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Short communication

# Thermoanalytical investigation of different hip joint arthropathies

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ABSTRACT

The main purpose of this study was to compare the altered metabolism in rheumatoid arthritis to normal hyaline cartilage by thermoanalytical methods. Further aim of this investigation was to elucidate the importance of water content and kinetic character of water loss.

A limited number of papers have been published earlier on the subject of thermal analysis of degenerative and healthy human hyaline cartilage. Rheumatoid arthritis has not been studied previously by thermogravimetry. The only use of thermoanalytical techniques was the measurement of the enthalpy change during denaturation of the rheumatoid and normal cartilage. All samples for this investigation were obtained during live surgeries.

According to the presented data, the water content of the hyaline cartilage is lower in patients with rheumatoid arthritis, than in the healthy donors. The reaction order turned out to be approximately 1 in all the cases, and the standard deviation was low. The TG curve's slope of the linear region showed, that the rate of water loss depends on the water amount remaining in the tissue. It can be concluded that the lower water content in the RA samples bound stronger to the matrix. The newly established compositional thermoanalytical measurement was sufficient for the study and differentiation of normal and rheumatoid human hyaline cartilage.



## 1. Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune inflammatory disease, affecting approximately 0.8% of the adult population worldwide [1]. RA is characterized by synovitis and serositis (inflammation of the lining surfaces of the joints, pericardium, and pleura), rheumatoid nodules, and vasculitis. RA most commonly results in the progressive development of various degrees of joint destruction, deformity, and a significant decline in functional status. Persistent symmetric polyarthritis that affects the hands and feet makes it different from other kinds of arthritis [2–4]. RA occurs in all races and ethnic groups. Although the disease often begins in middle age and occurs with increased frequency in older people, severity of RA may fluctuate over time. Like some other forms of arthritis, rheumatoid arthritis occurs much more frequently in women than in men. There is a high risk of disability and mortality in people with RA. Arthritis and related conditions, such as RA, cost the U.S. economy nearly $128 billion per year in medical care and indirect expenses, including lost wages and productivity. RA accounts for 22% of all deaths from arthritis and other rheumatic conditions [5].

Today research in animal models of arthritis and on tissue from patients undergoing joint replacement, provides remarkable insight into the disease process. Additionally, better characterization of the inflammatory process identified several molecules to be targeted for novel drug development. Cartilage is destroyed in RA by both enzymatic and mechanical processes. At least three components contribute to joint destruction: transformation of the synovium into a proliferative, tissue invasive pannus; generation of osteoclasts that lead to local resorption of bone; and effects of cytokines on cartilage cell function and survival [6].

Pathologic changes in molecular organization of cartilage composition and water content alter the exquisite balance of biomechanical properties [7]. Normal articular cartilage has a unique load-support mechanism, governed by its high water content and its low elastic moduli and permeability. In normal tissues, interstitial water provides over 90% of load-support [8]. The first alteration seen within days after joint destabilization is an increase in cartilage water content, due to loss of the collagen network's elastic restraint. Very shortly after the increase in cartilage water, newly synthesized proteoglycans are characterized by a higher proportion of chondroitin sulfate and a lower proportion of keratan sulfate, and proteoglycan aggregation is impaired. Once proteoglycan loss reaches a critical threshold, water content, which initially increased, falls below normal [9].

A limited number of papers [10–13] have been published previously on the subject of thermal analysis of degenerative [14–16], RA

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[17] and healthy human hyaline cartilage [18–20]. A new protocol [21] had to be established in preparation for this investigation since RA thermogravimetric properties have not been studied previously. Thermoanalytical techniques measure the change in physical or chemical properties of the sample as a function of temperature. There are many possible applications of thermoanalytical techniques: characterizations of active and inactive ingredients, routine analysis, and qualitative control.

The ability of thermogravimetric analysis (TGA) to generate fundamental quantitative data from almost any class of materials, has led to its widespread use in every field of science and technology. Compositional analysis is a key application: by carefully selecting the temperature programming and gaseous environment, complex materials or mixtures can be analyzed by selectively decomposing them or removing their components. This approach is regularly used to analyze moisture content of many substances. TGA is inherently quantitative, and therefore an extremely powerful thermal technique, but gives no direct chemical information. The ability to analyze the volatile products during a mass loss is of great value [22–24].

The main purpose of this study was to compare the altered metabolism in RA to normal hyaline cartilage by thermogravimetric method. In order to characterize the changes in human RA cartilage that promotes disease progression. Further aim of this investigation was to introduce thermogravimetric examination as part of thermal analysis alongside calorimetry, since water content of the cartilage has not been measured before by thermogravimetry. Addition purpose of this study was to establish the kinetic character of the effect of water loss by heating. Previous thermoanalytical studies used cadaver samples for the investigation as normal human hyaline cartilage. All samples that were extracted for this study were obtained during live surgeries. Therefore a new thermogravimetric and sample handling protocol had to be established before the detailed investigation could be performed.

## 2. Experimental

### 2.1. Materials

In order to conduct the thermoanalytical study, 39 samples were collected from live arthroplasty surgeries between October 2005 and April 2008. During hip arthroplasty procedures performed at the Orthopedic Department, University of Szeged, 28 RA samples were obtained and macroscopically normal cartilage from 11 knee. According to the criteria developed by the American College of Rheumatology, all patients were in severe progression Stage III group. There was no clinical meaningful difference in age between RA patients ($61 \pm 5.2$) and controls ($64 \pm 4.2$). There was no considerable sex differences between RA patients (75% females), and controls (70% females); Chi-square $P = 0.54$. Preoperatively, the diagnosis of the patient was established on basis of the patient history, clinical signs, laboratory tests, and radiological findings. The state of the hyaline cartilage was determined intraoperatively.

In osteoarthritis of both medial and lateral knee compartments, total knee arthroplasty is performed. When only one compartment is affected and ligamental stability is intact, unicondylar prosthesis is implanted. We were able to obtain normal cartilage samples from those patients where one compartment of the same knee was degenerated and the other one was normal. Therefore, the unaffected femoral condyle had to be sacrificed for the procedure because ligamental instability was the indication for total knee arthroplasty.

All tissues were yielded in accordance to legal regulation, international ethical concerns, and patients' consent. The Human Investigation Review Board of the University of Szeged has decided (2006.09.18.) that the experiments comply with the ethics of research and the declaration of the Medical World Federation.

**Table 1**
Mass loss and activation energy of normal and RA samples.

| Sample group | Sample number | TG step (°C) | Total mass loss (%) | $E_a$ (kJ/M) |
|---|---|---|---|---|
| Normal | 11 | 30.6–142.8 | 79.21<br>SD: 9.07 | 41.89<br>SD: 13.66 |
| RA | 28 | 29.3–138.5 | 71.96<br>SD: 12.16 | 52.49<br>SD: 15.47 |

After the operation, a disc (5 mm in diameter) was removed from the unhealthy and healthy cartilage surface. The sample was taken under sterile conditions, and excess bone was removed. The disc was first washed in sterile saline, then stored in 20 ml saline for transportation at room temperature. Before the examination all water from the surface was removed. Mean storage time was 6 h (min: 1 h, max: 26 h), 34 samples out of 39 were studied within 4 h of preparation. Five samples were stored over-night at 5 °C. Preemptive control examinations did not show any change in the calorimetric and thermogravimetric properties after storage for 26 h at 5 °C.

### 2.2. Methods

TGA is one of the oldest thermal analytical procedures and has been used extensively in the study of polymeric systems. The technique determines a material's thermal stability and the fraction of volatile components by monitoring the mass change that occurs as a sample is heated.

The thermogravimetric analysis was performed with the use of a MOM Derivatograph (MOM, Budapest, Hungary), and the TG, DTG, and DTA curves were determined. The temperature ($T$) curve shows the linear increase of temperature during the process. DTG curve represents the first derivative of the mass change curve. The DTA curve shows the same picture as a differential scanning calorimetry (DSC) examination, in which the temperature change of a sample is compared with the temperature change of a thermally inert material in order to give information about the endothermic or exothermic enthalpic transition or other reaction [24,25].

The heating was linear from 25 to 150 °C and the rate of heating was 5 °C/min. The thermally inert $Al_2O_3$ was used as reference material. In the first step, the total water loss and kinetic parameters were calculated. The kinetic parameters calculated by the software are the following: the reaction order ($n$), the activation energy ($E_a$), and the pre-exponential factor ($A$).

## 3. Results

TG and DTA curves of normal and RA samples are presented in Fig. 1. Much information can be obtained from the TG and DTA curves.

It was found, that the total water content of intact (normal) (TG N) cartilage is 79.21% (SD 9.07). To remove the cartilage extracellular water content, 41.89 kJ/M (SD 13.66) energy was needed.

Cartilage obtained from rheumatoid femoral head (TG RA) had a lower water content of 71.96% (SD 12.16). Extraction of the cartilage fluid content needed 52.49 kJ/M (SD 15.47) energy (Table 1).

Loss of water content in both groups are presented with a sharp step on the TG curve, starting on average temperature of 30 °C and ending at 138 °C. Linear part of the TG curve begun at around 61 °C and ended at around 95 °C (Table 2). Placing a line on this portion of the curve, the slope of the curve can be calculated which represents the speed of the water content loss (Table 2). The slope of the linear region correlated in both groups.

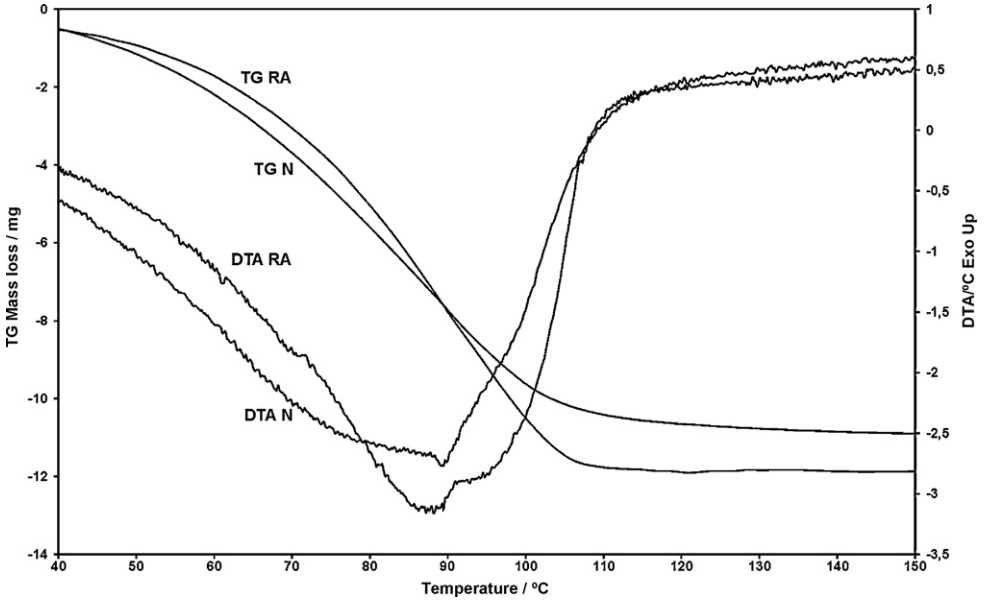


**Fig. 1.** Thermogravimetric curves of a normal and RA sample.

In case of the normal hyaline cartilage, 0.197 mg of fluid content release was observed (average mass of the normal samples was 14.79 mg) with increase of temperature by 1 °C, therefore 1.33% °C$^{-1}$ loss was detected. In the RA samples (average mass: 14.58 mg), 0.201 mg decrease was measured which represents 1.37% °C$^{-1}$ mass reduction. The resulting amount of mass lost in the linear region was recounted from these results (Table 2).

## 4. Discussion

This study represents a first attempt to use thermogravimetry in patient groups with RA to investigate disease activity. This approach opens new possibilities to the understanding of the complexity of the disease. In addition to the previously described decrease in the enthalpy change during calorimetric investigation of RA samples [17], manifest lowering of water content lowering, contributing to disease progression was observed in RA. Although the thermogravimetric change of the process initiated by the temperature change showed marked difference between the normal and pathological groups (Table 1) statistical analysis did not show significant alteration at $P \leq 0.05$. Consequently, further tests with increased number of samples are needed to clarify these results.

Previously, thermogravimetry has not been used for the investigation of rheumatoid hyaline cartilage. We found that the use of TGA as part of thermal analysis was a reliable method for differentiating normal hyaline cartilage from rheumatoid samples. Water molecules' binding mode may have an important consequence in pharmacokinetics. Based on the fact that thermogravimetric measurements showed the nature and quantity of water molecules in the investigated samples, preventive measures can be developed. These therapeutic steps can be adequately tested and monitored with thermogravimetric measurements.

In a collagen–water system, water molecules exist either as bulk water or as interior hydration water molecules. Based on a single endothermic DSC peak in cartilage, Bagratashvili et al. [26] found that the proportion of bound water in cartilage is around 4% using differential microcalorimetry and FTIR spectroscopy. Alternating breakage and reformation of weak bonds between water molecules and proteoglycans directs water movement in cartilage tissue. The thermogravimetric protocol did not differentiate the loss of the minimal bound water from bulk water. Therefore, the results presented in this paper are the total combined amount of water in the connective tissue.

Decrease in the water content of cartilage matrix was observed in all cases of rheumatoid cartilage, meanwhile water interstitial bonding was higher in these cases. Activation energy correlated considerably with water content in the samples. The reaction order turned out to be approximately 1 in all the cases, and the standard deviation was low (Table 2). The TG curve's slope of the linear region showed, that the rate of water loss depends on the water amount remaining in the tissue. Comparing the data in the presented tables (Tables 1 and 2) it can be concluded that the lower water content in the RA samples bound stronger to the matrix. The reaction order and the slope of the linear region correlated in both groups.

The combination of the clinical characteristics and the thermoanalytical data can increase the knowledge of the basic pathophysiology of the joint disease. Current knowledge of the pathophysiology of tissue changes that occur in RA cartilage has been gained mainly from studies of animal models [9]. The conclusions reached in such experiments do not necessarily translate to the human situation. Studies of humans tissue samples are therefore needed to enhance experimental data. In this study cartilage was obtained only from patients with Stage III RA. In Stage II, there is only slight cartilage destruction and in Stage IV there is terminal

**Table 2**
Reaction kinetic parameters of normal and RA samples.

| Sample group | Sample number | TG step linear region (°C) | Mass loss (%) | Reaction order ($n$) | Slope of linear region |
|---|---|---|---|---|---|
| Normal | 11 | 61.12–96.73 | −48.63 | 0.86<br>SD: 0.21 | 0.1973 |
| RA | 28 | 64.15–95.25 | −41.45 | 1.23<br>SD: 0.68 | 0.2010 |

progression with bony ankylosis. Neither of Stage II nor Stage IV are indication for surgery, therefore we were not able to differentiate between thermal changes as the disease progresses.

The relationship between the different pathomorphologic influences, and the role of extracellular matrix homeostasis will be necessary to reveal potential targets of therapy. While there is rapid progress in understanding the complexities of rheumatoid arthritis, a simple basic research technique like thermal analysis can be an effective method for controlling the relationship between biomarkers and disease progression.

Hopefully, the increasing insights into the disease process, the better approaches to tailor treatments to each patient, and availability of safer/more effective medicines will eliminate the suffering and mortality associated with RA in the coming years.

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