

patients experiencing a trial of labor was not analyzed. A rate of approximately 70 % has been reported with a success rate of approximately 75% of vaginal birth after cesarean.<sup>7</sup> This is a likely scenario if trials of labor are characterized by less frequent inductions of labor, more use of amniotomy, late epidural placement, and less repeat cesarean for dystocia performed in the latent phase of labor. A reduction in the primary cesarean section rate would lead to a reduction in that of the repeat cesarean section. Analysis of multicenter statistics has found that approximately 35% of cesarean sections were repeat procedures. Thus, reductions in the number of repeat cesarean deliveries would be expected to lower the overall cesarean delivery rate significantly. It has been estimated that if 80% of patients with a previous cesarean delivery attempted a trial of labor, an overall success rate of 75% would lead to a 21% reduction in the national cesarean delivery rate.<sup>8</sup>

In conclusion, the relative rates of indications for cesarean sections are heterogeneous; thus, some are amenable to overall rate reduction strategies. The combination of fewer women undergoing cesarean section for fetal distress and more women experiencing external cephalic version and a trial of labor and the enforcement of more strict criteria for the diagnosis of dystocia would result in more patients achieving a vaginal delivery.

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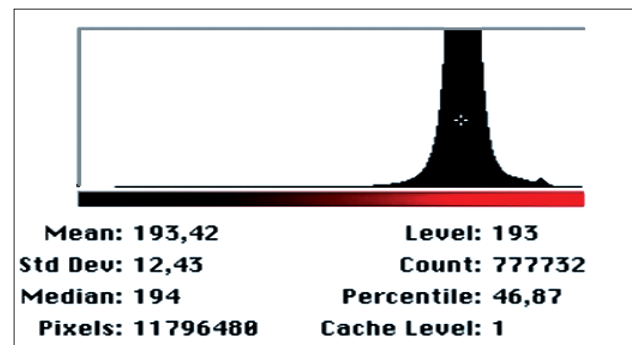
## Loss of zonal organization of articular cartilage after experimental subchondral trauma of the knee joint

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The healthy articular cartilage shows a superficial, a transitional, a deep radial, and a deep calcified zone, which in light microscopic findings can be distinguished, especially by Safranin-O-Staining representing the proteoglycan content. The commonly used histopathological grading systems show a lack of validity especially in the scoring of mild to moderate cartilage damage, and the physiological zonal formation is mainly not a parameter for the quality of the cartilage. Intra- and inter-observer reproducibility in these systems are not satisfying in the grading of osteoarthritis. In several studies, models have been described for the Photoshop-based image analysis for the quantification of proliferative activity and of hormone receptor expression in invasive breast cancer. Meanwhile, Photoshop-based image analysis has been used in different kinds of analysis of cartilage and bone samples, and also to demonstrate the medium term influence of subchondral damage on the articular cartilage, measured by Safranin-O for proteoglycans and by Alcian blue for glycosaminoglycans. Other possible options are PAS for glycoproteins and immunostaining, for example, collagen I and II. It can replace subjective evaluations of color intensities, but it is not meant as another grading system, and it should and cannot contain a classification of arthritis. The benefit of quantifying changes in articular cartilage is well established in animal models, as more attention is paid to the description of biomechanical factors of the subchondral bone leading to cartilage damage. Meanwhile, many studies have shown that MRI can accurately and non invasively evaluate post traumatic changes defined as bone bruises, sub and osteochondral fractures, and even provide information about the

stability of the lesion, as microfractures of bone trabeculae might lead to inadequate mechanical support of the subchondral plate and subsequent overloading of the cartilage itself.<sup>1</sup> In clinical and experimental studies, the histopathological and cryosectional appearance of the lesions could be identified as microfractures of cancellous bone and weight-bearing trabeculae, edema, and bleeding of fatty marrow. The purpose of the present study is to demonstrate meaningful reproducible image analysis of different kinds of staining concerning the above mentioned zonal formation and its loss in post trauma cartilage changes can be performed using inexpensive widely distributed graphics software (Adobe Photoshop). After staining, this technique is fully computerized without any manual interference at any step and is very reliable for the objective quantification of any pattern of osteoarthritis and assessing especially the loss of zonal formation. The present study was performed in the University Hospital Freiburg, Germany from June 2001 to July 2002. In a previous study,<sup>2</sup> an experimental animal model was introduced to produce pure subchondral damage without affections to the articular cartilage. In a preliminary cadaver test and later in a main study with living beagle dogs, reproducibility of the transmitted forces under identical conditions could be shown. In the present subsequent study in 12 adult beagle dogs, the medium term effect of a pure subchondral fracture of the lateral aspect of the trochlea on the initial healthy cartilage was examined. The study was performed in accordance with the German law for animal protection and had been approved by the Review Board for the Care of Animal Subjects at the Regierungspräsidium, Freiburg, Germany. The non-impacted left knee was examined as a control knee in every animal. The transarticular force is maintained via patella to the lateral aspect of the trochlea by a weight that is dropped onto the patella of a rigidly held knee joint of an anesthetized dog. The construction consists of a drop-tower, a drop weight of 2.1 kg with a force-transmitting rod-tip with a diameter of 1.9 cm (adapted to the dog's patella), a load cell (Kistler, Swiss Type 5001) and a force transducer (Kistler, Quarz). The transmitted forces are recorded on an oscilloscope with settings of 0.5 msec/div. and 0.5 V/DN (1V/1000 N). After the animal was anesthetized it lies in lateral recumbency with the hip abducted and 90 degrees flexed. The tibia is held in 100 degrees of flexion in the knee joint, the whole lower extremity is secured rigidly with the thigh positioned in a frame. Radiographs in sagittal and axial views in pre-impact state were performed for a correct perpendicular impact. The force is induced by the fall of the released weight over a prescribed distance. General anesthesia was induced with 0.3 mg/kg Midazolam (Dormicum), intravenous

(IV), followed by 5-6 mg/kg Propofol (Disoprivan), IV and after endotracheal intubation, maintained by the IV infusion of 0.3 mg/kg/min Propofol and repeated IV bolus of 0.05 mg/kg Fentanyl (Fentanyl-Janssen). At the end of anesthesia, and for the next 3 days they received Caprofen (Rimadyl,) subcutaneously 4 mg/kg once daily to achieve post-operative analgesia. Both knee joints were examined by MRI using a 1.5 Tesla imaging unit (Vision, Siemens, Erlangen, Germany) using a head coil and a small surface ring coil. The first MRI examination was 4 hours after impact and consisted of, besides the standard T1 and T2 weighted sequences, Fast T1-weighted (Spin Echo) SE-sequences (TR/TE 550/14 ms) in 3 perpendicular planes, T1-weighted SE sequences (TR/TE 420-535/12-17 ms) with slice thickness 3 mm and a 240 x 256 matrix (sagittal), TSE (Turbo Spin Echo) with spectral fat saturation T2-weighted (Turbo factor = 15, TR/TE 2900/120 msec), slice thickness 3 mm (sagittal), fat suppressed TIRM-sequences (Turbo Inversion Recovery Magnitude) with slice thickness 4 mm, TR/TE 6194/60 msec (sagittal) and 3D FLASH, (fast-low angle shot) fat-suppressed sequences, TR/TE 48/11 msec. Flip angle = 40°, 336 x 512 matrix, slice thickness 1.5 - 2 mm (sagittal). As described before, on follow-up 6 months after producing a subchondral fracture in each dog, osteochondral sections were taken from both the area above the former subchondral lesion (serial sections in cases without visible cartilage damage) and the corresponding area of the non-affected knee joint. After fixation and decalcification in ethylenediamine tetraacetic acid (EDTA), the sections were fixed, decalcified in EDTA, dehydrated, and embedded in paraffin (5 µm slice thickness). Hematoxylin-Eosin, van Gieson, Safranin-O, PAS and Alcian-blue staining was performed using standard methods. It is important to expose the tissues to fix, decalcify, embed, and stain the samples simultaneously, using the same materials



**Figure 1** - Diagram in Image menu indicating the pixel analysis of PAS Staining with mean, median and standard deviation. Higher total values indicate less staining intensity in a case of moderate osteoarthritis with a small standard deviation indicating a loss of zonal formation.

by one person to decrease non-specific staining. Conventional microscopic pictures were photographed using Axioskop (Zeiss, Oberkochen, Germany) with 10x optics and Kodak Elite 400. Using Adobe Photoshop, the digitized images (x50) of the Safranin-O and Alcian-blue stains were stored on an external data storage device. Firstly, it was evaluated how large a window was necessary for the accurate determination of coloring per pixel. Using the Magic wand tool in the select similar menu the cursor was placed on the darkest area, because the pixels thus chosen represent the "purest" narrowest color limitation, namely, the smallest variation from the selected color hue and color saturation. Subsequently, every image is transformed to a gray-scale and the total pixel number is determined. The area occupied by any specific color range can be specified and compared in a relative manner directly from the histogram. An optical density plot of the selected area was generated using the histogram tool in the Image grow menu and the mean staining intensity (in arbitrary unit, AU) was recorded. When the Histogram menu is selected, a display appears depicting gray levels (black/white) or luminosity (color) of all pixels within the selected area, including mean, median, and standard deviation, pixels, and so forth. Standard deviation of the images expresses the variability of the coloring and especially, in case of articular cartilage, the decrease in typical zonal formation in the analysis of hyaline cartilage. Statistical analysis was performed using the t-test. After producing the acute subchondral fracture the intact articular cartilage was demonstrated in the FLASH 3D®-sequences in all 12 affected knees with a comparison of the healthy control knee. The subchondral fractures (microfractures) with bleeding are shown by a decrease of signal intensity on TIRM and T1-weighted images due to a transformation of hemoglobin to deoxyhemoglobin or even methemoglobin. The comparability of these MRI-detected lesions with microfractures of the cancellous bone has been demonstrated in our previous study. In no case was any intra-articular structure affected by impact. In the safranin-O-stains, we found a decrease in standard deviation of the pixel analysis in 11 of 12 cases, 9 of them with a large amount of more than 10% showing a uniform staining in the different zones compared with the healthy cartilage. Exceptions were dogs 4 and 9 with no relevant differences in cartilage degeneration, and dog 8, which showed extended scar tissue leading to a large intensity difference compared with the surrounding tissue, and an increase in standard deviation. In general, decreases in standard deviation were less severe in those stains with scar tissue formation, as these areas show different signal intensities compared with "normal tissue." The results

of the measurement of standard deviation of Alcian-blue-staining revealed slightly smaller decreases of the standard deviation in the most of the 12 dogs with very small differences in dogs 4, 9, and 11. Diagrams in the Image menu reveal mean, standard deviation, and other statistical parameters for t-test (**Figure 1**). A *p*-value less than 0.001 was reached for Safranin-O-staining and Alcian Blue staining. Different kinds of cartilage and matrix components have already been analyzed using Adobe Photoshop. It has become a valuable supplement for well known histopathological grading systems of articular cartilage damage. Years before, the method had already become an accepted tool in the quantification of immunocytochemical hormone receptor studies in oncology and neuropathology and a very good correlation has been found between Photoshop based image analysis and biochemical techniques, for example, enzyme immunoassay.<sup>3</sup> However, although these approaches are suitable for comparative assessments, quantification of the absolute amount of proteoglycan, glycoproteins, or other molecules is not allowed, as the area occupied by one particular color range can be specified and compared in a relative manner. In our experiment, Photoshop-based image analysis has been used to demonstrate the medium term influence of subchondral damage on the classical zonal formation of articular cartilage, measured by Safranin O for proteoglycans in the specific zones, and is not so good with Alcian blue as it represents the amount the polysaccharide components of the proteoglycans. Other possible options are PAS for glycoproteins and immunostaining. Histology and biochemical analysis of cartilage shows that chondrocytes from the various zones differ in several parameters, for example, in matrix synthesis. The PAS staining reveals good comparisons of the total amount of staining but has a limited applicability for deviations in the different cartilage layers. In several studies, follow-up MRI or arthroscopy some months after subchondral damage has shown alterations of the hyaline articular cartilage, which had not revealed any evidence of damage during the initial examination. Johnson et al<sup>4</sup> reported an increased loss of joint homeostasis in patients who had sustained subchondral damage in addition to an anterior cruciate ligament (ACL) injury as well as a higher complication rate in such patients after ACL-reconstruction. Unfortunately, many times, subchondral edema, subchondral fracture, and even osteochondral fractures are not clearly distinguished. Therefore, Miller et al<sup>5</sup> found complete resolution of subchondral signal changes in their repeat MRI of patients with former isolated medial collateral ligament injury. The groups that correlated MRI with histological findings could distinguish lesions as



microfractures of cancellous bone and weight bearing trabeculae, bleeding of fatty marrow, and edema. They stated that edema was found only in a minority of the cases and that the frequently used diagnosis "bone marrow edema" should be replaced by "ill-defined signal intensity," "edema-like MRI imaging abnormality." The present experimental study shows clearly, that such subchondral bone lesions initiate changes in the zonal formation of articular cartilage indicating early osteoarthritic damage.

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## Beneficial effects of soy protein isoflavones on lipid and blood glucose concentrations in type 2 diabetic subjects

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Evidence is emerging that dietary supplementation with soyprotein have a beneficial role in type 2 diabetes.<sup>1,2</sup> Nutritional intervention studies performed in animals and humans suggest that the ingestion of

soyprotein with isoflavones and flaxseed rich in lignans improves glucose control and insulin resistance.<sup>3</sup> In animal models of obesity and diabetes, soyprotein has been shown to reduce serum insulin and insulin resistance.<sup>2</sup> In studies of human subjects with or without diabetes soyprotein also appears to improve hyperglycemia and reduce body weight, hyperlipidemia and hyperinsulinemia and supporting its beneficial effects on obesity and diabetes.<sup>1,2</sup>

A meta-analysis of 38 controlled clinical trials indicated that soyprotein was effective in lowering plasma cholesterol, low density lipoprotein (LDL) cholesterol, and triglycerides (Tg) concentration.<sup>4</sup> The purpose of the present study was to assess the effects of a processed defeated meal containing soyprotein 98 gr/ 100 gr and isoflavones 195 mg/ 100 gr taken one time daily as a beverage with regular meals for 3 months, on serum levels of total cholesterol, Tg, high density lipoprotein (HDL), LDL, fasting blood sugar (FBS), glycosylated hemoglobin weight and blood pressure.

Twenty-six type 2 diabetic subjects who were referred to Ahvaz Jondishapour University, diabetes center for uncontrolled diabetes, completed the study. Eleven patients had previous history of hypertension and 3 of microalbuminuria. All of the patients were treated with diet and anti diabetic drugs (18 sulfonylureas, 11 metformin, 4 combined sulfonylureas and metformin and 1 insulin). They took the prescribed medicine throughout the study without any changes. Subjects were asked to maintain their habitual diet and level of physical activity through out the study. All were in good general health and had a normal renal function. Informed written consent was obtained from each subject. All subjects received 25 gr/day defeated soyprotein meal providing a daily amount of 12 gr soyprotein with 50 mg isoflavones for 3 months. Subjects were instructed to mix their daily supplement in 200 ml water and consume as a beverage with their current meals. The participants were weighed monthly. Blood samples for measuring FBS, HbA1C, total cholesterol, Tg and HDL- cholesterol were obtained after an overnight fasting period before the study and monthly thereafter. The LDL cholesterol was calculated using the Friedwald equation. Blood pressure was measured before the study and at each monthly visit. Plasma glucose concentrations were measured by the glucose oxidase method and HbA1C by high performance liquid chromatography Serum Tg, total cholesterol and HDL-cholesterol were measured by pars Azemon kits on a RA-XY and RA-1000 Automatic Analyzer (Pars Azemon Company, Tehran, Iran). Statistical analysis was performed using the Statistical Package for Social Sciences version 11.5. Significant point was set at 0.05 level. A total of 26 (17 female and 9 male) type 2