ever since Freeman published his thoughts on cartilage fatigue and cartilage erosion in 1975 (42), and from when Radin, in 1982, published his observations regarding the protective effect of a soft surface (sawdust) compared to a hard surface (concrete), in preventing arthritic changes in the knees of walking sheep (43), several publications have appeared supporting the thought that repetitive exercise such as running might cause arthritic changes in the lower limb joints.

In 1989, Marti published his observational study, in which he demonstrated radiological arthritic changes in the lower limbs of runners, depending on intensity of running and the cumulative distance (44). This publication caused a great deal of confusion in light of the combined international efforts made at that time to encourage physical exercise in the general population and promote healthy behaviour, as the study created a valid excuse to avoid physical exercise by many who truly needed it for their wellbeing. A few years later, in 1994 and in 1995, publications by Kujala et al appeared, which demonstrated a relative risk (RR) of 1.73 of developing OA of the hip, the knee and the ankle in elite Finnish retired runners (30,45), and radiological changes supporting OA were demonstrated in 67 female runners by Spector et al in 1996 (46). Nonetheless, it should be emphasized that the observation of osteophytes on the radiograph does not necessarily reveal the presence of clinical OA (40-41,47-48), nor do radiographic changes suggesting osteoarthritis (marginal osteophytes, subchondral sclerosis and joint narrowing) correlate regularly with pain (41,49).

2. Controversial or problematic publications

This paper relates mainly to Weight Bearing Sports. Publications that do not discuss different sports separately; or which do not give details

regarding the type of activity or the age of the individual when taking on the activity; or those which do not give a complete report of previous injuries; or ones which do not contain a control group or contain a control group which is essentially different from the group being investigated; all constitute a basis which allows conclusions to be made only with caution. Other publications constitute a problem by the implications of their findings or if their findings are difficult to explain and are not logically feasible, requiring further investigation. The method of OA diagnosis presents other problems as osteophytes may not represent OA (40-41, 47-48), radiological changes may be totally asymptomatic (41,49) and "self reported" OA may have little true significance (41)

In 2000 Neidhart (50) pointed out the presence of cartilage metabolites in the blood of marathon runners. Does this suggest cartilage damage or perhaps increased metabolism and raised anabolism? Kessler et al demonstrated in 2006 the loss of cartilage and meniscus volumes during a 5 Km run while the meniscus continues to lose its volume in a run of up to 20 Km (51). According to the authors, duration is not related to the development of arthritic changes. In 2000, Cheng et al reported their findings in 16,961 individuals as they tried to determine whether physical activity such as running might cause arthritic changes (52). In those under 50 years of age, the authors found a Relative Risk of 2.4 for developing arthritic changes in men. No risk difference was found in men over 50 years of age nor in women. These results are difficult to explain especially as the diagnosis was made on the basis of "personal reports and doctors' diagnoses". The article did not give full details of the fields of sport; neither did it review previous injuries and past medical history. Similarly, Sandmark and Vingard in 1999 (53) reported a relative risk for osteoarthritis of 2.9 for men aged 55 to 65 years who were highly exposed to all kinds of sport. The limited age range should be noted as well as the observation that no increased Relative Risk was seen in women. The authors concluded that "Moderate daily general physical activity was not found to be a risk factor".

In 1999, Kujala et al published their findings on 269 orienteering runners who had been top athletes in their field 15 years prior to the study, compared

to 188 inactive individuals (54). Regardless of the theory under investigation, they found that the incidence of myocardial infarction was almost eight times higher in the control group. The authors indicated a rise in the incidence of arthritic changes in knees of old athletes but not in their hips. Interestingly, the athletes' disability was less severe than the control group.

In 1997, the article of Sarna et al was published, comparing 2,613 Finnish athletes that were active during 1920-1965 to a control group of 1,712 people (55). The athletes showed an increased longevity, an increased length of active lifestyle, a lower incidence of diabetes mellitus, and a slight increase in the incidence of arthritic changes. An increased incidence of arthritic changes was demonstrated in runners by McDermott and Freyne in 1983 (56), but only in those runners who were known to have abnormal anatomy (meniscal tears, varus deformity of the knees, congenital joint laxity). Similar findings were picked up by Buckwalter in his review in 2003 (8) and by Hunter and Eckstein in their very recent review (41). In 1991, Radin et al (57) suggested the "miscoordination" element as a cause of arthritic changes due to the force of the heel strike during stance and an excess of uncoordinated load. Shrier reviewed different clinical studies in 2004, and similarly to Radin's view in 1991, concluded that defective muscle function (i.e. power, coordination) is the prime aetiological factor in the development of arthritic changes in the joints during exercise, and not the actual activity itself (58).

3. Publications which found no relationship between running and osteoarthritic changes in the joints of the lower limbs

In 1986, 1993 and 1998, Lane published her findings on running club members compared to a control group (59-61). In the follow-up of 41 runners after five and nine years, she found no difference in the incidence of arthritic changes in the knees nor in the back. However, the nine year follow up showed a reduction in the narrowing of the joint space of the knee in female runners compared to the control group (61). The Radiographic Assessment of Osteoarthritis in Lane's paper was based on the methods developed at the San Francisco Conference on Radiographic Assessment of the Progression of Osteoarthritis

according to Altman (62). This included blind reading of serial knee weight bearing radiographs by two different radiologists, both unaware of when the radiographs were taken and to which study group they belonged. Hips were evaluated by non weight bearing radiographs. The radiologist' findings were compared, showing an agreement of 0.85. Both medial and lateral joint spaces of the knee were measured.

Kujala followed up 2000 Finnish athletes who were admitted to hospital for OA. These athletes were divided into athletes of aerobic sports (running and cross country skiing), team games and power sports (30,45). Even though in most athletes more hospital admissions than expected were noted, the actual diagnosis of OA was made in 14% of the athletes compared to 12% in the general population, an insignificant difference. Leaver examined 128 ultra-marathon runners in 1998 (i.e. running distances from 100 to 150 Km) and did not find an increased rate of OA (63).

Back in 1975, Puranen (64) followed up 60 marathon runners radiologically and did not find any difference in the rate of occurrence of arthritic changes compared to the normal population. In a prospective review that Panush carried out in 1986 with the aid of radiographs (65), she did not find an increased incidence of arthritic changes in the hip, knee, ankle or joints of the foot in 17 runners. Her follow-up observation concluded with the same outcome in 1995 (66). In 1990, Konradsen examined 30 runners who ran between 18 to 40 Km per week for 40 years, and did not find a higher rate of OA on ankle, knee or hip X-rays (67). Sohn compared 504 cross country runners to swimmers in 1985 (68), without the aid of X-rays, and did not detect a difference in the occurrence of OA in the hip or knee. Eastmond in 1979 (69), with the aid of X-rays, examined arthritic changes of the hip and knee in PE teachers in order to investigate arthritic changes caused by moderate intensity exercise. No difference was found between the PE teachers and the control group. Bird and Barton, in 1993 (70), examined a similar group of PE teachers and did not identify an increased incidence of arthritic changes. In 1993, White (71) looked at a further group of past PE teachers and besides not finding an increased incidence of arthritic changes, he actually observed

a lower rate of arthritic changes in the knee. A review of 216 individuals self reporting knee OA was performed by Sutton in 2001 (72) and revealed that a history of knee injury was the only detectable risk factor for arthritic changes, with a RR of 8.0.

In the reviewed material there are several other publications that also point towards the absence of an increased occurrence of arthritic changes in the knees or hips in long distance runners (49,73-76). Kohatsu and Schurman, in 1990 (77), demonstrated different aetiological factors for the development of OA of the hip: Being overweight constitutes a 3.5 fold increase in the risk of developing arthritic changes, hard physical work comprises a risk factor constituting 2.5 fold increase, previous injury increased the risk by a factor of 5.0, whereas physical exercise showed no risk at all. In 1994, Panush and Lane (78) created a table that included estimated risks for developing arthritic changes in the hip or knee in a variety of sports. The authors concluded that running produces "little or no" arthritic changes in the hip or knee.

In 1975, Puranen, in a letter (64) claimed that degenerative changes occur in half as many athletes as members of the general population (4% compared to 8.7%).

Of special interest is the paper by Racunica et al (79), who in 2007 showed by Magnetic Resonance Imaging (MRI) that vigorous physical activity in 297 adults aged 50 to 79 increased cartilage volume of the tibial plateau of the knee and reduced the incidence of cartilage defects. Regular walking was shown to reduce the risk of bone marrow lesions (79). Knee cartilage volume was measured using MRI, determining the cartilage volume by image processing at an independent work station using Osiris Software (University Hospital of Geneva, Geneva, Switzerland). The method was based on previous publications by Cicuttinini (80) and Wluka (81). Racunica et al's observations, similar to that previously shown by Frankel and Nordin (82), showed a three fold thickness of the cartilage in the active human joint. This may explain the findings of Rogers et al (83), who showed a positive effect of physical activity on self reported physician diagnosed osteoarthritis of the hip and knee, and the findings of Roos et al (84), who reported an increase of knee cartilage glycosaminoglycan content following moderate exercise in human subjects, as

revealed by MRI. Foley in 2007 (85) observed that physical work capacity was negatively associated with cartilage loss, and thus concluded that physical activity has probably a protective effect on joint cartilage.

4. Reviews

Most of the reviews we found on this topic discuss, first and foremost, the quality of the studies that were done, as well as the features of OA, the discrepancies between radiological and clinical findings, diagnostic criteria and the heterogeneity of the population. Retrospective studies are clearly defective due to the imprecise reporting of exercise levels and injuries. The reviews, often, cover sports in general and do not always differentiate between repetitive activity, such as running, and other types of sports.

Factors such as previous injury, pre-existing anatomical abnormality or obesity, are all reported as risk factors for the development of degenerative changes in the joints of the lower limbs, and appear as a leitmotif in modern literature (11,20,86-90), as well as the genetic predisposition that includes a Relative Risk (RR) of 2-5 (91). These variables may distort the results of the different studies. Lequesne et al, 1997 (92), did not find an increased incidence of arthritic changes in recreational activity, while in aggressive physical activity or in rigorous physical work they observed in the literature a RR of 1.5 to 5.0. In the same year, Buckwalter and Lane (89) did not find in their review any evidence of a link between moderate physical exercise and the occurrence of arthritic changes. In Hochberg's review in 2002 (88) the author detected "only limited evidence" of OA in the hip in athletes, including runners, while Lievens, in a very thorough review in 2003, found nine publications about runners, three of which were highly considered, and these showed "moderate evidence" of a link between running and arthritic changes in the hip (90). On the other hand, therapeutic exercise, including an aerobic and/or strengthening program, was shown in a recent review by Hernandez-Molina in 2008 (93) to have a beneficial effect on hip osteoarthritis. In 2002, Conaghan (11) reviewed a large number of publications, and concluded that there is only a low risk for individuals who are recreational runners.

Felson's review from 2004 (20), mainly discussed the aspect of excess weight as being a risk factor for the development of arthritic changes. Where running is concerned, Lane and Buckwalter's review in 1999 (94) includes a summary of the risk factors for OA of the knee and hip. The authors identified no risk in running for leisure, and a small risk when running competitively.

A more recent review by Zeller and Sukenic (95) did not reveal any significant rise in knee osteoarthritis in runners nor did an editorial by Mann et al reveal increased arthritic changes in the large joints of the lower limb in continuous low impact sports (96).

A more recent review by Hunter and Eckstein published in January 2009 (41) concluded that vigorous low impact sports would not cause OA whereas elite sports may cause a rise in OA most likely due to joint injuries and not the sport itself. The authors pointed out the positive effects of exercise on the synovial joint tissues.

5. *In vivo animal studies*

Considering the inconsistent data given in the literature, it seems that it would be better to turn to *in vivo* laboratory studies, which in controlled conditions may give answers to controversial issues (97). In 1995, Lapvet et al (98) examined the effect of running on the occurrence of arthritic changes in C57BL-type mice; 70-80% of which would develop arthritic changes. In this sample group, running led to a rise in the incidence of both moderate and severe arthritic changes, above the normally high incidence observed in this species. This finding underlined the idea that when an inherited pathology is present, be it genetic (91) or otherwise, caution is necessary when planning a training program. Newton et al in 1997 (99) allowed 11 dogs to walk for 75 minutes per day at a pace of three kilometers per hour for 5 days a week while carrying 130% of their body weight. This experiment lasted the dogs' whole life. The joint cartilage, ligaments, menisci and any presence of osteophytes was recorded. The data showed no difference between the study group and the control group. In 1992, Oettmeier et al (100) investigated the joint surfaces of running dogs. The subchondral bone and the joint cartilage

were found to be thicker than those in the control group, with less arthritic changes. In the same year, Helminen (101) and Arokoski (102) examined the joints of dogs that ran uphill for four kilometers per day for a year. The cartilage thickness increased by 3-23% and the proteoglycan content increased by 59%. The increase in the cartilage thickness and the concentration of glycosaminoglycans was also demonstrated by Kiviranta et al (103) in his assessment of dogs that ran 15 kilometers per day for 40 days. By over-training the dogs (20 or even 40 Km per day) the positive effect on the patella was maintained, with the increase in both cartilage thickness and proteoglycan concentration, no changes were noted in the medial compartment of the knee, and the lateral compartment suffered a drop in proteoglycan concentration with softening of the joint cartilage.

When using dogs as a model for osteoarthritis it should not be forgotten that dogs tend to develop early arthritic changes when they become ACL deficient, although they tend to do relatively well after meniscal repair or cartilage damage. Thus the dog model may not necessarily be the best model for osteoarthritis research.

In 1981, Palmoski (104) demonstrated in a canine model that commencing an intensive physical program without prior preparation, could damage the joint cartilage, as could excessively overloading cartilage which has degenerated due to unloading. However, when a gradual training program was used in young rabbits, the proteoglycan content increased, (105-106) the cellular activity was found favorable and cellular degeneration was reduced in running rabbits (107).

In 1998, Otterness et al (108) published their outstanding work done on hamsters: Joints of six young hamsters were compared to joints of 12 old sedentary hamsters and 12 old active hamsters. The cartilage on the femur was found to be smooth in the young hamsters and in the 12 old running hamsters, but was fibrillated and roughened in the 12 sedentary hamsters. The authors concluded that a sedentary lifestyle leads to a decrease in the proteoglycan content, decrease in the amount of synovial fluid, and to fibrillation and microscopic fractures of the joint cartilage.

DISCUSSION AND CONCLUSIONS

High impact physical activity, which entails sharp turns (i.e. rotational forces) and brakes, imposes great strain on the joints of the lower limbs, especially the knee and hip. These forces may traumatize the joint, change its stability by causing acute damage to the ligaments, or by modification of the anatomy through damage to the meniscus, cartilage or subchondral bone. These changes will lead to arthritic changes of the damaged joint. The literature implies that even in a situation where there are no obvious or "known" injuries, the incidence of degenerative changes in a joint that encounters high impact physical activity would be higher than the incidence in the general population who do not perform such activity. Even so, many argue that if the injury (solitary or multiple) could be avoided, one would not expect to see either early or late onset arthritic changes in the joints of the lower limbs.

The supporting evidence showing the development of arthritic changes in the lower limb joints; ankle, knee, or hip, as a result of low impact continuous sport, such as running, walking or cycling, has not been established. Even though some strongly supported studies indicate possible changes, either radiological alone or radiological and clinical, in particular concerning the knee or the hip, a significant number of studies did not detect any link between the activity and the arthritic damage, either radiologically or clinically, and a number of studies further revealed a protective effect on the joint cartilage.

In vivo animal studies, which are designed to allow the collection of objective data, which are far more accurate and better controlled than clinical observations in human beings, reveal the positive effect that repetitive low impact activity, such as running, has on the joint and the joint cartilage as long as the joint is not genetically impaired, is anatomically intact and is biomechanically balanced. In addition, the activity must be gradual, continuous, and not aggressive or excessive. When the animal being studied is exposed to activity for which it is not prepared, an activity of unusual intensity or an activity that exerts undue forces on degenerated or damaged joint cartilage for any reason including lack of use, it may very well be that the positive effect of

the activity will become a negative one and this will damage the joint.

There is borderline, though possibly existing evidence, to suggest that high impact activity may damage an intact joint in which no previous injury has occurred and in which there is no anatomical or genetic deficit. In the majority of joints showing arthritic changes, previous history reveals a single or recurring injury, and the damaged joint demonstrates arthritic changes which are secondary to that damage.

The evidence that repetitive exercise, such as running, walking or cycling, will damage a joint in which there is no anatomical, biomechanical or genetic abnormality, does exist but is controversial, and at best weak and unconvincing. This is true for gradual, non-explosive, non-intensive, and not out-of-the-ordinary activity for that particular individual.

Evidence showing improvement of joint structures by physical activity is today clearly evident, both in human subjects as well as in the animal model.

Methods of diagnosis of osteoarthritis are obviously evolving and the criteria for radiological diagnosis are changing and may not always concur with the evolving modern methods of cartilage mapping on MRI. New generation MRI machines may highlight changes in water content and the basic physical or chemical constitution of the joint cartilage and sub-chondral bone, long before traditional radiographic criteria for osteoarthritis reveal damage. Future research may illustrate the significance and value of these new modalities.

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REVIEW ARTICLE

SMOKING-RELATED MUSCULOSKELETAL DISORDERS

R.S. KUMAR and V.B. SELVAN

*Department of Orthopedics, Faculty of Medical Sciences, Madras University, Chennai, India**Received August 25, 2008 – Accepted January 8, 2009*

Cigarette smoking is the most preventable cause of premature death in this country and is responsible for one in five deaths. Millions of other people are living with serious illnesses caused by smoking. Smoking has been linked with many health problems including an array of orthopedic conditions and complications. In the past, there have been many individual reports that deal with these relationships separately but very few published comprehensive reviews. The aim of this study is to review the available literature to find out the effects of smoking on the musculoskeletal system and the subsequent orthopedic problems. We have used predominantly online facilities such as PubMed, Orthogate, and other orthopedic web links, as well as Google, Yahoo, and AltaVista search engines for this study. Papers in English have been quoted in the present review. The time period of the review goes from 1976 to 2005. All internationally-indexed journals with articles on smoking-related musculoskeletal disorders were used. The majority of articles were chosen from journals with good impact factors. A detailed discussion about smoking-related orthopedic problems is presented. There is a real and reproducible relationship between smoking and disease of the musculoskeletal system; therefore, smoking cessation helps our bodies regain their normal health. Smokers should discuss smoking cessation strategies with their physicians.

Tobacco smoking is the second major cause of death in the world. It is currently responsible for the death of one in ten adults worldwide (about 5 million deaths each year). If current patterns continue, smoking will be causing some 10 million yearly deaths by 2020. Half of the 650 million people that smoke today will eventually be killed by tobacco. Cigarette smoking continues to impose substantial health and financial costs on individuals and society. It's in everyone's best interest to prevent and reduce tobacco use.

Cigarettes contain 109 known chemicals, including nicotine, benzene, and tar which are harmful. Nicotine has a significant negative role on the musculoskeletal system. Nicotine in tobacco

products causes peripheral vasoconstriction and tissue ischemia and decreases oxygen tension. Moreover, nicotine depresses osteoblast activity, may inhibit revascularization of bone graft (1), has a negative impact on bone healing (2), and inhibits the expression of a wide range of cytokines including those associated with neovascularization and osteoblast differentiation (3). It increases the risk of bone fractures, reduces estrogen effectiveness, and can counter the antioxidant properties of vitamins C and E.

The objective of this study is to review the available literature to find out the effects of smoking on the musculoskeletal system and the subsequent orthopedic problems.

Key words: smoking, musculoskeletal disorders, osteoporosis, arthritis, fracture

Mailing address:

Dr VB Selvan
50, North Car Street,
600108 Vadapalani, Chennai, India
e-mail: dr_vbs@rediffmail.com

1973-6401 (2009) Print

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Smoking and osteoporosis

Lowering of bone mineral density in smokers was shown 20 years ago (4). Post-menopausal women who smoke lose significantly more cortical bone and have more spinal osteoporosis than non-smoking counterparts. Cigarette smoking may increase bone resorption at the fracture ends (5,6). In addition, smoking may negate the protective skeletal effects of estrogen replacement therapy (7). Smoking also lowers bone mineral density and results in decreased calcium absorption associated with secondary hyperparathyroidism and increased bone resorption (8). Subsequent studies have also demonstrated a direct relationship between tobacco use and decreased bone density. In addition, a relationship between cigarette smoking and low bone density in adolescence and early adulthood has been identified.

In order to elucidate the influence of nicotine smoking on bone mass in elderly women, bone mass was assessed by dual energy X-ray absorptiometry (DXA) in total body, hip and lumbar spine, as well as with ultrasounds of the calcaneus and phalanges of the hand. Gerdhem concluded that nicotine smoking has a negative influence on bone mass independent of differences in weight and physical activity (9).

It has been found that while estrogen replacement protects women from fracture, this protective effect is eliminated in women who smoke. The results support an anti-estrogenic effect of cigarette smoking that is consistent with the conclusions of other researchers. Other reports have suggested that smokers have less effective calcium absorption, opposite to the effect of estrogen, which is believed to enhance calcium absorption. The anti-estrogen effect of tobacco may help explain the increased risk for osteoporosis among female smokers. Postmenopausal smokers have lower estrogen levels than non-smokers, and smokers tend to reach menopause earlier than their non-smoking counterparts. This reduction in estrogen is likely to result in an increase in bone resorption, contributing to osteoporosis.

In men, smoking at any stage of life has adverse effects on the skeleton that was independent of weight, alcohol or caffeine use, implying other mechanisms of effect on bone. Among men, a consistently lower bone mineral density (BMD) at all skeletal sites was observed for smokers regardless of when in

their life they smoked, whereas in women who had used estrogen, BMD was lower in current and recent smokers than it was in non-smokers. Studies have shown that smoking reduces the blood supply to bones and that nicotine slows the production of bone-forming cells (osteoblasts) and impairs the absorption of calcium. With less bone mineral, smokers develop fragile bones (osteoporosis).

Smoking and risk of fracture

While the first suggestion of an association between tobacco smoking and osteoporosis was published in 1976 (4), several studies have examined the effect of tobacco smoking on bone mineral density and fracture risks. Smoking is widely considered a risk factor for future fractures. Several biological effects of tobacco may influence the risk of fractures in smokers. Smoking may exert adverse effects on bone strength through direct toxicity of nicotine and non-nicotine components of cigarette smoke on bone cells, as demonstrated *in vitro* (10-11). Further, smoking may indirectly affect bone strength through decreased intestinal calcium absorption (12), increased metabolism, decreased production of estrogen (13), or hypercortisolism (14). In addition, smoking may influence the fracture risk through other mechanisms unrelated to osteoporosis, such as poorer balance and physical performance due to neurovascular and peripheral vascular deleterious effects of smoking (15). Nicotine can counteract the antioxidant effects of vitamins C and E and lead to a significantly higher risk of bone fracture. Recent meta-analyses on the effects of smoking on the bone revealed that current smokers sustained decreased bone mass and increased fracture risk at 50 years of age and older. These relationships remained significant after adjusting for the effects of age, years since menopause, and body weight, regardless of sex. The raised risk of fractures was observed to be consistent at all sites (16). Ex-smokers showed an intermediate risk between those of non-smokers and current smokers, which implies the beneficial effect of smoking cessation (17-18).

It has recently been indicated that intrauterine exposure to tobacco smoke retards skeletal growth resulting in increased risk for fracture later in life, though this needs to be confirmed by further studies (16). It is estimated that smoking increases

the lifetime risk of developing a vertebral fracture by 13% in women and 32% in men. Kanis, et al. quantified the risk of smoking on an international basis and explored the relationship of this risk with age, sex, and bone mineral density (BMD) (19). Risk ratios were significantly higher in men than in women for all fractures. Smoking is estimated to increase the lifetime risk of hip fracture by 31% in women and 40% in men (18). Cigarette smoking is a risk factor for hip fracture among postmenopausal women; this risk decreases after smoking cessation (20). A meta-analysis based on these studies recently concluded that postmenopausal bone loss is greater in smokers than in non-smokers and that tobacco smoking increases the lifetime risk of hip fracture in women by about 50% (21). A similar adverse effect of smoking is suspected to be present in men, and recent bone mineral density studies have raised the concern that men may be more sensitive to the deleterious effect of smoking on bone than women (22-23). Smoking cessation reduces the risk of hip fracture in men after 5 years, while the deleterious effect of smoking seems to be more long-lasting in female ex-smokers. The association between BMD, smoking, and risk of fractures was studied (20), and low BMD accounted for only 23% of the smoking-related risk of hip fracture. Adjusting for body mass index had a small downward effect on risk statistics for all fracture outcomes. For fractures associated with osteoporosis, the risk ratio increased with age, though not for hip fractures. Current smokers run lower risks compared to chronic smokers. While the association between tobacco use and decreased bone density is fairly strong, most studies suggest at least a slight association between cigarette smoking and fractures, especially hip and vertebral fractures.

It appears that smoking has an independent, dose-dependent effect on bone loss, which increases the risk of fractures, and may be partially reversed by smoking cessation. Given the public health implications of smoking, it is important that this information be included in smoking prevention and cessation campaigns (24).

Smoking and healing problems

Current data show associations between smoking and a number of complications of the fracture healing process. Current smokers are more than twice as likely

to develop an infection and develop osteomyelitis. Ex-smokers are more likely to develop osteomyelitis but are at no greater risk for other types of infection. It is concluded that smoking places the patient at risk for an increased time to union and complications. A history of smoking appears to increase the risk of osteomyelitis and the time to union (25)

Smoking decreases unions, slows healing (26-28), and increases complications such as the rate of flap failure (29-30). In one study smokers' time to union was significantly longer, and they suffered more complications. Smokers had a 4.1-fold risk of tibial shaft fracture caused by low-energy injury compared with non-smokers (31). An accelerated failure time model showed that the more comminuted or open the fracture, the higher the number of cigarettes smoked, and the older the patient, the more time was needed for the clinical union of the tibia shaft fracture. Females appeared to run further risks for delayed healing in this group (26). Smoking is negatively associated with the healing of open tibia fractures. In smoking patients, the time until consolidation is on average significantly longer and more osteitis is suffered. Smokers remain longer in the hospital and undergo more re-operations (32).

The pathophysiological effects of smoking are multidimensional, including arteriolar vasoconstriction, cellular hypoxia, demineralization of bone, and delayed revascularisation. Nicotine seems to play a significant role in causing a lack of oxygen in the tissues. Increasing amounts of research link smoking to impaired healing of fractures. It is felt that smokers have a significant deficiency of oxygen (hypoxia) at the cellular level where the fracture is trying to heal. Fractures heal normally in areas which have a good blood supply and an adequate oxygen supply in the region of the fracture. Microvascular and trauma surgeons have documented the adverse effects of smoking in the healing of skin flaps and the increased complication rates in the treatment of nonunion. In addition, spine surgeons have noted the adverse effects of smoking in fusion rates (33). An increased rate of pseudarthrosis has been documented following posterolateral lumbar spine grafting in patients who smoke (34). Smoking has a significant negative impact on healing and clinical recovery after multilevel anterior cervical decompression

and fusion with autogenous interbody graft for radiculopathy or myelopathy (34).

A review of the smoking habits in 426 patients who had been followed prospectively for 2 years after a lumbar spinal fusion procedure was conducted. The aim was to analyze the effects of pre- and postoperative smoking on clinical and functional outcomes after a lumbar spinal fusion. Smoking was shown to have a negative effect on fusion and overall patient satisfaction. Smoking cessation increased fusion rates near to those of nonsmokers or ex-smokers (35).

In a study on spinal fusions in the lower back, the success rate was 80-85% for patients after their surgery. The success rate dropped to under 73% for smokers. More than 70% of non-smokers and ex-smokers were able to return to work, but only about half of the current smokers were able to resume working. Another study on spinal fusions in the neck showed successful fusion in 81% of non-smokers but in only 62% of smokers. Nicotine appears to have affected more than just local areas (3) because nicotine inhibits the expression of a wide range of cytokines, including those associated with neovascularization and osteoblast differentiation.

Problems have also been observed in another orthopedic procedure in which a bone graft fuses a particular area of the body. Examples of this could be the fusion of an arthritic ankle joint with joints in the foot (36-38) or in the hand. In fact, some orthopedic surgeons have insisted that patients stop smoking before elective bone graft surgery is done. These surgeons feel that the incidence of delayed healing of bone graft procedures is so high that it would not be worth proceeding with the bone graft surgery while the patient is still smoking. This delayed healing process in smokers has been observed by orthopedic surgeons in almost every type of fracture. Nicotine has been shown in previous studies to decrease production of fibroblasts (the main cells responsible for tissue repair). In addition, the carbon monoxide found in tobacco smoke reduces cellular oxygen tension levels, which are vital for cellular metabolism and tissue healing (39).

To investigate the success of exchange reamed femoral nailing in the treatment of femoral nonunion after intramedullary (IM) nailing and to analyze factors that may contribute to the failure of exchange

reamed femoral nailing, Hak David and coauthors did a retrospective study among smokers and nonsmokers. They found a detrimental impact on the success of exchange reamed nailing in smokers. All eight of the non-smokers healed after exchange reamed nailing, whereas only ten of the fifteen smokers (66.7%) healed after exchange reamed nailing. Tobacco use appears to have an adverse effect on nonunion healing after exchange reamed femoral nailing (40).

McKee et al. have done a retrospective review of 84 adult patients (86 limbs) who underwent Ilizarov reconstruction to determine the effect of smoking on outcome and complication rates. There was a higher incidence of nonunion in the smoking group. They concluded that smokers had a higher percentage of poor results due primarily to higher complication rates (41). Smoking is a significant, potentially remediable risk factor for failure following Ilizarov reconstruction, and cessation strategies are of paramount importance prior to initiating treatment.

In one other study, smokers had a significantly higher rate of nonunion than did non-smokers (18.6% vs. 7.1%). However, smoking cessation seems to improve the healing process in most cases, except for long-term, heavy smokers who have permanent artery damage due to smoking. Delayed or impaired healing of skeletal trauma in patients who smoke has been attributed to vascular responses of nicotine absorption and/or the direct effect of nicotine or other smoke components on bone cells. *In vivo* studies indicate variability in the osteosynthetic response to nicotine versus smoke inhalation. It has been hypothesized that components of cigarette smoke other than nicotine may be responsible for the adverse skeletal effects of smoking.

The negative effects of smoking have gained increased interest among plastic and microvascular surgeons because smokers have been shown to suffer higher rates of flap failure, tissue necrosis, and hematoma formation. In particular, smokers presenting with an open tibial fracture will suffer the negative effects of their smoking habit because these fractures are inextricably connected with soft-tissue injury. Their fractures will need a significantly longer time to heal than those of non-smokers and will have a higher incidence of nonunion. If microvascular surgery is to be performed,

persistent smoking significantly increases the rate of postoperative complications, with wound infection, partial flap necrosis, and skin graft loss being most common (42).

Smoking cessation has both short- and long-term beneficial effects. Nowadays, there is sufficient evidence to insist that patients with an open tibial fracture immediately refrain from smoking to promote bone healing and to lower the complication rate. In the case of elective reconstructive procedures, patients should refrain from smoking at least 4 weeks before surgery. In both situations, cessation should continue during the entire rehabilitation period.

Smoking and increased morbidity

Smoking has been shown to increase morbidity and mortality in surgical procedures. Smoking is a significant risk factor for the development of postoperative pulmonary complications after major surgical procedures. Moller et al. studied 811 consecutive patients who had undergone hip or knee arthroplasty, recording smoking and drinking habits as well as any postoperative complications occurring before discharge from the hospital. They found that smoking was the single most important risk factor for the development of postoperative complications, particularly those relating to wound healing, cardiopulmonary complications, and the need for postoperative intensive care. Delayed discharge from the hospital was usual for those suffering a complication. In those patients requiring prolonged hospitalization (>15 days), the proportion of smokers with wound complications was twice that of non-smokers. (43)

A study was performed to assess the effects of smoking on the incidence of short-term complications, resource consumption, and hospital stay length of patients undergoing arthroplasty of the hip and knee. Patients who smoked were found to have statistically longer surgical times and higher charges adjusted for age, procedure, and surgeon than patients who did not smoke. Patients who smoked also had longer anesthesia times. Preoperative screening for nicotine use can predict operative time and healthcare resource consumption. The exact reasons why patients who smoked had higher hospital charges remain elusive. Probable reasons include the higher degree of operative

complexity (severity of illness). In addition, the ex-smokers had a better short-term outcome than the patients who were currently smoking. This indicates the importance of smoking abstinence before joint replacement surgery and other surgical procedures. Regardless of the exact causes, it is more expensive to treat patients who smoke; therefore, contracting for orthopedic care should include a history of smoking (44).

According to Lindstrom et al. (45), preoperative cessation of smoking seems to reduce the frequency of complications. Tobacco smokers suffer from postoperative complications after surgery more often than non-smokers. This has been proven in general, orthopedic, and plastic surgery. In recent years, preoperative smoking cessation has been evaluated in several studies. It has been shown that smoking cessation four to eight weeks prior to surgery significantly reduces wound healing complications. There are still some unanswered questions concerning the necessary length of preoperative smoking cessation to affect the complication rate. There is also a lack of evidence concerning smoking cessation before emergency surgeries and the cost-effectiveness of smoking cessation intervention programs. Therefore, further studies on preoperative smoking cessation are needed. Smokers had slightly higher post-treatment self-reported pain and disability ratings, mixed and limited. Overall, there is evidence for the widely-held belief that smoking negatively affects tertiary rehabilitation (46).

Smoking and spinal problems

There is a definite link between smoking and low back pain that increases with the duration and frequency of the smoking (47). In several studies, smoking has been associated with the occurrence of spinal pain--mostly low back pain, but also pain in the neck and prolapsed cervical intervertebral discs (48). Exposure to secondhand smoke during childhood may increase the risk of developing back and neck problems in later life.

Associations between back pain prevalence and lifestyle factors (smoking and obesity) were analyzed. Back pain prevalence rose with increasing levels of smoking, with a relative risk of 1.47 for persons reporting 50 or more packs of cigarettes smoked per year. This association was strongest in

persons under the age of 45 years, for whom the corresponding relative risk was 2.33. (49)

Vogt investigated the association between the smoking status of spinal patients, the duration and severity of symptoms, and the patients' self-reported health statuses. Smokers and non-smokers reported spinal symptoms for similar durations, but the smokers reported more severe symptoms, which were present for a greater proportion of time each day. Also, the smokers had lower physical and mental health status scores than did non-smokers (50).

Numerous studies confirm that smoking is a strong risk factor for back pain and is associated with an increased risk of a prolapsed disc. Several explanations for the association have been proposed. Smoking might provoke disc herniation through coughing or lead to pathological changes in the intervertebral disc through alterations in its nutrition, pH, or mineral content. Another possibility is that smoking has a pharmacological effect on pain perception (51-55).

The most widely-accepted explanations for the association between smoking and disc degeneration is the malnutrition of spinal disc cells by carboxy-hemoglobin-induced anoxia or vascular disease. Nicotine, a constituent of tobacco smoke present in most body fluids of smokers, is known to have detrimental effects on a variety of tissues. It may also be directly responsible for intervertebral disc (IVD) degeneration by causing cell damage in both the nucleus pulposus and annulus fibrosus. Experimental investigation has been done to determine the effect of nicotine on intervertebral spinal disc nucleus pulposus (NP) cells cultured *in vitro*. The study was to evaluate the effects of nicotine on cell proliferation, extra cellular matrix production, and the viability of NP cells. There was significant inhibition of cell proliferation and extra cellular matrix synthesis. Hence nicotine in tobacco smoke may have a role in the pathogenesis of disc degeneration.

Spinal surgeons have shown the adverse effects of smoking in fusion rates (33). An increased rate of pseudarthrosis has been documented following posterolateral lumbar spine grafting in patients who smoke (34). Smoking had a significant negative impact on healing and clinical recovery after multilevel anterior cervical decompression and fusion

with autogenous interbody grafts for radiculopathy or myelopathy (34). A review of the smoking habits in 426 patients who had been followed prospectively for 2 years after a lumbar spinal fusion procedure was conducted. It was to analyze the effect of pre- and postoperative smoking on clinical and functional outcomes after lumbar spinal fusion. Smoking was shown to have a negative effect on fusion and overall patient satisfaction. Smoking cessation increased fusion rates to near those of non-smokers (35)

In a study on spinal fusions in the lower back, the success rate was 80-85% for patients who never smoked or who quit smoking after their surgeries. The success rate dropped to under 73% for smokers. More than 70% of non-smokers and ex-smokers were able to return to work, but only about half of the current smokers were able to resume working. Another study on spinal fusions in the neck showed successful fusion in 81% of nonsmokers, but in only 62% of smokers. The effects of nicotine appear to have more than just local effect because nicotine inhibits the expression of a wide range of cytokines, including those associated with neovascularization and osteoblast differentiation (3).

Smoking and inflammatory polyarthropathies

Anecdotal suggestions and retrospective studies indicate an inverse relationship between the incidence of osteoarthritis and individuals who smoke. As a possible explanation, studies confirm that nicotine up-regulates glycosaminoglycan and collagen synthetic activity of articular chondrocytes at physiological levels seen in individuals who smoke (56). Rheumatoid arthritis (RA) is considered to be a multifactorial disease, resulting from the interaction of both genetic and environmental factors, which contribute to its occurrence and expression. The main genetic risk factor for RA is the shared epitope (SE) of HLA-DR, while smoking is an important environmental risk factor particularly for rheumatoid factor (RF) positive RA (57). The disease risk of RF-seropositive RA associated with one of the classic genetic risk factors for immune-mediated diseases (the SE of HLA-DR) is strongly influenced by the presence of an environmental factor (smoking) in the population at risk (58), and there is also a strong association between smoking and rheumatoid nodules in early seropositive RA. (59). Smoking also

seems to influence the disease outcome. Data suggest that the disease outcome in female RA patients with a history of smoking is significantly worse than in those who have never smoked. According to a recent study, maternal smoking in pregnancy is regarded as a determinant of rheumatoid arthritis and other inflammatory polyarthropathies during the first 7 years of life. There is a potential effect of fetal exposure to tobacco smoke on the risks of RA and IP (inflammatory polyarthropathies) and juvenile rheumatoid arthritis in girls (60). Gender interacts with smoking by an unknown mechanism leading to a differential risk of RA (61).

Smoking and soft tissue problems

Cigarette smokers have an increased risk of rotator cuff tears; a strong relationship was found between smoking and history of rotator cuff tear. Rotator cuff (shoulder) tears in smokers are nearly twice as large as those in non-smokers.

Smoking also has a negative impact on surgeries involving muscles, such as rotator cuff repairs (62). One study compared the results of 235 patients treated at two different medical institutions. Results in non-smokers were significantly better than results in smokers. Non-smokers experienced less pain and higher degrees of function after surgery than smokers.

Dupuytren's contracture is a deforming, fibrotic condition of the palmar fascia. The cause of Dupuytren's disease is still unknown in spite of significant recent advances in identifying the type of cell responsible for initiating the process (63). Smoking is linked statistically to Dupuytren's disease and may be involved in its pathogenesis by producing microvascular occlusion and subsequent fibrosis and contracture (64).

Miscellaneous

Perthes' disease is an idiopathic necrosis of the capital femoral epiphysis, and the inhalation of secondhand smoke is a significant factor. The risk of Legg-Calve-Perthes disease (LCPD) in children exposed to smoke is more than five times higher than in children who are not exposed. It seems that secondhand smoke is a factor either directly or indirectly associated with LCPD (65). Parents who smoke at home put their children at risk of

developing LCPD. Statistical analysis shows an extremely strong association between smoking in the home, and the presence of secondhand smoke seems to be a significant risk factor in the development of LCPD (66). Secondhand smoke exposure while *in utero* and during childhood appears to lower stimulated tissue plasminogen activator activity and, additionally, may depress heritable low stimulated tissue plasminogen activator activity, leading to hypofibrinolysis. Hypofibrinolysis may facilitate thrombotic venous occlusion in the head of the femur, leading to venous hypertension, hypoxic bone death, and LCPD (67).

There are known associations between alcohol intake, cigarette smoking, occupation, and other factors with the development of idiopathic osteonecrosis of the femoral head (68-70).

Smoking has a detrimental effect on athletic performance. Because smoking slows lung growth and impairs lung function, there is less oxygen available for muscles used in sports. Smokers suffer from shortness of breath almost three times more often than non-smokers. Smokers cannot run or walk as fast or as far as non-smokers.

CONCLUSIONS

Experience has shown that there are many cost-effective tobacco control measures that can be used in different settings and that can have a significant impact on tobacco consumption. The most cost-effective strategies are population-wide public policies, like bans on direct and indirect tobacco advertising, tobacco tax, price increases, smoke-free environments in public settings and workplaces, and large, clear, graphic health messages on tobacco packaging. Smoking has been linked with many health problems; surgeons have long known about the relationships that putatively exist between smoking and an array of orthopedic conditions and complications. Although scientific and clinical information on smoking and its consequences suggests differing degrees of correlation between smoking and orthopedic conditions, most available data do suggest a real and reproducible relationship. In the past, there have been many individual reports that deal with these relationships separately but very few published comprehensive reviews. This

summary of the current literature regarding the relationship between smoking and musculoskeletal diseases provides information that can be used clinically. Every tissue in the human body is affected by smoking, but many effects are reversible. Avoiding or quitting smoking can reduce the risk of incurring many conditions.

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CLINICAL STUDY

THE USE OF DIGITISED RADIOGRAPHS IN DETERMINING THE CONSISTENCY OF THE AO AND FRYKMAN CLASSIFICATIONS OF FRACTURES OF THE DISTAL RADIUS.

A.F. MCGRATH, I.R. STEVENSON, I. MCFADYEN¹ and A.J. JOHNSTONE²

Royal National Orthopaedic Hospital, Stanmore; ¹Gloucestershire Royal Hospital; ²Robert Gordons University, Aberdeen, United Kingdom

Received November 5, 2008- Accepted July 20, 2009

For any system used to classify fractures, high levels of intraobserver reproducibility and interobserver reliability are desirable. We compare the consistency of the AO and Frykman classifications of fractures of the distal radius with an assessment of the digitized radiographs of 100 fractures by 15 orthopedic surgeons and 5 radiologists using a Picture Archiving and Communications System (PACS), allowing manipulation of the image. This process was repeated 1 month later. Intraobserver reproducibility was moderate for both the AO and Frykman systems, while interobserver reliability was only fair for both the AO and Frykman systems. In each case, reproducibility using the Frykman system was slightly greater. The assessor's level of experience and speciality was not seen to influence accuracy. The ability to electronically manipulate images does not appear to improve reliability over the use of traditional hard copies, and their sole use in describing these injuries is not recommended.

Distal radius fractures are common, accounting for approximately one sixth of all fractures, and are the most commonly occurring fractures in adults. With the expected expansion of the aging population, their incidence is likely to increase.

If a radiological fracture classification is to serve as a useful discriminator (1), creating standards by which treatment can be recommended and outcomes compared (2), it should ideally be easy to use and have a high degree of both interobserver reliability and intraobserver reproducibility (3).

The multitude of eponyms relates to fractures of the distal radius, Colles (4-5), Barton (6), Smith (7), Pouteau (8) and Goyrand among them, each of whom describes one or more specific features

characterized by clinical evaluation or laboratory dissection, demonstrating the difficulty in formulating a comprehensive and useable system for these injuries.

While the Frykman (9) classification is the most popular, it is limited in that it does not quantify displacement, shortening, or the extent of comminution. The more comprehensive AO system (10-11), while useful as a reference, is limited in its complexity with 27 possible subdivisions.

Computerized tomography has been shown to give only marginal improvement in the consistency of classification in published literature (12-13), we explore the effect of electronic manipulation of radiographs alone.

Key words: distal radius fractures, Frykman Classification, AO Classification, interobserver, intraobserver reliability, consistency

Mailing address: Mr. A.F. McGrath,
Royal National Orthopaedic Hospital,
Stanmore,
HA7 4LP United Kingdom
Tel: ++44 790 4976790
Fax: ++44 0208 9095273
e-mail: anthonymcgrath@nhs.net

1973-6401 (2009) Print

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MATERIALS AND METHODS

Radiographs of 100 fractures of the distal radius were selected to represent a spectrum of fracture configuration and severity. These included an anteroposterior and a lateral view for each fracture, standardized with regards to positioning of the wrist, distance, and direction of the X-ray, and radiographic technique. 15 orthopedic surgeons and 5 radiologists were recruited as assessors; 5 were specialist registrars (surgeons in training) in trauma and orthopedics, and the others were consultants in their respective specialties.

Each assessor was given a printed description of the Frykman (Table I, Fig. 1) and AO (Table II, Fig. 2) classifications for use during the assessment and a short lecture on each system.

Computerised Radiography and PACS

P- Picture viewing at diagnostic, reporting, consulting and remote workstations

A - Archiving images using short or long-term storage devices.

C- Communications using local or wide area networks or public communication services.

S- Systems that include modality interfaces and gateways.

Computerised radiographs were obtained by using photostimulable phosphor plate radiography. Plates (Agfa-Gevaert, Mortsel, Belgium) 24 x 30 cm in size were read in a compact digitizer (Pathspeed MP3010; GE Medical Systems) with a matrix of 2048 x 2577 pixels.

Digital images were postprocessed at a quality assurance workstation (Pathspeed VIPS; GE Medical Systems) with proprietary software (Mimosa 1.1.06; Agfa-Gevaert). These 12-bit images were sent to the PACS short-term storage device (a redundant hard-disk array) and to the optical disk archive (Pathspeed, version 8.1; GE Medical Systems) and retrieved as 12-bit images by means of a commercially available Web browser (Pathspeed Web, version 8.1; GE Medical Systems). The workstation (Pathspeed 2A; GE Medical Systems) consisted of a computer (Pentium II; Intel, Santa Clara, Calif) that was running at 400 MHz, with 512 MB synchronous dynamic RAM. The display was calibrated and controlled by using proprietary software (MediCal Pro, version 2.02.04; Barco Medical Imaging Systems). Measurement was performed with a narrow-angle luminance meter (Minolta LS-100; Konica Minolta, Tokyo, Japan) with a display of a Society of Motion Picture and Television Engineers test pattern, these monitors had a maximum luminance of 311 and 312 candelas (cd) per square meter.

Radiographs could be manipulated digitally, including size, contrast, brightness, orientation, and negative image display. Intra- and inter-observer reproducibility was analyzed using Kappa statistical methods. A comparison was then made between the reproducibility of radiologists and surgeons, and then between that of consultant orthopedic surgeons and trainees. We did not assess accuracy, as that would require a definitive classification for each fracture. It is important to note that agreement does not necessarily reflect accuracy.

Table I

Type I:	Extraarticular
Type II:	Type I with ulnar styloid fracture
Type III:	Involvement of the radiocarpal joint
Type IV:	Type III with ulnar styloid fracture
Type V:	Involvement of the distal radioulnar joint
Type VI:	Type V with ulnar styloid fracture
Type VII:	Involvement of the radiocarpal and radioulnar joints
Type VIII:	Type VII with ulnar styloid fracture

Table II

A1 extra-articular fracture of ulna, radius intact
A2 extra-articular fracture of radius, simple and impacted
A3 extra-articular fracture of radius, multifragmentary
B1 partial articular fracture of radius, sagittal
B2 partial articular fracture of radius dorsal rim
B3 partial articular fracture of radius, volar rim
C1 complete articular fracture of radius, articular simple, metaphyseal simple
C2 complete articular fracture of radius, articular simple, metaphyseal multifragmentary.
C3 complete articular fracture of radius, multifragmentary

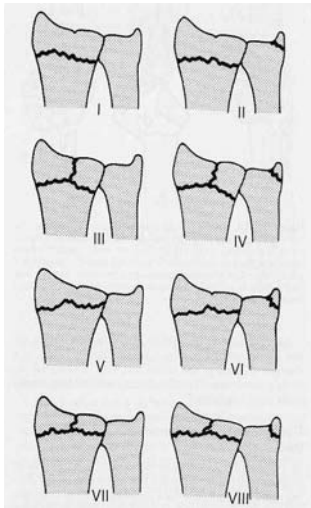


Fig. 1

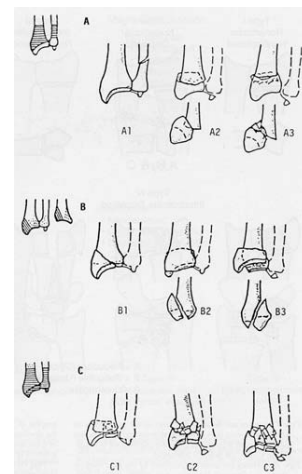


Fig. 2

Statistical methods

The kappa coefficient for multiple raters was calculated according to the guidelines proposed by Landis and Koch (14-15). Values less than 0.00 indicate poor reliability; between 0 and 0.2 represent slight agreement; 0.21 to 0.4 fair; 0.41 to 0.60 moderate; and 0.61 to 0.8 substantial agreement. Values above 0.8 are considered excellent or almost perfect. This analysis involves adjustment of the observed proportion of agreement between observers by correction for the proportion of agreement that could have

occurred by chance. Kappa coefficients for agreement among the orthopedic surgeons were compared to those for the 5 radiologists using the Student t-test that incorporated the standard errors of kappa for these 2 groups. A similar method was employed to compare the performance of orthopedic consultants to trainees.

RESULTS

One assessor claimed to regularly use the AO

Classification, while 20 were “familiar” with it. All 25 assessors reported familiarity with the Frykman Classification, and 10 had used it regularly. 14 assessors denied routinely using any system. We did not perform category-specific testing for the expanded classification of groups or subgroups within the AO system.

Median interobserver reliability was fair for both the AO ($\kappa = 0.31$, range 0.2 to 0.38) and Frykman ($\kappa = 0.36$, range 0.30 to 0.43) systems. Median intraobserver reproducibility was moderate for both the AO ($\kappa = 0.45$, range 0.42 to 0.48) and Frykman ($\kappa = 0.55$, range 0.51 to 0.57) systems. In each case, the Frykman system was statistically ($p < 0.01$) more accurate. Level of experience and specialty were not seen to influence accuracy ($p < 0.01$).

DISCUSSION

Classification systems for fractures are extremely important in the practice of trauma and orthopedics. They are a means by which fractures, and fracture dislocations may be described, and they provide important guidance on treatment and the results of treatment.

The Bone and Joint Committee of the prestigious International Forum for Social Sciences and Health failed at successive congresses to reach a consensus as to which existing classification system best describes fractures of the distal radius. Ideally, this would distinguish groups that behave in predictable ways, require certain treatments, and parallel clinical outcomes. This system would ideally have a high degree of reproducibility.

Existing systems are based on 4 general characteristics (16): the extent of comminution (17-18), the radiographic appearance or degree of fracture displacement (19-20), articular joint involvement (21-23), and the mechanism of injury (24-25).

Our analysis comparing the Frykman and AO systems is, to our knowledge, the largest group of assessors reviewing the most radiographs reported in literature. This was done using digitized radiographs allowing the manipulation of images as described. At best, the interobserver error in the popular Frykman classification was only fair. As is typical of comparative studies, intraobserver reproducibility

scored higher as it reflected reproducibility independent of agreement. Incorrect responses that are repeated will show good intraobserver reproducibility but often low interobserver reliability.

Our results demonstrate that using classification systems in isolation to determine treatment and compare results following treatment cannot be recommended (26). Interestingly, this is consistent with classification systems designed to quantify injuries at other anatomical sites (27-32), highlighting the need for caution in interpreting clinical outcomes for treatment of injuries at these sites.

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CLINICAL STUDY

LONG-LASTING EFFECTS OF PROPRIOCEPTIVE TRAINING ON BALANCE IN ELDERLY WOMENO. BRUNETTI, R PANICHI, G. CERULLI¹, F.M. BOTTI and V.E. PETTOROSSO*Department of Internal Medicine, Section of Human Physiology; ¹Department of Orthopedics and Traumatology, University of Perugia, Perugia, Italy**Received October 9, 2008 – Accepted July 20, 2009*

The influence of physical exercise on the body's balance, flexibility and leg power was studied in two groups of elderly women trained with different cycloergometers: a circular cycloergometer for resistive exercise and an elliptical one for proprioceptive and resistive exercise. The subjects were tested during training (21 weeks) and detraining (21 weeks) periods. Their balance, as shown by stabilometric indexes (ellipse area and mean velocity of center of pressure), tended to improve during training in both groups, but the effect of proprioceptive exercise was greater than that of resistive. In addition, the enhancement of balance control in the elliptical cycling group remained throughout the detraining period, while it faded away in the circular cycling group. On the contrary, leg power and flexibility showed only a transient improvement in both groups. This result indicates that elliptical cycling can be useful for an efficient balance training, implying specific learning in the central circuits controlling posture.

Human maximal aerobic power, muscle mass, strength, power and trunk flexibility all decrease with age, while fat mass increases as a result of generalized functional and structural degradation in elderly people (1-4). Moreover, the capacity for explosive force production declines drastically with increasing age (5). Associated with these changes, there is a reduction in balance control (6-9) that causes a tendency to fall (10-15), severely limiting the number of possible activities in daily life. Besides the muscle performance and body flexibility degradation, the central nervous system deterioration (12, 16-20) may also be relevant to equilibrium. Elderly people naturally tend to restrict and slow down body movements so that they become disaccustomed rapid body movements and abrupt perturbations. It has been suggested that specific

balance training could be useful for enhancing balance control, but this may be challenged by the reduction of plasticity in the central nervous system. However, most of the experimental studies seem to suggest that it is still possible to enhance the balance control in elderly people by training them with proprioceptive exercises (21-25). These studies do not clearly show whether the effect is due to proprioceptive learning or to muscle power and body flexibility improvement, and, most of the studies showed that all effects fade away during detraining (26-33).

Therefore, the purpose of this study is to find out if specific balance exercises, implying intense visuo-vestibular and proprioceptive system activation, could induce long-lasting enhancement in the efficacy of equilibrium control. Since balance depends on

Key words: balance training, cycloergometers, proprioceptive exercise

Mailing address: Dr. Vito Enrico Pettorossi
Section of Human Physiology,
University of Perugia,
Via del Giochetto,
06100 Perugia, Italy
Tel: ++39 075 5857376 Fax. ++39 075 5857371
e-mail: vitopett@unipg.it

multiple sensory systems (visual, vestibular, and somatosensory), training enhances the efficiency of these sensory channels may be useful for inducing efficacious proprioceptive learning.

To this purpose, we examined the effects of training exercises in the postural stability of healthy elderly women. Two different types of cycloergometers were used: a circular cycloergometer requiring only resistive work and an elliptical cycloergometer requiring continuous postural adjustments. In order to compare the effects of the different exercises on physical performance and balance control, we studied leg power, flexibility, and balance indexes by measuring the ellipse area and velocity of the center of pressure.

MATERIAL AND METHODS

Subjects

This study was a double-blind randomized controlled clinical trial conducted on twenty four voluntary and sedentary women over 60 years recruited from the University of the Third-Age of Perugia. Sedentary living was defined using PASE, a self-administered questionnaire for the elderly. Our experimental protocol was designed according to the Helsinki declaration (1964) and approved by the local ethical committee. All subjects included in the study gave their informed consent. Treatment data collection and evaluation were performed by two different teams. Those who supervised the training session and those who performed the analysis were unaware of the research expectation and were blinded from the study assignment.

The exclusion criteria for this study were the presence of cardiovascular, respiratory, neurological, or musculoskeletal pathologies, and/or other chronic conditions. To be eligible for the study, participants could not be taking any pharmacological therapy which is known alter movement or space perception.

Participants were randomly assigned to one of two groups, each formed by twelve women. The two groups were sufficiently homogeneous in age, height, weight, flexibility, leg power, heart rate, mean velocity, and ellipse area of the center of pressure (Table I). No statistical differences were observed between the groups. The groups were also similar in terms of diet and level of habitual activity, as ascertained by a specific questionnaire. All of the subjects continued their normal daily activities and diets without introducing any extra form of physical training; this was controlled by periodical phone interviews.

Three subjects dropped out, two from group EC and one from CC, after the second week due to the onset of cardiovascular and respiratory problems, so they are not included in the test. The homogeneity of groups was maintained after excluding these subjects ($P > 0.05$).

Exercises

One group performed exercises with a circular cycloergometer (ProForm 890 Performance, ICON Health & Fitness) (CC), and the other group with an elliptical cycloergometer (ProForm 485 E, ICON Health & Fitness) (EC). In the CC group, cycling was circular and required minimal postural adjustment, while in the EC group, cycling was elliptical, and the handlebars oscillated back and forth conversely from the foot oscillation, continuously eliciting reflexes and motor strategy adaptation for balance control (Fig. 1).

Subjects pedalled in an upright position at a cadence of ~ 60 rpm. The intensity of the exercise was modulated by changing the cycloergometer resistance. The intensity and the frequency of cycling were adjusted to obtain 65% of the peak heart-rate (HR_{peak}) for the first month, 70% for the second, and 75% for the third month. In this way, the subjects of both groups made similar efforts. The HR_{peak} of each subject was established as the maximal frequency recorded during a preliminary exercise test. This test consisted of three 5-minute workload stages increasing from 25 to 50 to 75 watts.

Training and detraining procedure

The 21-week exercise program consisted of 35 min. of pedalling a day (10 min. warm up, 15 min. exercise, and 10 min. cooling down) three times a week. The elliptical cycling was performed during the first week with the handles fixed to facilitate balance-keeping, then the handlebars were allowed to move elliptically. Detraining consisted of a 21-week period during which subjects refrained from all exercise training and performed only normal activities. Before and after the training, all subjects performed various static stretching movements involving all major joints and muscle groups.

Testing

The evaluation of physical parameters and balance indexes were performed before training to establish baseline values. These tests were repeated a 7, 14, and 21 weeks during the training and detraining periods.

Leg power

Muscular power (expressed in wattage) of the legs was assessed using a modified version of the Wingate Anaerobic Power Test on a Monark bicycle ergometer (Ergometer Monark 818 E). The Wingate test has been

shown to be a valid measure of muscle power in women (7, 36). The test consisted of a 3 to 5 min. warm-up period of low-intensity cycling at 60-70 rpm, followed by 15 sec. of maximal pedalling against a resistance set at 7.5% of the subject's body weight. The highest value obtained during the first 10 seconds was taken to reflect peak leg muscle power.

Flexibility

The flexibility index was determined by the Sit and Reach test. The test is performed on an electronic platform like a flexometer used as per the research protocol of the Canadian Standardized Test of Fitness (34). The subjects, sitting on the ground with their legs stretched out and feet resting on the platform at 2 cm. from the lateral edge, had to bend as far forward as possible and to press a series of parallel pairs of buttons on the platform with both forefingers for 3 sec. Flexibility was evaluated in cm.

Balance indexes

Equilibrium was investigated using a balance platform (Bertec Corporation, USA) with each subject's heels touching and feet wide apart (30°). The upper limbs were relaxed and hung parallel to the sides. The actual parameter being measured by the balance platform was the center of body pressure (CoP). Each subject stood, with her head relaxed and looking straight ahead; the test was performed with eyes both open (OE) and closed (CE). The parameters estimated were mean velocity in mm/s and ellipse area in mm². The tests were carried out in sequence without moving the feet. The duration of each period was 52.2 sec.

Data analysis

All the pre-stimulation values of interest were measured at 7 days and at 24 hours prior to the onset of training, there were no statistically significant differences between the two groups. No statistical difference was observed between the results from early and late preliminary tests either. Therefore, for the present analyses, results recorded 24 hours before training were considered to be baseline values. The results obtained from the post-treatment period were then compared to the baseline values. We performed a test-retest reliability evaluation on sway and muscle performance at different times. The intraclass correlation coefficients (ICCs) were used as statistical methods for assessing test-retest reliability. The intraclass correlation coefficients ranged from 0.70 to 0.85 indicating adequate reliability of all measurements.

The responses were statistically analyzed by a Mixed Model Analysis with post-treatment values as the dependent variable; group, time, and group time as the fixed effects of main interest; baseline as the fixed effect covariate; and a random subject for the repeated measures.

Baseline and first or second measurement data collected from the drop-out subjects have been included.

Post hoc analyses were performed using the Tukey test to compare the two groups and the treatment times. The level of significance was established at a *P* value of <0.05.

RESULTS

Flexibility

The baseline flexibility indexes were 8.6 ± 1.2 cm in the CC group and 8.9 ± 1.1 cm in the EC group. These values were not statistically different between the groups. After 21 weeks of exercise, the values significantly increased to 13.1 ± 1.2 (52% increase) in group CC and to 13.9 ± 1.2 (56% increase) in the EC group. The increase in the EC group was significantly higher than that of the CC group ($p < 0.05$). After 21 detraining weeks, the flexibility indexes of groups CC and EC tended to decrease to

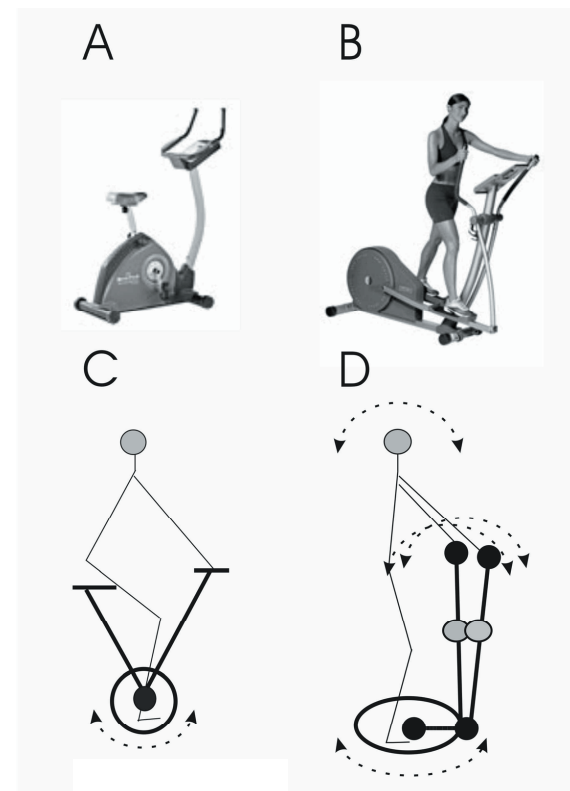


Fig. 1. Photograph of real equipment (A,B) and drawing (C,D) of cycloergometer: A,C: circular cycling; B,D: elliptical cycling. The dotted lines indicate the displacement of the head, hands, and feet during cycling.

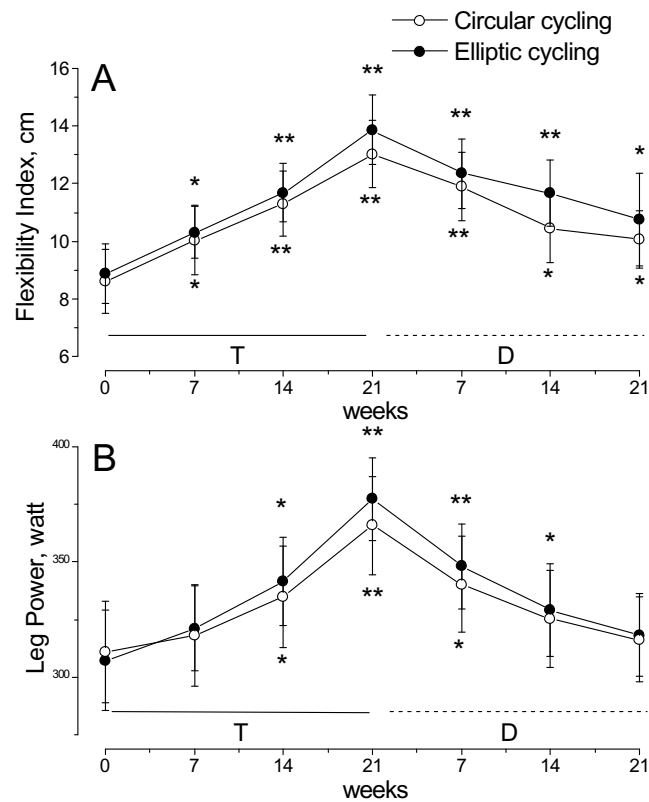


Fig. 2. Changes in the flexibility index (cm) (A) and leg power (watt) (B) in circular (open circle) and elliptical (closed circle) cycling groups during training (T) and detraining (D) periods. All values are indicated as means \pm SD, and time is expressed in weeks. Significant difference compared to pre-training value (* $p < 0.05$; ** $p < 0.001$).

10 ± 1.2 and 11 ± 1.9 , respectively (Fig.2A). The values observed at the end of the detraining period were significantly lower than those at the end of exercise ($P < 0.05$).

Leg power

Baseline maximal leg power was 308 ± 21 watt in group CC and 305 ± 22 watt in group EC. These values were not statistically different between the groups. After 21 weeks of training, the leg power showed a significant increase, reaching an 18% enhancement in group CC (365 ± 18 watt) ($p < 0.05$) and 24% in group EC (378 ± 21 watt) ($p < 0.02$). The increase in group EC was significantly different compared with the CC group increase ($p < 0.05$). During the detraining period, leg power decreased and, after 21 weeks, reached values close to the pre-training ones in both groups (CC 317 ± 21 ; EC 318 ± 19 watts) (Fig. 2B). The values observed at the end of the detraining period were significantly lower than

those at the end of exercise ($P < 0.05$).

Balance.

Balance was analysed by measuring mean velocity (mm/s) and ellipse area (mm^2) of CoP in the open eye (OE) and closed eye (CE) state (Fig. 3). The pre-training values of mean velocity were not statistically different between the groups (CC: 14.8 ± 0.6 mm/s OE, 16.7 ± 0.6 CE; EC: 14.4 ± 0.7 mm/s OE, 16.9 ± 0.7 CE). After 21 weeks of training, the velocity values significantly decreased in both groups but by different amounts. In the CC group, values decreased to 13.8 ± 0.5 mm/s (7% decrease) in OE and 15.9 ± 0.7 mm/s (5% decrease) in CE, while in the EC group, values decreased to 12.7 ± 0.5 mm/s (12%) in OE and to 14 ± 0.7 mm/s (18%) in CE (Fig. 3). The values of the EC group were significantly lower than those of the CC group ($p < 0.01$). After the detraining period, the values of the CC group increased compared to the end-

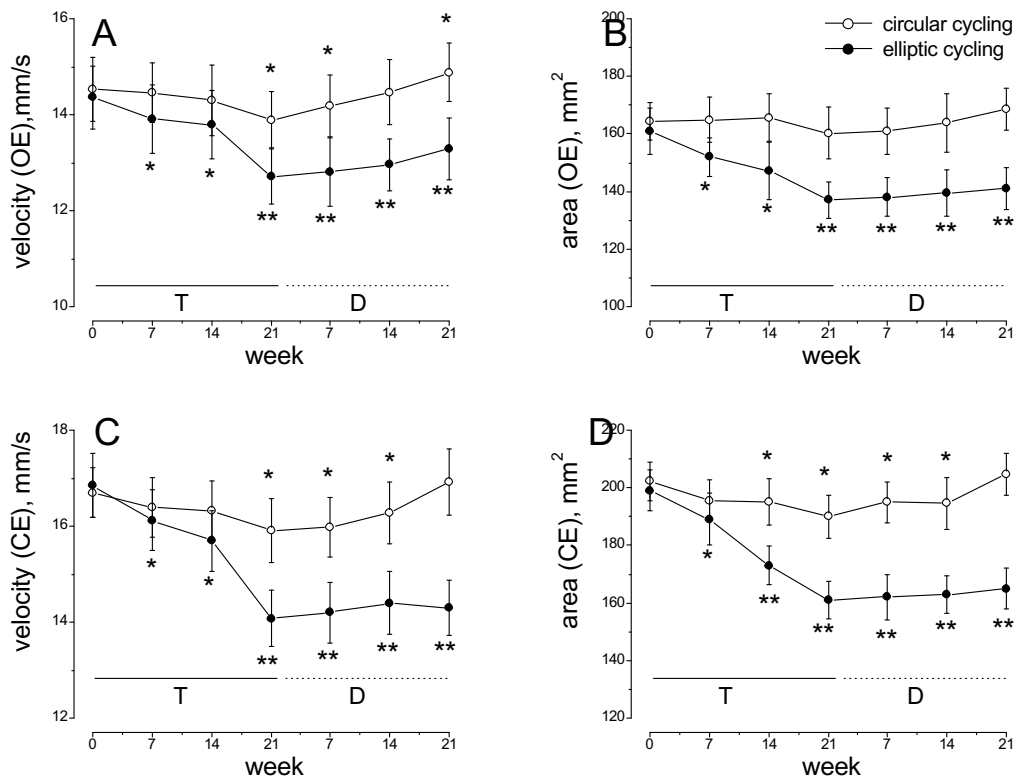


Fig. 3. Changes in velocity (mm/s) (A,C) and ellipse area (mm²) (B,D) of CoP in circular (open circle) and elliptical cycling (close circle) groups during training (T) and detraining (D) periods. All values are indicated as means \pm SD, and time is expressed in weeks. Significant difference compared to pre-training value (* $p < 0.05$; ** $p < 0.001$).

training ones: 14.8 ± 0.8 mm/s in OE and 16.9 ± 0.7 mm/s in CE, returning to the pre-training levels. On the contrary, in the EC group, values remained close to the training-end level: 13.3 ± 0.6 mm/s in OE and 14.3 ± 0.6 mm/s in CE (Fig. 3A, C).

A second parameter used for evaluating balance control was the ellipse area of CoP in OE and in CE conditions. The pre-training values of the ellipse area were not statistically different between the groups (CC: 165 ± 6 mm² in OE, 202 ± 7 in CE; EC: 161 ± 6 mm² in OE, 199 ± 6 mm² in CE). After 21 weeks of training, the ellipse area significantly decreased in both groups. In the CC group, values slightly decreased to 160 ± 7 mm² (3%) in OE and 190 ± 7 mm² (6%) in CE, while in the EC group, values decreased to 137 ± 7 mm² (15%) in OE and to 161 ± 6 mm² (20%) in CE (Fig. 3B,D). At the end of the detraining period, the values of the CC group increased compared to the end-training ones: 168 ± 7 mm² in OE and 204 ± 8 mm² in CE, returning to the pre-training level. Conversely, in the EC group,

values remained close to the end-training level: 140 ± 8 mm² in OE and 165 ± 7 mm² in CE.

Comparison between ellipse area and leg power or flexibility changes

We plotted the percent change of the elliptical area versus the percent changes of leg power and flexibility in the CC and EC groups. These plots provided information about the ratio of changes in balance and other physical performances. The closed-eye condition was chosen because, in this condition, balance is more sensitive to vestibular and proprioceptive inputs, not being influenced by vision. During training (Fig. 4A,C), values changed and the two groups paralleled each other, but the slope was less steep in the EC group because of a larger change in the balance index. During detraining (Fig. 4C, D), the ratio of the percent changes between the two groups was remarkably different; the EC group showed a near invariant of the balance index, while the CC group's balance value was returning to its pre-training value.

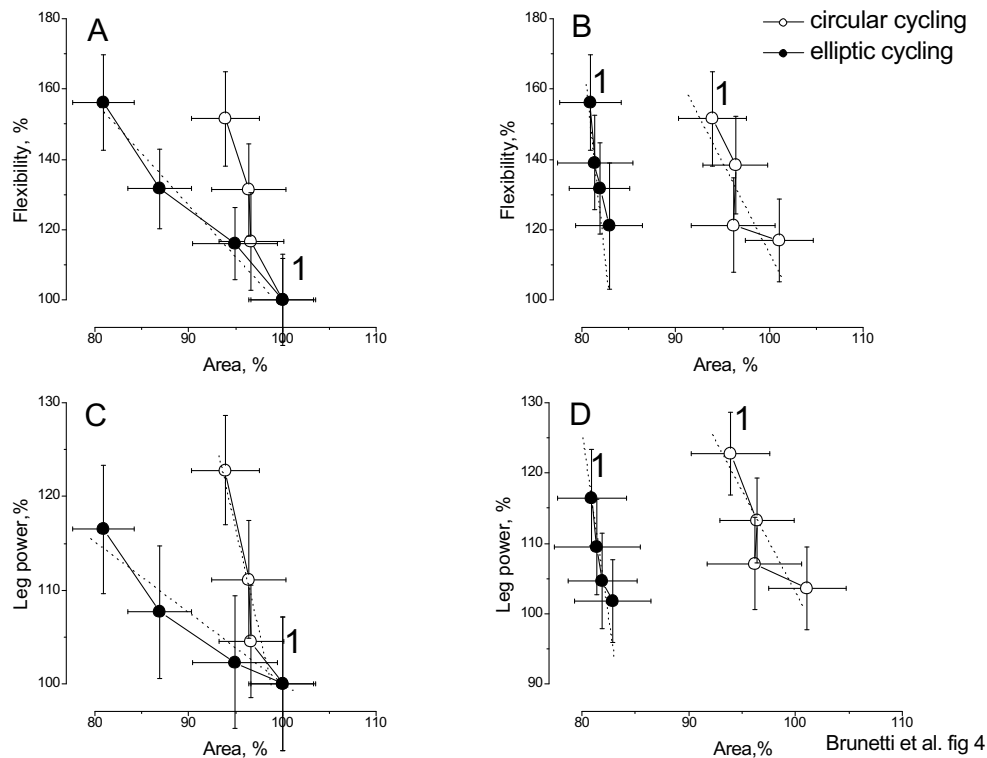


Fig. 4. Percent change of CoP ellipse area versus percent change of flexibility (A,B) and leg power (C,D) during training (A,C) and detraining (B,D) periods in circular cycling (open circle) and elliptical cycling (close circle) groups. The pre-training (A,C) and the final training values are indicated by number 1; the other values correspond to the subsequent week tests. All data are expressed as means and \pm SD. The dashed line is the linear fitting of all data points.

DISCUSSION

The effects on balance control induced by simple resistive exercises (CC group) and those induced by combined proprioceptive and resistive exercise (EC group) were markedly different. In fact, at the end of the training period, the balance indexes of the EC group were remarkably lower than those of the CC group. The difference was even more evident after the detraining period, since the balance indexes remained low in the EC group while they increased to a level even higher than those observed pre-training in the CC group. These effects were observed in both open- and closed-eye conditions, yet changes were to diminished in the open-eye condition because of visual contribution to body stability. Therefore, only the subjects who underwent intense proprioceptive exercise maintained their improvement of balance.

As far as flexibility and leg power were concerned,

both groups showed a progressive increase in values during the training, but the values decreased during the detraining period, thus confirming other studies (31). Therefore, during the detraining period, the change in balance indexes, flexibility and leg power significantly differed only in the EC group. This provides evidence for a distinctly greater effect on proprioceptive training than on other physical performances.

One could question this statement because the different effects observed in the two groups could be due to the fact that the two exercises were not equivalent in terms of muscle activation and body flexibility. Although the overall intensities of the two exercises were similar, the elliptical exercise involved more muscles and joints. It may be possible that the improvement of balance might depend on the enhanced flexibility and muscle force of other body segments. However, we discard this idea since

Table I Pre-training values of Subjects

	CC n° 12	EC n° 12
Age (year)	62.2 ±5.1	63.5 ±4.6
Height (cm)	164.7 ±5.4	165.4 ±4.8
Weight (kg)	67.3 ±5.3	65.1 ±4.6
Flexibility index (cm)	8.9 ±1.05	8.6 ±1.2
Leg power (W)	308.6 ±20.9	305.7 ±22.1
Heart Rate (bpm)	76.4 ±7.3	79.6 ±6.8
CoP velocity (mm/s)	14.8 ±0.6 (OE)	14.4 ±0.7 (OE)
	16.7 ±0.6 (OC)	16.9 ±0.7 (OC)
CoP area (mm ²)	165.4 ±6.2 (OE)	161.9 ±8.7 (OE)
	202.5 ±7.3 (OC)	199.2 ±8.5 (OC)

Characteristics of subjects: the table shows pre-training values of age, height, weight, flexibility index, leg power, heart rate, CoP velocity, and ellipse area of elderly women that underwent different physical training; CC: circular cycling and EC: elliptical cycling. The values are indicated as mean ± SD. No significant differences were observed between the groups.

it is likely that both muscle power and elasticity of all body segments would decline in a similar way during the detraining period. Therefore, the remaining higher balance level of the EC group should be considered a specific effect of proprioceptive training on the central nervous system.

In our opinion, the remarkable efficacy of elliptical cycling in balance improvement depends on the fact that this training causes continuous displacements in the postero-anterior and latero-lateral directions, causing oscillation of the center of mass in different planes and requiring adequate postural correction in response to balance perturbation. In addition, the elliptical cycling caused a continuous visuo-

vestibular stimulation because of greater head oscillation and, via gamma motoneurons, an enhancement of proprioceptive input.

Interestingly, subjects were trained in dynamic perturbation, while the balance test was a static test measuring balance. The influence of the dynamic proprioceptive stimulation on static balance performance may suggest that proprioceptive learning is not specific and that its effects may extend to all systems controlling body position and movement.

In conclusion, the elliptical exercise represents powerful proprioceptive training while resistive circular training does not require significant postural

adjustment and induces only limited effects. This is in agreement with previous studies, showing that only transient effects on balance can be obtained using resistive training alone (32). Our results strongly suggest that proprioceptive exercise is able to enhance the ability to respond to spontaneous body oscillation, reduces the stabilometric values, and consolidates its effects through a learning process.

Proprioception and visuo-vestibular systems are certainly activated in balance control. Therefore, if all proprioceptive systems are activated with a certain intensity, the central nervous system is able to retain the information and induce learning for quick posture adaptation to regain balance. The evidence for this learning process in older people is very strong, demonstrating that proprioceptive memory is not only powerful in young people but may maintain great efficacy even in elderly subjects. Finally, it is important to underline that any enhancement in balance control is reflected in daily life, subjects can become more confident in their motor performance and balance control.

ACKNOWLEDGEMENTS

We thank Mr. M. Roscini for his valuable technical assistance, as well as ICON Health & Fitness, Italy for the use of the cycloergometers.

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CLINICAL STUDY

ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION IN FEMALES: A PROSPECTIVE RANDOMIZED COMPARISON BETWEEN HAMSTRING AND PATELLAR TENDON GRAFTS.

F. GIRON, M. LOSCO, D. LUP, R. BUZZI and P. AGLIETTI

First Orthopedic Clinic, University of Florence, Florence, Italy

Received September 24, 2008 – Accepted July 20, 2009

This study was performed to assess differences in the outcomes of ACL reconstructions performed in female patients using either a patellar tendon (BPTB) or a double-looped hamstring (DSTG) autograft fixed with modern devices at a minimum 3-year follow-up. The study design was a case series. Fifty-two female patients with chronic isolated ACL tears were randomly selected to receive a DSTG or BPTB graft for ACL reconstruction. All patients were prospectively evaluated by an independent observer using the new International Knee Documentation Committee form, the Functional Knee Score for Anterior Knee Pain Score, the KT-1000 arthrometer, and the Cybex NORM dynamometer. A radiographic study was performed to investigate tunnel widening. The average side-to-side difference in anterior tibial translation was 2.1 mm in the BPTB group and 2.4 mm in the DSTG group. The final IKDC result was A (normal knee) in 65% of the BPTB and 60% of the DSTG knees. One failure (4%) was present in the BPTB group and two failures (8%) in the DSTG group. Muscle strength deficits at Cybex Norm were within 10% for extensors and within 5% for flexors in both groups. No statistically significant differences were found in terms of subjective satisfaction, objective evaluation, stability, or muscle strength recovery. The BPTB group showed a higher incidence of postoperative kneeling discomfort ($p < 0.05$) and a larger area of decreased skin sensitivity ($p < 0.001$). The DSTG group showed a higher incidence of femoral tunnel widening ($p = 0.02$). We conclude that by using strong and stiff fixation devices, ACL reconstruction in females is not influenced by graft choice.

Clinical outcome studies on anterior cruciate ligament (ACL) reconstruction mostly include male patients (1-4) as men are usually more involved in sport activities than women. In recent years, however, the number of women practicing sports has increased, and this has led to a dramatic growth in the incidence of ACL lesions in women (5-6). It has also been reported that when sports are practiced by both sexes, the frequency of ACL lesions is higher in women than in men (7-12). The causes

of this increased predisposition of women to be affected by an ACL lesion are multifactorial (6,8). The most discussed etiologies include differences in generalized laxity, hormonal influence, muscle strength, and anatomy, such as lower extremity alignment, notch width, and ligament size (6,8,13).

Many published studies have investigated the outcome of ACL reconstruction using patellar or hamstring tendon grafts, but all have been performed on a mixed population (14-22). Only a few articles

Key words: ACL, hamstring, patellar tendon, female, graft fixation

Mailing address: Dr. Francesco Giron
First Orthopedic Clinic,
University of Florence,
Largo P. Palagi 1,
50139 Firenze, Italy
Tel: ++39 055 7948187 Fax: ++39 055 4224063
e-mail: francescogiron@gmail.com

compare the ACL reconstruction outcomes in men and women (19,23) separately. Moreover, there is still a debate about which graft would be better for females. For many years, the patellar tendon has been advocated as the “gold standard” in ACL reconstruction; however several disadvantages have been reported, such as donor-site morbidity, residual patellar pain, patellar fracture, and patellar tendon rupture. This has led to an increasing interest in using hamstring tendon autografts. The theoretical advantages of using hamstring tendon grafts are a lower incidence of patello-femoral problems, a low morbidity, easier rehabilitation and, at least, a better cosmetic appearance of skin incisions (24).

The purpose of this study was to prospectively compare the clinical and radiographic results of ACL reconstruction in women using both the middle third of the bone-patellar tendon-bone (BPTB) and the double loop semitendinosus and gracilis tendons (DSTG) grafts at a minimum 3-year follow-up. We hypothesized that, in the short term, the use of a DSTG autograft fixed with modern fixation devices would achieve comparable results in terms of knee laxity and functional outcome to that of a BPTB autograft.

MATERIALS AND METHODS

A prospective comparative cohort study was carried out on 52 consecutive female patients undergoing primary ACL reconstruction. The patients were randomly selected to receive either a BPTB or a DSTG ipsilateral autograft. All patients fulfilled the following inclusion criteria: 1) complete, isolated and chronic (injury surgery interval > 6 weeks) ACL lesions; 2) closed physes and less than 40 years old; 3) healthy contralateral knee; 4) no previous surgeries in the index knee (apart from partial meniscectomy); 5) chondral lesions less than Outerbridge grade III (25); 6) no patellofemoral symptoms; 7) absence of systemic illnesses. Ethical approval was obtained from the Internal Review Board. All subjects were informed of the study design and gave informed consent.

Initially, the BPTB group included 27 patients and the DSTG group included 25. In the BPTB group, three patients were lost at follow-up (two after the 4-month evaluation and one after the one-year evaluation), and one withdrew consent after surgery, so the final number of patients was 23. In the DSTG group, all 25 patients returned for follow-up evaluation.

Patients were comparable in age, sex, body weight,

height, generalized laxity (26), operated side, pre-injury sports activity level, mechanism of injury, time from injury to operation (Table I) and preoperative anterior knee laxity (Table II). There were no significant differences in any of these categories.

The majority of injuries were non-contact injuries occurring during sport activities. There were 5 competitive athletes in each group; the remaining subjects were recreational athletes. All but 3 patients in each group played pivoting sports before the injury. One patient in the BPTB group underwent partial medial meniscectomy before her ACL injury. All knees showed positive Lachman and pivot shift tests (Table III) with a pathological increase of side-to-side difference in anterior tibial translation (ATT) evaluated with the KT-1000 arthrometer at 134N (Table IV). An asymptomatic patellofemoral crepitus was present preoperatively in 4 knees in the BPTB group (15%) and in 5 knees in the DSTG group (20%).

All the procedures were performed by the senior author (AP) using a tourniquet. Complete ACL tear was confirmed at arthroscopy.

The surgical technique for both types of graft has been described previously (14). The grafts and the fixation devices employed were the only difference in the surgical technique.

The medial meniscus was torn in 11 knees (41%) in the BPTB group and in 6 (28%) in the DSTG group, while the lateral was torn in 3 knees (11%) in the BPTB and in 2 in the DSTG group (8%). A medial partial meniscectomy was performed on 7 knees (26%) in the BPTB group and on 4 knees (16%) in the DSTG group. A lateral partial meniscectomy was performed on 2 knees (8%) in each group. Four reparable medial (2 BPTB and 2 DSTG) and 2 lateral meniscal tears (longitudinal in the red-red zone of the posterior horn) were treated with an inside-out technique (27). Meniscal repair sutures were tied after ACL reconstruction was completed. A stable longitudinal lesion (<1 cm) of the medial meniscus was left untreated in 1 knee (4%) of each group.

The BPTB autograft was harvested through a longitudinal skin incision centered over the medial aspect of the patellar tendon. The central third of the PT, 9mm-wide, was removed with a rectangular bone plug (20-25 mm in length) at each end. At a work-station, the tendon portion of the graft was freed from fat, and the bone blocks were trimmed in order to fit the diameter of a 9-mm bone tunnel. At the end of surgery, the paratenon was sutured to close the defect, while the bone defects were filled with autologous bone chips collected during graft preparation and tunnel drilling. Semitendinosus and gracilis tendons were harvested through a vertical anteromedial skin incision over the pes anserinus. The distal ends of the

tendons were left attached to bone. Proximal tendon ends were cleaned from retained muscle and fat tissue and tapered with No 1-0 Vycril absorbable sutures in a criss-cross fashion. Once prepared, the graft diameter was 7 mm in 17 knees (68%) and 8 mm in 8 knees (32%).

The tibial guide wire was inserted using the One-Step Tibial Guide (Arthrotek, Warsaw, ID, USA) (28), keeping the knee in extension to avoid graft-roof impingement. The femoral guide pin, through a transtibial approach, was placed 5 mm anterior to the posterior cortex to allow for a 1 to 2 mm posterior cortical wall after reaming at about 11:00 o'clock (right) or 1:00 o'clock (left). All tibial tunnels and femoral sockets were reamed according to graft size.

Fixation on the femoral side was transcondylar using a Tunneloc screw (Arthrotek, Warsaw, ID, USA) in the BPTB group and a Bone Mulch screw (Arthrotek, Warsaw, ID, USA) in the DSTG group. Tibial fixation was achieved in extension using a soft threaded interference screw (Soft Lock Cannulated Screw, Smith and Nephew, Acufex, Mansfield, MA) for the BPTB group and a Washerloc (Arthrotek Inc., Warsaw, ID) for the DSTG group, applying a low manual tension (approximately 20 N). Postoperative coronal and sagittal radiographic views were obtained at the end of each reconstruction to assess the correct placement and fixation of the graft.

The same brace-free rehabilitation protocol (14) was used in both groups. Full range of motion and strength were recovered after 4 and 8 weeks, respectively. Running was allowed after 3 months and return to sport-specific training after 4 months.

All the patients were evaluated before surgery; every 2 weeks up to the second postoperative month; monthly up to 4 months after surgery; and then after 12, 24, and 36 months by an independent and blinded observer (F.G.). At each control during clinical evaluation, both knees of the patients were covered with a stockinette so that the examiner was unaware of the operated side and the graft employed. Subjective and objective evaluations were performed using the Visual Analogue Score (29) (VAS), the Knee injury and Osteoarthritis Outcome Score (30) (KOOS), the new International Knee Documentation Committee (IKDC) evaluation form (31), and the Functional Knee Score for Anterior Knee Pain (FKSAKP) (32).

Side-to-side difference (SSD) in anterior tibial translation was assessed with the knee flexed 30° using a KT-1000 arthrometer (Medmetric, San Diego, California) at 133-N and manual maximum forces (33).

The presence of skin hypoaesthesia possibly related to the surgical dissection of the infrapatellar branch of the saphenous nerve was evaluated asking the patients to delineate the boundaries of the area using a dermatographic pen. The two major axes were measured, and the overall

area was calculated in square centimeters.

Concentric muscle strength recovery of extensors and flexors of the knee and of internal and external rotators of the foot was investigated using an isokinetic dynamometer (Cybex NORM, Lumex Inc, Ronkonkoma, NY) according to the protocol reported in a previous study (14).

X-ray series, including an antero-posterior weight-bearing film (AP), and a lateral (LL) view in full passive extension with condyle superimposition, were also obtained in all patients at 4 months, 1 year, 2 years, and 3 years after surgery. All images were centered with an image amplifier.

Tibial tunnel enlargement was investigated according to L'Insalata, et al. (34) in coronal and sagittal views. Using a caliper, the distance between the sclerotic margins of each tunnel was measured at its widest point together with the diameter of the transcondylar Bone Mulch or Tunneloc screw. This was completed independently by two observers (D.L., M.L.). The size of the drill bit used for tunnel reaming was recorded at the time of surgery, and the diameters of the two different screws used for femoral fixation were known and constant. All measurements were corrected for magnification by comparing the measured and the real diameters of the transcondylar screws. Change in tunnel size was calculated as a percentage of the diameter of the drill bit used. The incidence of femoral tunnel widening in the sagittal plane was not assessed in both groups due to the superimposition of the fixation device on the femoral tunnel margins in the lateral radiographic projection.

All statistical analyses were conducted on Stat Win II software (StatSoft, Tulsa, OK). We used the Student's T Test to compare differences between groups and used the Chi-Square Test and Fisher's Exact Test for categorical variables. In all tests, an alpha level of 0.05 was considered significant.

RESULTS

There were neither intra- nor postoperative complications in this series.

VAS, KOOS, and IKDC questionnaires showed a progressive and significant score increase during the review period compared to the preoperative condition. No significant differences in the postoperative subjective assessments were found at any of the follow-ups between the two groups (Table III).

All patients regained a "normal" range of knee motion within 4 months of the index surgery. At the 3-year follow-ups, the pivot shift test in the BPTB

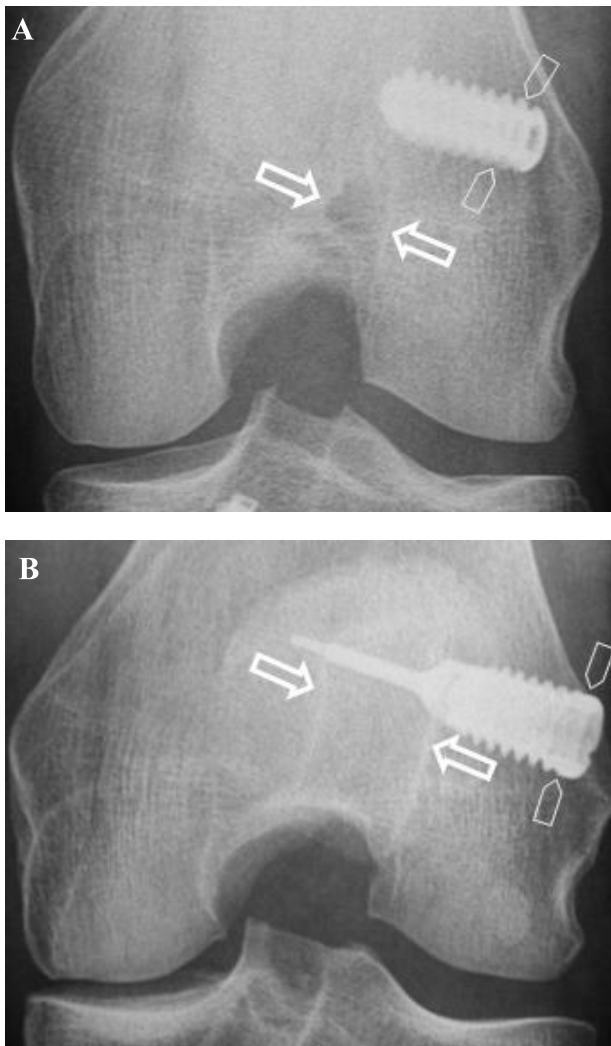


Fig. 1 Example of femoral tunnel enlargement in frontal x-ray projection after ACL reconstruction with BPTB graft (A) and with DSTG (B)

group was negative in 18 (78%) knees, glide in 4 (17%), and clunk in 1 (4%), whereas in the DSTG group, it was negative in 19 (76%) knees, glide in 4 (16%), and clunk in 2 (8%). At the 3-year follow-ups, the KT-1000 side-to-side difference in anterior tibial translation at 134N was comparable in both groups. A significant decrease ($p < .001$) in KT-1000 side-to-side anterior tibial translation at 134N was recorded before operation and at the last follow-up (Table IV). An anterior tibial translation within 5 mm was recorded in 95% of the knees of the BPTB group and 92% of those in the DSTG group. No statistically significant differences were recorded

between the two groups.

We observed one failure (4%) in the BPTB group and two failures (8%) in the DSTG group. The failures were defined as a positive pivot shift graded as clunk and a KT-1000 side-to-side difference in anterior tibial translation greater than 5 mm. These patients reinjured themselves during resumed sport activities.

The final IKDC scores at 3 years post-surgery in the BPTB group were graded normal (A) in 15 (65%) of the patients, nearly normal (B) in 7 (30%), and abnormal (C) in 1 (4%). In the DSTG group, 15 (60%) of the patients were graded A, 8 (32%) graded B, and 2 (8%) graded C. The difference between the two groups was not statistically significant.

At the last follow-up, a moderate patello-femoral crepitus was recorded in 9 (40%) knees in the BPTB group and in 8 (32%) in the DSTG group. If compared to the preoperative status, these two percentages showed a 100% increase in patello-femoral crepitus in both groups. The FKASKP was the same for the two groups (mean value 45).

If we specifically analyze the section which evaluates the ability to kneel, we notice that the percentage of BPTB patients presenting moderate trouble in their patellar tendons (52%) is higher and statistically relevant ($p < .05$) to that of the patients operated with DSTG (27%). Investigating the consequences of the possible lesion of the infrapatellar branch of the saphenous nerve during surgery, we found that 86% of the patients of the BPTB group complained about disturbance in anterior knee sensitivity in a side region near the incision, while only 38% in the DSTG group made similar complaints. This difference is statistically significant ($p < 0.001$). The average skin area of uncomfortable sensitivity was 41 cm² in the BPTB group (range 10-87 cm²) and 25 cm² in the DSTG group (range 6-79 cm²).

The isokinetic test performed with the dynamometer Cybex Norm showed that the strength recovery of flexor/extensor and internal/external rotator muscles was almost complete by the 2-year follow-up (Table 4), so an isokinetic muscle strength test was not performed at the last follow-up. In both groups, a greater strength deficit was evident in extensor muscles at each of the three angular velocities tested (60°/sec, 120°/sec, and 180°/sec)

Table I Preoperative demographic data

	BPTB	D-STG
Patients	27	25
Age (years)	24 (range 17-36)	27 (range 17-40)
Side involved (right)	9	12
Body weight (Kg)	60 (range 48-78)	56 (range 48-69)
Height (cm)	165 (range 155-181)	165 (range 161-176)
Generalized Joint Laxity ^{Carter&Wilkinson}	9	7
Type of injury		
Contact	-	-
Non contact	18	22
Motor vehicle accident	6	3
ADL	3	-
Injury surgery interval (months)	27 (range 2-96)	24 (range 2-100)

Table II Preoperative knee laxity

	BPTB	D-STG
Lachman test (at 25° of knee flexion)		
+	3	2
++	22	20
+++	2	3
Pivot shift test		
+	1	-
++	24	22
+++	2	3
Generalized Joint Laxity	9	7

Table III Postoperative subjective evaluation at 3-year follow-up.

	BPTB	D-STG	Significance
VAS score	7±1.8	7.5±1.6	n.s.
KOOS score			
pain	89±11	95±10	n.s.
symptoms	89±9	90±9	n.s.
activities of daily living	93±8	97±5	n.s.
sport and recreation function	75±22	80±23	n.s.
knee-related quality of life	75±22	75±22	n.s.
IKDC score	75±17	81±17	n.s.

than in flexors. No statistically significant differences were found between the two groups.

Before injury, 74% (20 out of 27) of the patients in the BPTB group and 80% (20 out of 25) of the patients in the DSTG group were involved in level

I or II sports activities. At the 3-year follow-up, the BPTB group included 4 patients (17%) active in level I, 3 (13%) in level II, 10 (43%) in level III, and 6 (26%) in level IV. In the DSTG group, there was 1 patient (4%) active in level I, 5 patients

Table IV *KT-1000 anterior side to side difference (SSD) measurement in anterior tibial translation preoperatively and at follow-up*

	BPTB	D-STG	Significance
KT-1000 anterior SSD at 134N			
Preop	7.4 (4-12) mm	7.2 (5-12) mm	n.s.
Follow-up	2.1 (-1-6) mm	2.4 (0-7) mm	n.s.
Significance preop vs postop	p<.001	p<.001	
Stratified KT-1000 anterior SSD at 134N at follow-up			
-1-2 mm	15 (65%)	15 (60%)	n.s.
3-5 mm	7 (30%)	8 (32%)	n.s.
6-10 mm	1 (4%)	2 (8%)	n.s.

Table V *Postoperative muscle strength recovery at 2-year follow-up*

	BPTB	D-STG	Significance
Extensor deficits at			
60°/sec	+5.2	-2.5	n.s.
120°/sec	+7.6	+6.0	n.s.
180°/sec	+9.8	+8.6	n.s.
Flexor deficits at			
60°/sec	+3.6	+4.6	n.s.
120°/sec	+4.4	+2.6	n.s.
180°/sec	+2.6	+2.8	n.s.
Internal rotation deficits			
30°/sec	+1.4	+3.6	n.s.
60°/sec	+2.4	+4.5	n.s.
120°/sec	+8.8	+8.9	n.s.
External rotation deficits			
30°/sec	+3.8	+2.2	n.s.
60°/sec	+5.1	+5.4	n.s.
120°/sec	+8.4	+6.9	n.s.

were (20%) in level II, 10 (40%) in level III, and 9 (36%) in level IV. A significant difference was found between the pre-injury and postoperative number of patients active in level I or II sports. Postoperatively, a significantly lower number of subjects of both groups were able to return to higher level sport

activities (BPTB $p=0.003$; DSTG $p<0.001$).

Postoperative radiographic evaluation showed an incidence of tibial tunnel widening in the sagittal plane in 58% of the BPTB group and 60% of the DSTG group; the average tibial tunnel widening was 26% (range 20-50%) in the BPTB group and

28% (range 20-50%) in the DSTG group. None of the patients showed radiographic evidence of graft impingement.

In the coronal plane, a tibial tunnel widening was found in 37% of the knees in the BPTB group and in 39% of the DSTG group; the average widening of the tibial tunnel was 24% (range 20-40%) in the BPTB group and 27% (range 20-50%) in the DSTG group. The difference in the incidence and in the average of tibial tunnel widening between the two groups in both planes was not statistically significant.

Femoral tunnel widening was observed in 20% of the cases in the BPTB group and in 54% in the DSTG group (Fig. 1). The average tunnel widening in the femur was 25% in the BPTB group (range 20-30%) and 24% in the DSTG group (range 20-50%). The incidence of femoral widening was statistically significantly higher in the DSTG group ($p < 0.001$). The incidence and the amount of tunnel widening showed no changes at one year follow-up. No correlations were found between tunnel widening and postoperative knee laxity.

DISCUSSION

Although it is clearly documented that women are more likely to suffer ACL injuries than men (7-10), little has been presented in literature on the surgical results of ACL reconstruction especially in female patients.

The purpose of the present study was to prospectively compare the outcome results of ACL reconstruction with semitendinosus and gracilis tendons versus patellar tendons in a female population. The main finding of this study was that, after a 3-year minimum follow-up, no statistically significant differences were found between the two groups in terms of subjective assessment, objective evaluation, knee laxity and muscle strength recovery. The BPTB group showed a higher incidence of postoperative kneeling discomfort ($p < 0.05$) and a larger area of decreased skin sensitivity ($p < 0.001$). The DSTG group showed a higher incidence of femoral tunnel widening ($p = 0.02$).

Previous prospective comparative studies have not found any statistically significant differences in ACL reconstruction results between hamstring and patellar tendon grafts (14-22). However, they

were performed in a mixed population of males and females. Only a few papers have attempted to study whether there are significant differences between males and females after ACL reconstruction (15,19,23,35-36). In 2001, Aune et al. (15) compared a group of 37 patients who had undergone reconstructive surgery with DSTG grafts to a group of 35 patients in whom BPTB grafts had been used. The 2 groups were composed of the same percentage of men and women; the average follow-up of their study was 2 years. The objective and arthrometric evaluations did not uncover any differences in postoperative knee laxity between the two groups.

Corry et al. (35) compared 82 patients who underwent ACL reconstruction with BPTB grafts with 85 patients where DSTG grafts were used; they did not find significant differences between the 2 groups in terms of knee laxity, range of motion recovery or symptoms. The women operated on with DSTG grafts, in particular, appeared to have significantly looser KT-1000 measurements than the ones operated on with BPTB grafts. In their study, metal interference screws were used for graft fixation in all groups. This decision was ascribed to a lesser bone density in the female proximal tibial metaphysis that may allow slippage of the hamstring tendon graft.

In a later paper (37), the same authors reported better knee stability results by reinforcing tibial fixation of the DSTG graft with a supplementary staple. Nevertheless, they reported excellent subjective and objective results in both men and women after 7 years, investigating gender differences in the outcome of ACL reconstruction with DSTG and interference screw fixation (36). Although significantly greater laxity upon physical examination was still present in female patients as compared to males, the magnitude of this difference was small and had no effect on activity level, graft failure, subjective or functional assessment.

Gobbi et al. (19) prospectively compared the results of ACL reconstruction using BPTB and DSTG grafts in males and females at a 3-year follow-up and found similar results. There were no differences in subjective assessment or in standard knee evaluation scores between the two groups. However, in the DSTG group, female patients showed a significant increase of knee laxity upon computerized analysis

and a higher deficit of peak torque at 60 degrees/sec in flexion and extension during isokinetic tests at 1 year as compared to men.

Noojin et al. (23) evaluated gender differences after ACL reconstruction with DSTG grafts and found inferior results with an increased incidence of pain and failures in females as compared to male patients at an average follow-up of 3 years.

However, to date, only Barrett et al. (24) have carried out a prospective randomized study comparing the ACL reconstruction results using BPTB and DSTG grafts in only female patients. They evaluated 39 women in the DSTG group and 37 in the BPTB group suffering from an acute ACL lesion. The failure criteria applied were: a positive 2+ Lachman test, positive 1+ pivot shift test, and a side-to-side difference in anterior tibial translation at a KT-1000 manual maximum greater than 5mm. At an average follow-up of 41 months for the DSTG group and 52 months for the BPTB group, no significant differences were recorded between the two groups in postoperative effusion, range of motion, patellar crepitus, KT-1000 measurement, or Lysholm scores. The only finding was an increased incidence of graft failure in the DSTG group (23%) as compared to BPTB group (8%), but the difference was not statistically significant. The Tegner score demonstrated that fewer patients in the DSTG group were able to return to preinjury sports activities compared to those in the BPTB group. Knee pain was significantly greater in the DSTG group ($p=0.034$). They concluded that, even though the results of the BPTB graft were not statistically significant, they were better than the ones obtained with the DSTG graft. This result was ascribed to the type of graft fixation employed during the time interval of the study. Suture post fixation was probably the weak link in hamstrings tendon graft fixation. Both groups had similar fixation, but the more rapid healing of the bone plugs in the bone tunnels may account in part for the decrease failure rate noted in the BPTB group. It must be noted also that in the Noojin (23) and Gobbi (19) papers, similar suspended devices were employed to fix the grafts.

In the present study, we compared the outcome results of ACL reconstruction between BPTB and DSTG grafts using very strong and stiff fixation devices. Our hypothesis was that in female patients,

the use of modern fixation devices designed for hamstrings tendon grafts would assure comparable results between the two grafts in terms of postoperative knee laxity after ACL reconstruction. The 3-year results confirmed our hypothesis showing no statistically significant difference between the two groups in terms of subjective assessment, objective evaluation, or instrumented knee laxity. Contrary to another report (24), the percentage of graft failures was comparable (4% in the BPTB group and 8% in the DSTG group). Both the Lachman and the pivot shift tests were acceptable in over 90% of cases in both groups. 65% of the patients in the BPTB group and 63% of the patients in the DSTG group showed a "normal" anterior tibial translation, whereas 30% of the patients of the BPTB group and 32% of the DSTG group had a "nearly normal" anterior tibial translation. No other relevant differences were recorded during the pivot shift test which was absent in 78% and glide in 17% of the BPTB group and absent in 76%, and glide in 16% of patients in the DSTG group. These differences were not large enough to be statistically significant.

The only significant differences recorded in this study were a higher incidence of postoperative kneeling discomfort ($p<.05$) and a larger area of decreased skin sensitivity ($p<.001$) in the BPTB group, a higher incidence of femoral tunnel widening ($p=.02$) in the DSTG group, and a significantly low number of patients in both groups were able to recover high level (I or II) sport activities.

Despite the incidence of a moderate but asymptomatic patello-femoral crepitus doubling in the postoperative period, there were no significant differences between the two groups. Moreover, the results of the investigation on the incidence of postoperative anterior knee pain with the FKSAP were equal in both study groups. These data are very different from those reported by Barrett et al. (24) who found a significantly higher number of patients with pain in the DSTG group. A more accurate analysis revealed that 52% of the patients in the TR group complained about moderate discomfort when kneeling in comparison with only 27% of the patients in the DSTG group ($p<.05$). These data agree with those reported in other papers (14-15,35), although those studies included a mixed population. A possible explanation of this important difference

could be damage to the infrapatellar branch of the saphenous nerve during the harvesting of the BPTB graft at the time of surgery. In the BPTB group, in fact, a large number of patients complained about paresthesias, and the area of hypoaesthesia was significantly larger ($p < .001$) than that recorded in patients of the DSTG group.

Despite the subjective KOOS and IKDC, assessment scores did not reveal a significant difference between the two groups regarding sports and leisure activities, quality of life in relation to the knee, or symptoms relating to the level of physical activity; only a small number of patients in both groups were able to participate in high level (I or II) sports activities postoperatively. In Barrett's study (24), only the patients in the DSTG group showed a lower postoperative preinjury Tegner score, but the authors were not able to explain this discrepancy. In our study, we tried to investigate the reason most of our patients were not able to return to the same preinjury level of sport activities, and we found that this was not related to the knee function but mainly to the fear of risking a new knee injury. Probably in females, ACL lesions have more psychological implications than in males.

The postoperative isokinetic strength test with the dynamometer Cybex Norm showed no statistically significant differences in muscle strength recovery between the two groups of study. On average, at the 2-year follow-up, the postoperative muscular strength deficit between the healthy and the operated knees was less than 10% both for flexor and extensor strength. A larger deficit was found in the extensor strength in both groups, but on average it was less than 10%.

Tunnel enlargement has been reported in numerous studies (38-40) after ACL reconstruction. The origin of this radiological finding remains controversial, and a multifactorial etiology has been postulated (41). Tunnel enlargement usually occurs within the first 12 postoperative weeks and stops within the first postoperative year. However, its presence has been shown to not correlate with an increase in knee laxity or in the failure rate (41). Our results are in line with a previous report (18). We found more tunnel widening in the DSTG than in the BPTB group, but this increase reached a statistical significance only on the femoral side ($p < .02$). The tunnel widening

was present in 54% of the knees in the DSTG group compared to 20% in the BPTB group and did not progress after the 1-year follow-up.

This study has several limitations that warrant review before definitive conclusions can be drawn. The number of patients recruited in this study was small, and that could affect the power of the study. Nevertheless, the results of this study are comparable to those of a previous study on ACL reconstruction with BPTB and DSTG grafts that we published on a mixed population (14). The majority of our patients were recreational rather than professional athletes, this could have affected the level of sports participation after surgery due to the fact that our patients were less motivated to recover the preinjury sports activity level. However, despite these limitations, the value of this study lies in the fact that comparable outcome results can be achieved after ACL reconstruction using either BPTB or DSTG grafts as long as new and more effective graft fixation devices are employed to fix hamstring tendons graft. To our knowledge, this is the first study able to draw this kind of conclusion. Previous studies (15,24, 35-36) were performed using fixation devices with inferior biomechanical properties (42-43), and this could have affected the results.

In conclusion, we can state that the use of modern fixation devices in female patients allowed us to achieve comparable results in terms of knee laxity after ACL reconstruction with both grafts.

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CLINICAL STUDY

TREATMENT OF A DEEP CARTILAGE DEFECT IN THE KNEE JOINT OF A 15-YEAR-OLD FEMALE PATIENT WITH THE TRANSPLANTATION OF CULTIVATED AUTOLOGOUS CHONDROCYTES. CLINICAL AND MAGNETIC RESONANCE IMAGING EVALUATIONS

M. HANDL, P. KOS, T. TRC, M. FRICOVA¹, J. ADLER² and E. STASTNY

Orthopaedic Clinic; ¹Clinic of Imaging Methods, 2nd Faculty of Medicine, Charles University, Prague; ²Tissue Bank, University Hospital Brno, Bohunice, Czech Republic

Received August 6, 2008- Accepted July 20, 2009

This case concerns a severe cartilage defect of the femoral lateral condyle which was successfully treated by transplanting cultivated autologous chondrocytes in the form of a solid chondrograft. A 15-year-old female had a one-year history of left knee pain induced by an injury. The clinical examination showed clicking and pain on maximum flexion and extension. Magnetic resonance imaging and arthroscopy confirmed the diagnosis – cartilage detachment of the femoral lateral condyle with no chance of refixation in situ. A cartilage sample was harvested from the non-weight-bearing zone of the femur, then chondrocytes were cultivated. During surgery, the solid chondrograft was fixed by tissue glue. A knee brace and crutches (with no weight bearing for 8 weeks) were prescribed. Comparing results after surgery to the pre-operative data, clinical scores increased. Magnetic resonance imaging (MRI) was performed and evaluated at 2 weeks, 2 months, 6 months, and 12 months. An accurate fixation of the graft in the defect was observed. The originally high graft signal intensity on TSE PD sequences gradually decreased to the level of the surrounding cartilage, reflecting the chondrograft's maturation. The regression of subchondral bone oedema was also a positive sign of the integration.

The number of knee joint injuries in children and adolescents is increasing. Among such injuries, acute deep chondral lesions are frequently represented. Treating their more severe grades and consequences is difficult for the routine orthopaedic practice (1). Intra-articular pain and restricted function often accompanied by joint effusion are frequent clinical symptoms. Clicking in the joint is predominant for chronic lesions.

A 15-year-old female had a one-year history of left knee pain. After her initial injury, she had been diagnosed and treated non-operatively. Our clinical

examination showed persistent pain and clicking inside the joint in maximum flexion and extension between the two extreme positions. Arthroscopy confirmed a 2.1x2.8x0.3 cm defect of the femoral lateral condyle cartilage (Fig. 1A) detached from the subchondral bone (Outerbridge score IV) with no chance of refixation in situ.

A full cartilage sample the size of one grain of rice was harvested from the non-weight-bearing zone of the trochlea femoris. A solid chondrograft was formed from cultivated chondrocytes over a period of 36 days. The transplantation followed, employing

Key words: knee joint, children, adolescent, injury, chondrocyte, cartilage transplantation

*Mailing Address: Milan Handl,
Orthopaedic Clinic,
2nd Faculty of Medicine, University Hospital Motol,
V Úvalu 84, 150 18,
5 Prague, Czech Republic
Tel: ++420 224 438 856 Fax: ++420 224 432 820
e-mail: milan.handl@lfmotol.cuni.cz*

1973-6401 (2009) Print

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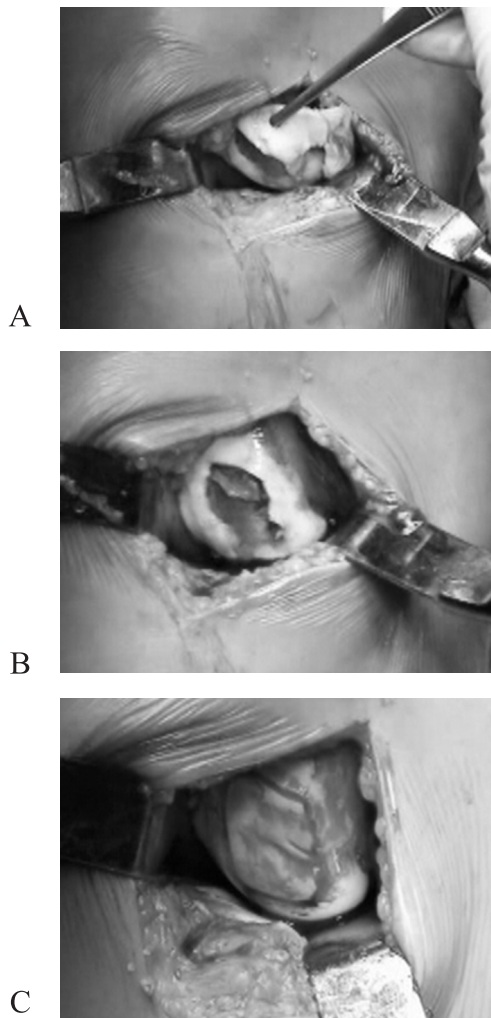


Fig. 1. *A)* The full-thickness defect of the cartilage on the femoral lateral condyle – partially removed by tweezers from the bottom during the surgery. *B)* Preparation of the bottom of the defect for positioning the chondrograft. *C)* The final position of the transplanted cartilage.

the lateral parapatellar approach. Debridement of the subchondral bone, adjustment of cartilage margins (Fig. 1B), and fine drilling of the defect bottom were performed. The chondrograft was precisely sized and fixed by fibrin glue (Fig. 1C). Afterwards, the patient used a knee brace with regulated ROM and crutches with no weight-bearing for 6 to 8 weeks. The pre-set flexion range was 0-30 degrees by the end of the 3rd week, 0-60 degrees after 4 weeks, and 0-90 degrees by the end of the 6th week. After 8 weeks, full motion and weight-bearing were allowed.

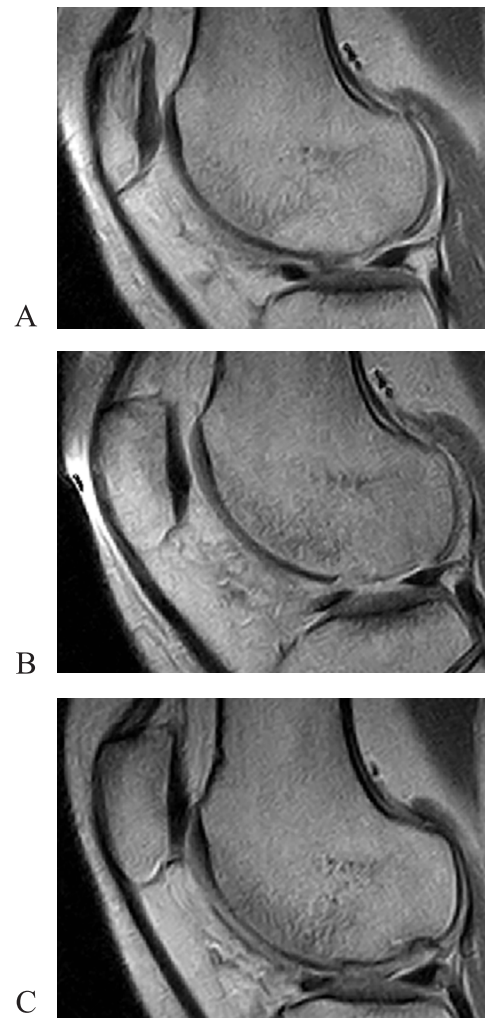


Fig. 2. *A)* MRI preoperatively – a sagittal TSE image. Hyperintensity, thinning and surface irregularity of the articular cartilage on the weight-bearing portion of the lateral femoral condyle represent cartilage detachment. *B)* MRI – 2 weeks postoperatively – sagittal TSE image after autologous matrix-based chondrocyte transplantation shows filling of the defect at the repair site and a high signal intensity of the graft (arrows show the borderline). *C)* MRI – 12 months postoperatively – sagittal TSE image 12 months after the surgery. Note a decrease in signal intensity of the implant from a fluid-like signal after 2 weeks to isointensity to native cartilage after 12 months.

Clinical results were evaluated by comparing pre-op and post-op statuses at 6- and 12-month intervals. The Meyers score improved from 5 to 15 and 18 respectively, the Tegner score from 0 to 6 and 8 respectively, and the Lysholm-Gillquist

score from 15 to 65 and 95 points respectively. MRI examinations were performed preoperatively and at 2 weeks, 2 months, 6 months, and 12 months postoperatively on 0.2T (E-scan, Esaote) and 1.5T (Gyrosan, Philips) units. The signal intensity of the repair tissue, its surface, and its integration with the adjacent native cartilage and the presence of subchondral bone oedema were evaluated (Fig. 2A). In early follow-ups, MRI showed an incomplete defect filling, which improved over time (Fig. 2B). At 12 months postoperatively, there was only a slight step-like incongruence on the posterior edge of the implant. In the early post-surgical stage, a higher graft signal intensity could be seen when comparing the surrounding cartilage at TSE PD, particularly T2 STIR sequences. After 12 months, the graft signal matched that of the surrounding cartilage; the edges of the graft also became less defined (Fig. 2C). An oedema at the subchondral bone could be detected in the pre-surgery stage under the pathologically-changed cartilage. The oedema became more significant in the early post-surgery stage due to the bone's trauma during the surgery. During the subsequent examinations carried out 6 and 12 months after the surgery, a significant regression of the bone oedema was observed.

DISCUSSION

Methods used for the invasive treatment of chondral defects are dominated by drilling and spongiolizing the subchondral bone according to Ficat or Pridie, or by microfracturing according to Steadman (2). Hangody's mosaic plasty or osteochondral autograft transfer are associated with a risk of growth plate damage and therefore their use in children is limited. Another method is Brittberg and Petersson's use of cultivated chondrocytes in a solution and covered by the periosteum (3). Nowadays, the transplantation of cultivated autologous chondrocytes in the form of a solid chondrograft, prepared according to variety of standardised schemes (4), is becoming more frequently used.

Clinical examinations and their correlation with MRI results were used for our evaluation. MRI enables an exact assessment of the graft's integration and dynamics as well as enabling the

specialist to distinguish between a possible failure of the transplanted tissue and another intraarticular pathology. In the early postoperative period, the graft may even have a fluid-like signal intensity, but in about 6 to 12 months, the implant's signal should resemble the native hyaline cartilage and the interface should be indiscernible. Such changes reflect the chondrograft's maturity, namely changes in its extra-cellular matrix. Regression of the subchondral bone's oedema can also be considered as a positive sign of the integration. The repair tissue should restore the contour of the articular surface. An improvement of both underfilling and overfilling of the defect site over time have been reported in literature. A worsening of the defect fill would be a poor prognostic sign (5).

The ideal means to confirm the efficacy of this treatment would be a second-look arthroscopy together with a biopsy and histopathological examination. However, due to ethical reasons, we consider the positive clinical outcomes accompanied by satisfactory MRI results fully sufficient to prove the treatment's success.

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BASIC SCIENCE

ASSESSMENT OF NERVE REGENERATION IN A NEW NEURAL SURGICAL TECHNIQUE BY COMBINED EMG AND ENG ANALYSISC. DE MARIA, D. S. POGGI ¹, S. BURCHIELLI ² and G. VOZZI

Interdepartmental Research Center "E. Piaggio", Faculty of Engineering, University of Pisa, Pisa; ¹Orthopaedic Clinic, Faculty of Medicine, University of Perugia; ²Institute of Clinical Physiology, CNR, Pisa, Italy

Received September 10, 2008 – Accepted July 20, 2009

The standard method used to repair large lesion gaps in the peripheral nervous system is to replace the nerve autograft in the nerve gap maintaining the same natural direction of nervous fibers. This study aims to show the extent and quality of nerve regeneration when the tissue gap is bridged using an autograft rotated 180° degrees in respect to its original position, exchanging the proximal and distal endings. We set up an experimental protocol for the rabbit model to evaluate the quality and speed of nerve regeneration in both classical and inverted grafts by means of electromyography and intra-operative electroneurography.

Disruption of peripheral nerves (neurotmesis) due to trauma is a frequently occurring clinical problem. Partial or total transection of the nerves leads to degeneration of the axonal segment distal to the lesion (Wallerian degeneration) (1). Although such interference obviously has an effect on the axon, the innervated end-organ and the dendritic tree of the motoneuron may also be influenced. The proximal segment and the cell soma survive when the transection is at a reasonable distance from the soma. The soma generally reacts by a chromatolytic reaction, a swelling of the soma due to increased metabolic activity which may be reversible. Sometimes, however, the neuron dies, possibly due to a lack of trophic substances derived from the target organ, and this occurs particularly at young ages and when the lesion is close to the soma. The axons of motoneurons in the peripheral nervous system, in contrast to those in the central

nervous system, have a great capacity to regenerate: this could be explained by the neurotrophic factors in peripheral tissue that promote neurite outgrowth. Surgical realignment is required in the case of total nerve disruption, and the ideal surgical technique of nerve repair should provide rapid and complete regeneration without side effects (2). Several techniques for peripheral nerve repair have been developed. These methods are divided into two main groups: direct repair (neurorrhaphy) and graft repair techniques (3-4). Direct repairs can be further subclassified into epineurial repair (epineurial suturing), grouped fascicular repair, and fascicular repair (perineurial suturing). In the epineurial suturing method, the proximal and distal nerve ends are mobilised to avoid tension at the coaptation site. After surgical transection and careful alignment of both nerve stumps, single epineurial sutures are applied along the circumference of the nerve. The

Key words: nerve regeneration, EMG

Mailing address:
Giovanni Vozzi
Via Diotallevi 2,
56126 Pisa, Italy
e-mail: g.vozzi@ing.unipi.it
Tel: ++39 050 2217056
Fax: ++39 050 2217051

1973-6401 (2009) Print
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intraneural contents remain undisturbed, giving minimal intraneural fibrosis. The individual fascicles and their bundles, however, may be disorientated, which in turn may result in misalignment of axons at the site of coaptation. In the perineural suturing nerve repair technique, under microscope guidance, fascicles are aligned, and the perineurium is sutured. The main disadvantage of the perineural suture technique is the presence of intraneural foreign material, which will lead to intraneural fibrosis. Another difficulty is in finding the proper alignment of fascicles in the proximal nerve stump with respect to those in the distal nerve stump. If a gap is too large, a graft should bridge the nerve gap (5). The most widely-used technique for bridging defects in peripheral nerves is the use of autologous (non-) vascularized perineural nerve grafts because in autografts, the fascicular pattern is preserved and its Schwann cells survive. The purpose of these grafts is to mechanically guide the axonal outgrowth towards the distal nerve stump. The sural nerve (a sensory nerve) is the most commonly-used donor nerve. The size of the defect, the fascicular architecture in the graft, the limited number of donor nerves, and the necrosis of the graft (in the case of large diameter grafts) are problems commonly encountered. Furthermore, harvesting of the graft causes sensory deficit at the donor site and risks neuroma formation.

To avoid these problems, nerve conduits for reconstruction have been developed, such as vessels (regular veins, inside-out veins, and arteries), synthetic materials (silicone and polyglycolic acid), biological conduits (collagen), and muscle conduits. Veins may collapse because of their thin walls and the absence of pressure from inside, and the surrounding scar tissue can cause constriction. By using muscle tissue to bridge a nerve gap, nerve fibers can easily divert from the muscle tissue, thereby forming a neuroma in continuity. Also non-degradable nerve guides can be applied, but their main disadvantage is that the material remains *in situ* as a foreign body, potentially causing a chronic reaction with excessive scar tissue formation. Biodegradable nerve guides therefore promise to be a successful alternative, their aim being to direct the outgrowing nerve fibers towards the distal nerve stump while preventing neuroma formation and the ingrowth of fibrous tissue into the nerve gap. The nerve guide gradually

degrades with a minimal foreign body reaction (6). Still, the functional outcome of peripheral nerve trauma is often disappointing, and this makes the optimization of therapeutic interventions important. Recovery is most often impaired by persistent functional loss and by neuropathic pain that lasts for weeks or even years. Besides being extremely unpleasant, it is resistant to most therapeutic strategies and clearly reduces the quality of life (2).

In this paper we studied the extent and quality of nerve regeneration in a new surgical method in which the tissue gap is bridged using an autograft rotated 180 degrees with respect to its original position, exchanging the proximal and distal endings. We set up an experimental protocol for the rabbit model to evaluate the quality and speed of nerve regeneration in both classical and inverted grafts by means of electromyography and intra-operative electroneurography.

MATERIALS AND METHODS

Animal Experimentation

The experiments were carried out on 7 New Zealand male rabbits (1.3 kg \pm 0.1). The experimental protocol was performed in accordance with D.L.116/92 and EEC 609/86 guidelines for the protection of animals used for scientific purposes. Each animal was anaesthetised with Zoletil® (30 mg/Kg) and Sulfate Atropine (0.5 mg/Kg). The anaesthesia was maintained with Diprivan® in continuous infusion through the marginal ear vein. Each posterior limb was subjected to trichotomy, disinfection, and basal electromyographical analysis. The peroneal nerve was isolated after cutting the femoral biceps in the median area, so the adductor and the semimembranous muscles were separated from the leg with a retractor (Fig.1). After isolation, the electroneurographical signal was recorded. In each subject, the common peroneal nerve of one hind limb was grafted according to the classical procedure, while in the other hind limb, the nerve graft was rotated 180° with respect to its original position (Fig. 2,3).The suture was performed firstly on the proximal then on the distal nerve ending, applying 2/3 separate stitches using Ethilon® 9/0 thread. After suture, electroneurography was repeated. The surgical incisions were sutured using Dexon® 2/0 thread, and the electromyography was performed. In the post-operative period, each rabbit underwent antibiotic therapy with Baytril® (2.5 mg/kg) for 7 days and analgesic therapy with Finadyne® (1mg/kg) for 3 days . The animals were subdivided in to three groups: Group 1, survival



Fig. 1. Rabbit common peroneal nerve

30 days (n=2), Group 2, survival 56 days (n=2), and Group 3, survival 96 days, (n=3). The EMG recordings of each animal were obtained pre- and post-operatively and at fixed times (7, 14, 30, 45, 60, and 90 days after surgery) under general anaesthesia. Intra-operative ENG recordings were obtained directly from the nerve pre- and

post-operatively and before the animal was sacrificed.

EMG and ENG analysis

EMG and ENG signals were recorded by a Biopac® system using stainless steel electrodes with a sampling rate of 500 Hz. The signal was filtered with a 50Hz analog notch filter and digitally filtered in the Matlab® environment (4Hz high-pass) before being rectified (Fig.4). We also evaluated the signal envelope obtained by running an average filter over 100 samples. For standardizing the evaluation of signals, the beginning and end of muscle and nerve activity were defined as a detectable increase of the EMG and ENG amplitude over the background signal, and all values were normalized to the EMG and ENG average amplitude of pre-operative acquisitions. To obtain a concise and homogenous assessment, we evaluated both numerical parameters (amplitude, root-mean square [RMS], maximum amplitude of enveloped signals, and median frequency) and qualitative parameters (the presence of twitch in the absence of stimulation and the absence of twitch after a stimulation). Statistical comparisons were performed using the Mann-Whitney U-test. A p-value of <0.05 was chosen as the level of significance. The p-values were calculated for the average values of all 7 injured animals. All values are presented as average \pm standard error of the mean (SEM).

RESULTS

Figure 5 shows changes in the enveloped EMG amplitude (a) and in the RMS (b) value,

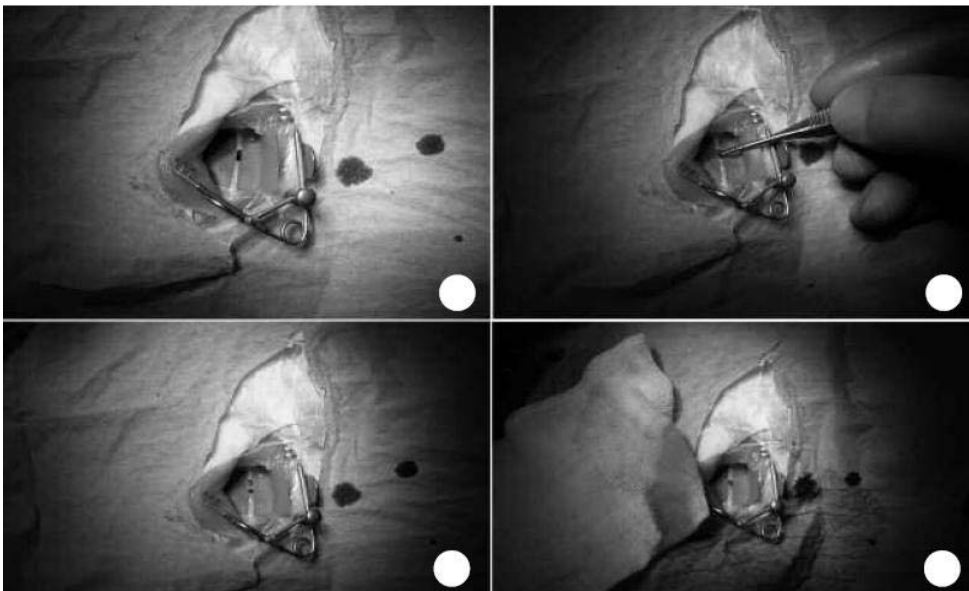


Fig. 2. Traditional grafting technique

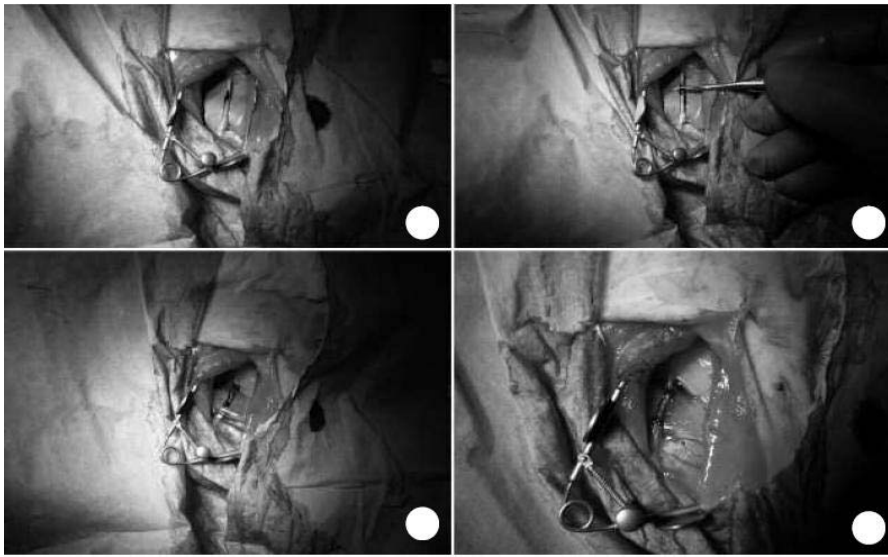


Fig. 3. New surgical method with autograft rotated 180° degrees from its original position

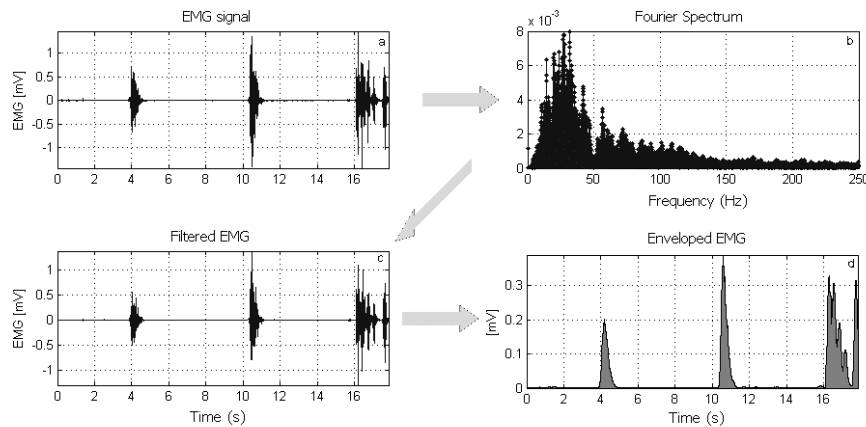


Fig. 4. Signal analysis: EMG signal (A); EMG spectrum (B); filtered EMG (C); enveloped EMG (D)

representing the energy over a given interval (7) normalized to the amplitude of the measurement just prior to surgery (baseline signal). Each of these parameters is different in the two kinds of surgical techniques, with a higher value in the hind limb with the “inverted” graft on day 30 ($p < 0.05$), and a faster recovery towards baseline values on day 45 ($p < 0.05$). At day 90, EMG measurements showed the same behavior, confirmed by ENG signals. The RMS value of electroneurographical signal just before animal sacrifice is the same in both legs. The median frequency of EMG signals from the hind

limb with the inverted nerve implanted is closer to the original median frequency compared to the other hind limb ($p < 0.05$). This difference disappears in the last measurements. Together with this overall assessment, we evaluated follow-ups of each single subject; there were no cases in which the operated hind limb, following the gold standard method, showed a better recovery than the hind limb with the “inverted” graft. In addition, we found three cases in the classical surgery group which presented no muscle response to stimulation, and no case in the other group. However, there were no cases of

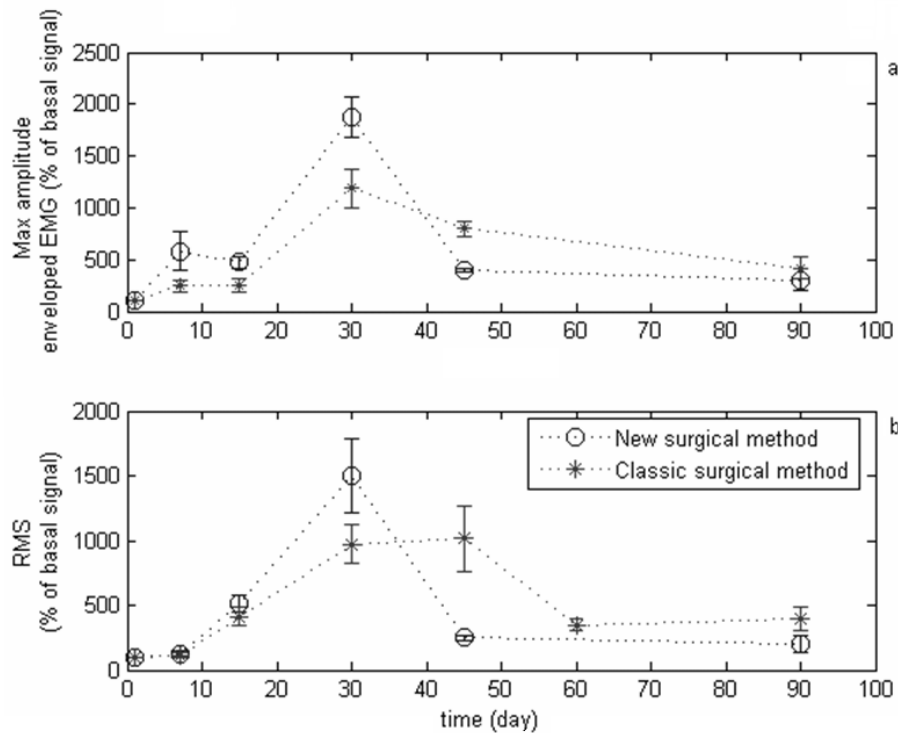


Fig. 5. Changes in the enveloped EMG amplitude (A) and in RMS value (B)

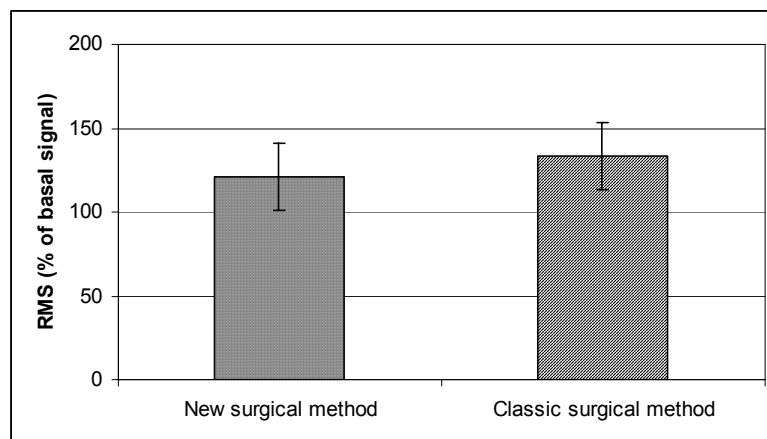


Fig. 6. Changes in RMS value of ENG signals just before animal sacrifice

autotomy (self-mutilation), which, in an animal model, can be considered a sign of neuropathic pain (2).

DISCUSSION

The surgical method assessed in this study has

already led to outstanding results in humans, such as complete and rapid nerve regeneration and reinnervation in sensory areas previously presenting defects (8). Animal models have confirmed these results as have the instrumental data. The rationale for this new surgical method is based on the requirements for nerve regeneration and

the reconstructive principles of nerve repair. The regeneration of peripheral nerves is influenced by neurotropism, "neurotrophism," fascicular end-organ specificity, and contact guidance. Neurotropism is a substance's innate ability to influence axonal growth direction, as opposed to axonal maturation, which is termed neurotrophism. Contact guidance refers to the ability of surrounding tissue to influence the direction of regeneration (9-11). The graft is a guiding structure allowing axons to proceed in the proper direction. The Schwann cells have been proven to survive nerve graft transplantation. These cells are associated with the perineurium of the fascicular graft and most likely contribute to the regeneration process. They aid in phagocytosis of myelin and axonal debris. Moreover, the laminin of the Schwann cell basal lamina promotes neurite growth, and the proteins produced by the Schwann cells produce a neurotrophic effect. The presence of intact Schwann cells can be one of the main reasons for superior results of autologous graft compared to the use of various combinations of neurotrophic factors and materials for grafting (12). Furthermore, the proposed methodology has also confirmed that the neural polarity expressed as the concentration gradient of chemical factors plays a fundamental role in the process of nerve regeneration, as demonstrated by the research by Weiss (12-14) that showed the flow of mediators that inside neuronal cells are principally concentrated at synapsis. They are responsible for the plasticity of the neural cell that continuously adapts and modifies itself through the production of electrical signals that are the basis of physiology of the nervous system. There are several factors related to evaluating efficacy that must be considered in the development of new treatment alternatives. The definition of efficacy, or treatment success, is not always evident, and many of the presently used evaluation techniques function as surrogate endpoint descriptors for specific subparts of the process (e.g. sensory recovery) or are products of a subjective review (14).

In the case of animal models, several methods have been proposed in literature to assess the recovery after a nerve transaction. The peroneal function index (PFI) (15) and the toe-spreading reflex (16) are two common indices used to evaluate functional recovery. The PFI is an index of the

functional condition (recovery) of a rat's peroneal nerve based on measurements from its walking tracks. The toe-spreading reflex, instead, can be observed by holding the animals by the loose skin of their backs and then suddenly lowering them in the air without letting them touch a surface. When this happens, the animals spread their toes reflexively in an attempt to enlarge the surface area of their feet for a safer landing. The muscles of the rabbit involved in this reflex are the peroneal II, III, and IV muscles innervated by the peroneal nerve. In our study, we focused on the electrophysiological assessment by evaluating EMG and ENG signals and by analyzing histological samples (8). This choice is based on the fact that outgrowing nerve fibers, after nerve injury, aselectively reinnervate the muscle they originally were in contact as well as with other muscles. This leads to dramatic changes in muscle activation patterns which are recorded as abnormalities in EMG signals (7).

In conclusion, nerve repair using inverted grafts is, in our opinion, the technique to be used for chronic nerve injuries with a loss of tissue matter. This is just the first step in the experimental analysis of this surgical technique. It will be important to clarify in the future why the 180°-rotated neural graft accelerates the nerve regeneration process. It will also be important to study the chemical, bioelectrical, and histological changes with particular attention to the cell component at the superficial level of the sections of the nerve trunk. The application of these data may well lead to the development of new surgical methods for bone marrow damage in man.

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BASIC SCIENCE

EVALUATION OF GROWTH ABILITY OF OSTEOGENIC PROGENITORS FROM AMNIOTIC FLUID ON TITANIUM PLATES: PERSPECTIVES FOR BONE ENGINEERING

A. PANTALONE, I. ANTONUCCI^{1,2}, L. STUPPIA^{1,2}, G. PALKA^{1,2,3}, V. SALINI and C. A. ORSO

Orthopedic and Traumatologic Division, ¹Department of Biomedical Sciences, "G. d'Annunzio" University Chieti-Pescara; ²Aging Research Center, C.E.S.I., "G.d'Annunzio" University Foundation Chieti-Pescara; ³Human Genetics Division, Pescara Hospital, Pescara, Italy

Received May 29, 2008- Accepted July 31, 2009

Amniotic fluid represents an important source of stem cells to be used for regenerative medicine; stem cells generated from amniotic fluid have apparent advantages of accessibility and pluripotentiality compared to embryonic stem cells and other kinds of adult stem cells respectively. The present study reports the ability of osteoblastic cells derived from rat and sheep amniotic fluid to grow on titanium plates commonly used in orthopedic implantology. Scanning electron microscopy analysis revealed an efficient cell growth on this surface, which suggests that stem cells from amniotic fluid could be an important instrument in regenerative medicine applied to orthopedic implantology.

Amniotic fluid is known to contain stem cells (AFS) able to differentiate into different lineages and to produce cell types inclusive of all embryonic germ layers (1-9). As a consequence, these cells have been classified as pluripotent stem cells. However, differently from embryonic stem cells, AFS are not tumorigenic after transplantation in mice (4-5). Amniotic fluid can thus be considered as a powerful source of stem cells to be used in regenerative medicine for therapeutic purposes (6-8). Osteoblastic cells derived from AFS could be useful for bone regeneration after traumatic or degenerative damage (10). The aim of the present study is to evaluate by scanning electron microscopy (SEM) the ability of rat and sheep AFS-derived osteogenic cells to grow on titanium plates commonly used for orthopedic implantology, in order to demonstrate their usefulness in preclinical studies of bone regeneration.

MATERIALS AND METHODS

Isolation and culture of MSCs from amniotic fluid (AFMSCs)

Amniotic fluid samples were obtained from female rats at day 14 and sheep at day 91-125 of pregnancy, respectively. AF samples were centrifuged for 10 minutes at 1800 rpm. Pellets were resuspended in Iscove's modified Dulbecco's medium supplemented with 20% FBS, 100U/ml penicillin, 100 µg/ml streptomycin (Sigma), 2mM L-glutamine, 5ng/ml basic fibroblast growth factor (FGF2) and incubated at 37° C with 5% humidified CO₂. Non-adherent cells were removed after 4 days while adherent cells were left to grow in the same medium, which was changed every 4 days. At confluence (about 10 days after the primary culture), cells were treated with 0.05% trypsin and 0.02% EDTA, then counted and replated in 25 cm² culture flasks.

Osteogenic differentiation

AFMSCs cells during the second stage were

Key words: amniotic fluid, stem cells, osteoblastic cells, titanium surface

Mailing address: Vincenzo Salini,
Orthopedic and Traumatologic Division,
G. d'Annunzio University
Via dei Vestini 35
66013 Chieti, Italy
Telephone: ++39 0871 358263
e-mail: v.salini@unich.it

1973-6401 (2009) Print

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transferred into an osteogenic medium consisting of the medium described above with the addition of 150 µg/ml βGlycerophosphate, 50 µg/ml ascorbic acid, and 10⁻⁸ M dexamethasone. To visualize calcium sediments, the cells were stained at different times (19, 22, and 30 days from withdrawal) with Alizarin Red S solution, according to the techniques presented by Gregory et al. (11). Mineralization was demonstrated by the presence of red depositions.

Culture on titanium plates

After differentiation, osteoblastic cells were seeded onto a titanium implant surface commonly used for orthopedic implantology (titanium plate-Compact MF Synthes). When 70% confluence was observed (after 2-3 days of culture), cells were prepared and analysed by SEM. The entire culture protocol was repeated twice.

RESULTS

After 10 days of culture, at 70-80% confluence, AFMSC cells were treated with trypsin and EDTA, collected and transferred to the osteogenic medium. After 18 days of culture in osteogenic medium (day 28 from withdrawal), the cells showed 70-75% confluence, and the presence of aggregates or nodules of calcium mineralization was appreciable. Alizarin Red staining confirmed the presence of biomineralization.

In order to evaluate the growth ability of osteoblastic cells on titanium surface, cultures were carried out on an implant surface and evaluated using SEM analysis (Fig. 1). Adherent cells were found to cover the whole surface of the titanium plate (Fig. 2). Cell aggregates were arranged almost uniformly and formed a single layer cell culture on the plate surface (Fig. 3). Scanning electron microscopy revealed a good osteoblastic proliferation and a great integration of stem cells isolated from rat and sheep amniotic fluid on titanium plates (Fig. 4).

DISCUSSION

Pluripotentiality, the ability of a cell to form all the three embryonic layers, is generally considered to be confined to embryonic stem cells of the preimplantation embryo, embryonal carcinoma cells, and embryonal germ cells of the primitive gonad. The recent report by De Coppi et al. is a breakthrough in amniotic fluid stem cell research (4); clonal cell lines that are positive for mesenchymal

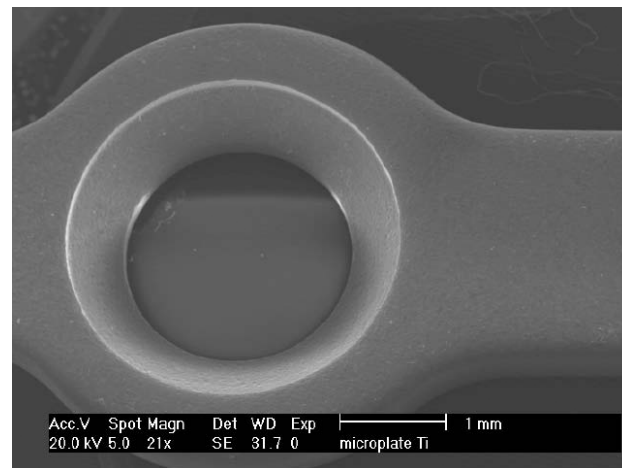


Fig. 1: Scanning Electron Microscope analysis of osteoblastic cells cultured on titanium plate. 21x.

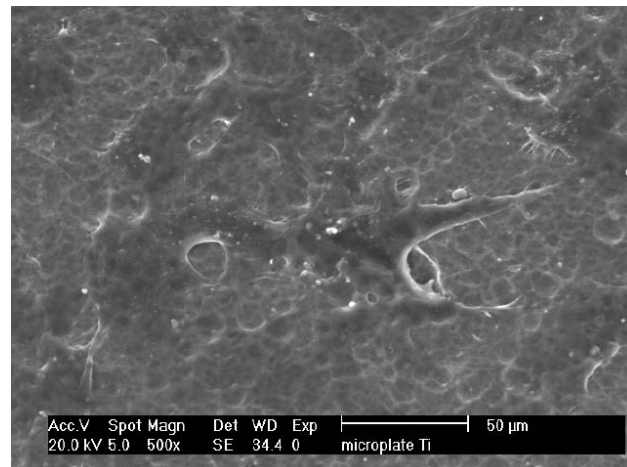


Fig. 2: Scanning Electron Microscope analysis of osteoblastic cells cultured on titanium plate. 500x.

markers and negative for hematopoietic markers (CD34, CD35) have been isolated. Over 90% of these cells also express the transcription factor Oct-4, specific marker of pluripotent stem cells. Thus, amniotic fluid can be directed into the three primary embryonic lineages--mesoderm, ectoderm, and endoderm. This differentiation potential into cells of all three embryonic germ layers together with the observed high proliferation rate are two clear advantages over most of the known adult stem cell sources. Importantly, unlike embryonic stem cells, AFS cells do not form tumours: a low risk of tumorigenicity could be advantageous for possible therapeutic applications. In addition, embryonic stem cell research raises profound ethical issues,

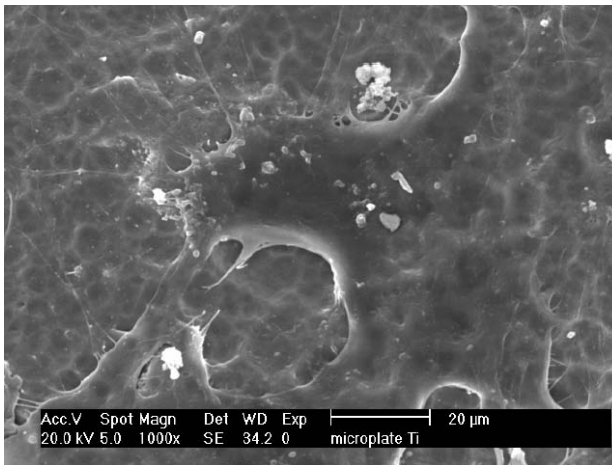


Fig. 3: Scanning Electron Microscope analysis of osteoblastic cells cultured on titanium plate. 1000x.

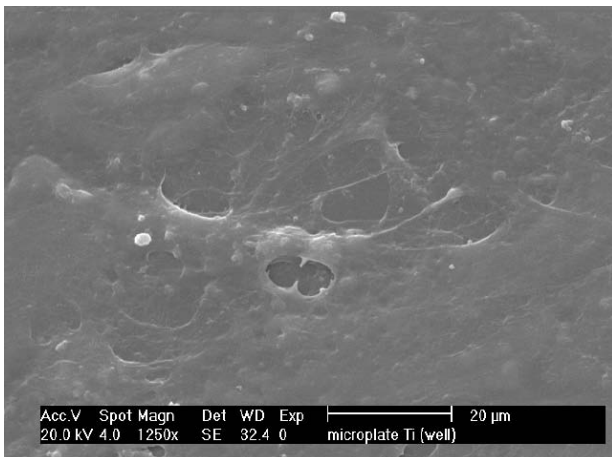


Fig. 4: Scanning Electron Microscope analysis of osteoblastic cells cultured on SLA titanium plate. 1250x.

regarding when human life begins and the moral status of the few-day-old embryos.

All these data provide evidence that amniotic fluid represents a new and very promising source for stem cell research. Amniotic fluid stem cells are an intermediate stage between embryonic stem cells and lineage-restricted adult progenitors.

Osteoblastic progenitors can be successfully obtained from bone marrow stromal cells. In orthopedic reconstructive surgery the use of autologous bone marrow stromal cells is generally considered to be effective for the treatment of major bone defects (14-15). However, AFS can be easily obtained from routine clinical amniocentesis specimens that would otherwise be discarded. Thus,

it is possible that banking these stem cells may provide an important source both for autologous therapy in adulthood and for transplant in HLA matched recipients in the future.

In this study, we demonstrate the ability of rat and sheep AFS, differentiated into osteogenic cells, to grow on titanium plates commonly used in orthopedic implantology, which is an important consideration in view of a possible therapeutic application of these cells. Electron microscopy showed a good growth and adherence of osteoblastic cells on this surface. This result indicates the excellent biocompatibility of osteoblastic cells obtained from amniotic fluid with the titanium scaffold. These preliminary *in vitro* results open a new field for the *in vivo* application of AFMSCs, differentiated in osteogenic lineage, in the treatment of fractures and their complications as well as in diseases with bone excision.

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