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Abnormal lipid metabolism in collagen-induced arthritis rat model: *In vitro*, high resolution NMR spectroscopy based analysis

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Collagen-induced arthritis (CIA) was induced in female Wistar rats by intradermal injection of porcine immunization grade native collagen type II (Chondrex). Development and progression of CIA was monitored by studying histopathological, radiographical and biochemical features of arthritic manifestations in the knee joints, hind limb and blood plasma. In addition, oxidative stress status of arthritic animals was determined by measuring lipid peroxidation and the antioxidant enzymes: catalase, superoxide dismutase and glutathione peroxidase. High resolution proton NMR spectroscopy was employed for the analysis of lipid components in the lipid extracts of the joint tissue and plasma of collagen-induced arthritic and control rats. Triglyceride levels showed significant decreases in plasma (1.7 times) but were unchanged in the joint tissue of CIA rats as compared to control. One-dimensional proton NMR spectra showed a 6.2 times reduction in the quantity of choline-containing phospholipids in the plasma of CIA as compared to control rats. There was a 1.6 times elevation of choline-containing phospholipids in the joint tissue of CIA rats as compared to controls. Induction of arthritis showed a 4.0 times reduction in the level of total cholesterol in the plasma and 1.6 times elevation in the joint tissue of CIA rats as compared to controls. The ratio of saturated fatty acids to unsaturated fatty acids was 1.5 times significantly higher in joint tissue and 2.1 times significantly higher in plasma of CIA rats as compared to controls. The results demonstrated significantly altered lipid patterns in the joint tissue and plasma of collagen-induced arthritic rats as detected by one- and two-dimensional NMR spectroscopy compared with controls.

Keywords: Cholesterol, Collagen-induced arthritis, Lipid extraction, Lipid components, Metabolism, NMR spectroscopy, Oxidative stress, Phospholipids

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation of the synovial joints and destruction of articular cartilage[1,2]. The inflamed arthritic joint exhibits a markedly abnormal metabolic profile characterized by low-oxygen tension, decreased glucose concentrations, increased utilization of lipids for fuel, and high level of free fatty acids and ketone bodies[3]. Further the metabolites of synovial fluids may be markedly different from those of serum[3]. Rheumatoid arthritis patients were reported to have decreased concentrations of serum lipids, total serum cholesterol, LDL and HDL cholesterol, free-fatty acids and phospholipids[4,5]. Rheumatoid arthritis is thus accompanied by alterations in circulating metabolites[6]. Oxidative stress is thought to play an important role in the etiology of RA. Increased levels of lipid peroxidation in the erythrocytes and blood plasma of patients with RA and oxidative-stress associated decrease in phospholipids have been reported in children with chronic arthritis[7]. In an animal model of arthritis (k/BxN mice) abnormal generation of reactive oxygen species and lipolysis have been observed[6]. Decreased levels of cholesterol and phospholipids in the erythrocytes membranes and reduced levels of total lipids and cholesterol in the serum of RA patients have also been found[5].

The abnormality of lipid metabolism in patients with rheumatoid arthritis has been characterized in several studies. Reduced levels of total lipids, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, apolipoprotein A1 and HDL-cholesterol were observed in the serum of patients with RA as compared to healthy blood donors[4,8-11]. Anomalous decreases in the total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides have also been observed in the serum of adjuvant-induced arthritic rats[12].

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Proton NMR spectroscopy (^{1}H NMR spectroscopy) is a versatile technique and permits quick assessment of the nature and levels of a large numbers of biomolecules simultaneously[3]. This technique has been applied in many clinical studies and has major advantages over gas chromatography and liquid chromatography for the analysis of lipid components, and identification of lipid components is almost unequivocal even if a few distinct resonances are resolved[13,14].

There have been a number of proton NMR spectroscopy based studies on rheumatoid arthritis[3,6, 15]. These biochemical and NMR spectroscopy-based studies of the plasma and synovial fluid of arthritic patients and animal models have indicated the possibility of abnormal lipid metabolism in arthritis[3,6,15]. However, in these studies, absolute quantification of lipid components: triglycerides (TG), choline-containing phospholipids (PLc), total cholesterol (T.Chol) and the ratio of saturated fatty acids (SFA) to unsaturated fatty acids (USFA) in the plasma as well as in the joint tissue were not made.

Collagen-induced arthritis (CIA) in the rat is an autoimmunity-based disease and in many ways resembles clinical rheumatoid arthritis[1]. The collagen-induced arthritis rat model has thus been considered a relevant animal model of rheumatoid arthritis and has been used in many studies to investigate the pathogenesis of inflammatory arthritis,[2,16] and to evaluate the pharmaco-therapeutic potential of antiarthritic medication [17-20].

The main objective of the present study is thus to make a comparative analysis and quantitative estimation of lipid components in the plasma and joint tissue of CIA rats by high resolution ^{1}H NMR spectroscopy with a view to provide more precise data on altered metabolism of lipids in arthritis.

Materials and Methods

Induction of CIA—Female Wistar rats (5-7 weeks of age, weighing 150-180 g) were used. Rats were housed separately in cages, fed with standard rodent chow and water *ad libitum* and maintained at 12:12 h L:D cycles at 25±2 °C and 42±5%, RH. All experimental protocols were approved by the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) and the Institutional Animal Ethical Committee (IAEC number 18/2009). Animals were randomly divided into 2 groups of 10 animals each. One group served as control and another group comprised CIA rats. The induction of arthritis in animals was performed as per Sharma *et al*[21]. Rats were allowed to acclimatize for 7 days before initiating the experiment. Briefly, porcine immunization-grade native collagen type II (cII) (Chondrex) was dissolved overnight (2 mg/mL in 0.05 M acetic acid). Collagen solution was emulsified with an equal volume of complete Freund's adjuvant (Sigma). Each rat was immunized with a dose of 200 µg intradermally at multiple sites followed by a booster dose with the same antigen preparation on day 7. The gradual onset of arthritis started approximately 2 weeks after primary immunization and rats were observed daily for clinical signs of arthritis. Control rats were injected with saline intradermally at multiple sites.

Paw diameter measurement and arthritic index—Using a dial thickness gauge, hindpaw edema of the rats was monitored every 3rd day throughout the experiment. The arthritis Index (AI) was calculated by using the formula described by Coelho *et al*[22].

$$\text{AI (\%)} = \frac{\text{Hind paw diameter day (x) - Hind paw diameter on day (0)}}{\text{Hind paw diameter on day (0)}} \times 100$$

Macroscopic scoring of CIA—Clinical score, a composite index of disease severity and the number of limbs affected was assessed[23] every 4 days to monitor disease progression. The scores from each of the 4 limbs were then added to obtain an arthritic score. Since, CIA is more pronounced in the hind-limbs, the scoring of rat hind-limbs only has been presented here (data of arthritic score of all the 4 limbs are not shown). Two independent observers, blinded to the experimental groups, performed the scoring.

Body weight examination—Body weight was measured every 3rd day. The change in body weight (%) of each individual animal after the onset of arthritis on day 12 was calculated as follows:

$$\text{Change in body weight (\%)} = \frac{\text{Body weight on day (x) - Body weight on day (12)}}{\text{Body weight on day (12)}} \times 100$$

On the 30th day after arthritis induction, blood plasma was drawn from CIA and control rats by retro-orbital puncture. Rats were sacrificed by cervical dislocation and the joints were excised, trimmed of the excessive fat and connective tissue. Joint tissues were snap-chilled in liquid nitrogen and stored at -80 ºC till further use.

Radiological evaluation of CIA—After sacrificing the rats, the hind limbs were removed and the right hind leg was fixed in 10% buffered formalin for radiological evaluation of arthritic manifestations. All radiographs were taken with X-ray film (Kodak Diagnostic Film, Ready-Pack, X-OMATt, Kodak, Rochester, NY, U.S.A.) using MBR-1505R (Hitachi Medical Corporation, Tokyo, Japan). Settings for radiography were 5 mA, 40 kV and 1 sec exposure. Films were placed 60 cm below the X-ray source.

Histological assessment of arthritis damage—Knee joint was excised from the fixed hind leg, its skin and muscular parts were trimmed off and subjected to decalcification in 5% HNO_3 for 7-10 days, processed for paraffin embedding, sectioned at 10 µm followed by staining with haematoxylin-eosin and studied using light microscopy.

Biochemical estimations in plasma and joint tissue—Blood was collected in EDTA-coated vials followed by centrifugation at 2000 rpm for 10 min. Clear plasma was aliquoted and stored at -80 °C for biochemical studies. To prepare tissue homogenate, knee joint was excised, trimmed off excessive fat and muscular tissue, and homogenized in 0.05 *M* Tris-HCl buffer (*p*H 7.4) with protease inhibitor cocktail (3 µL/10 mL buffer) to yield a 10% (w/v) homogenate. The homogenate was centrifuged at 1000 *g* for 10 min. The resultant supernatant was transferred into pre-cooled centrifugation tubes and centrifuged at 12,000 *g* for 30 min. The supernatant (cytosolic fraction), after discarding any floating lipid layer was used for the estimation of lipid peroxidation (LPO) and superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GP_x) activities. Homogenates were kept at -80 ºC until the experiments were performed.

Estimation of lipid peroxidation—Thiobarbituric acid-reactive substance (TBARS), an index of lipid peroxidation was estimated by the method of Ohkawa *et al*[24]. The amount of TBARS was determined spectrophotometrically at 532 nm and expressed as nM of malondialdehyde (MDA)/mg protein. Tetramethoxy propane (TMP) was used as a standard.

Determination of superoxide dismutase, catalase and glutathione peroxidase activities—Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activity was determined spectrophotometrically at 37 ºC as per Singh *et al*[25]. Enzyme activity was expressed as units/mg protein.

Lipid extraction from the joint tissue and plasma of collagen-induced arthritic rat—Lipid extraction was performed from the joint tissue and plasma (0.5 mL) specimens of CIA rats as well as control rats by using methanol and chloroform sequentially in 1:3 ratio[26]. All dried and lyophilized lipid extract samples were re-dissolved in 0.5 mL cold (due to high volatility of chloroform) $CDCl_3$ and taken in 5 mm NMR tube (Wilmad no. 528, USA). The ^{1}H-NMR experiments were performed using a Bruker Avance 400 MHz spectrometer (Bruker Biospin, Zurich, Switzerland) at 25 °C.

^{1}H NMR spectroscopy parameters—One-dimensional proton NMR experiments using single pulse sequence were performed. Spectral width used was 8012 Hz with time domain data points of 32 K. Flip angle of the radio frequency pulse was 458 and total relaxation delay was 7.5 s (relaxation delay 5 s and acquisition time 2.5 s). Typically, 128 scans were accumulated for each sample and the resultant data were Fourier transformed after multiplying by exponential window function using a line broadening function of 0.3 Hz and an FT size of 32 000 points.

Sealed and reusable glass capillary with known concentration of TSP [3-(trimethylsilyl) propionic-2, 2, 3, 3-d4 acid, sodium salt] in D_2O was used as a standard reference. The NMR spectra for all the samples were recorded under uniform conditions.

Assignment of the lipid components in the lipid extract of joint tissue and plasma—Assignments of the lipid components in the lipid extract of joint tissue and plasma were completed with the help of one-dimensional single pulse sequence and two-dimensional (2D) total correlation spectroscopy (TOCSY) employing the Bruker's standard pulse program library. Assignments were also confirmed by literature and spectra of standard lipid components[26-28].

Two-dimensional (2D) total correlation spectroscopy (TOCSY) parameters—Total correlation spectroscopy (TOCSY) experiment was recorded by using following parameters: Spectral width = 8012 Hz, time domain data points= 2048; number of scans = 24; dummy scan = 16; relaxation delay = 1.5 second and spin lock time = 70 ms.

Quantification of lipid components (TG, PLc, T.Chol and SFA/ USFA) in joint tissue and plasma lipid extract—One-dimensional ^{1}H NMR spectra was used for the quantification of the lipid components in lipid extract of joint tissue and plasma. Total triglycerides (including triacylglycerol and diacylglycerol) were estimated by taking the integral area of – CH_2 proton of glycerol backbone at 4.14 ppm. In plasma, there was an overlap of phospholipid

glycerol backbone signal to the –CH2 proton of glycerol backbone at 4.14 ppm and accurate quantity of triglycerides was measured by subtraction of phospholipid quantity from the actual measured triglyceride quantity with overlapped signal. Integral area of $N^+(CH_3)_3$ signals of phospholipids at 3.30 ppm and C18 methyl signal of cholesterol at 0.68 ppm were obtained, relative to the TSP [3-(trimethylsilyl) propionic-2, 2, 3, 3-d4 acid, sodium salt] (a known quantity of TSP was dissolved in deuterated water and a definite volume was taken in a reusable sealed capillary tube) signal at 0.0 ppm, for the quantitative estimation of choline-containing phospholipids (PLc) (including phosphatidylcholine, sphingomyelin, plasmalogen, and platelet-activating factors) and total cholesterol (T.Chol) (including cholesterol and cholesterol esters) respectively. Using the integrals, the quantities of PLc and T.Chol were calculated by using computerized program for the quantification of lipids[26-28,32,33]. Ratio of saturated fatty acids (SFA) to unsaturated fatty acids (USFA) was calculated by taking the division of the sum of the integral areas of all the saturated fatty acid signals to the sum of all the unsaturated fatty acid signals.

Statistical analysis—Mean levels of TG, PLc and T.Chol in CIA and control rats were compared by Student's *t*-test for independent groups. The *P* values < 0.05 were considered significant. Mean level of the ratio of SFA/USFA in CIA and control rats were compared by Mann-Whitney 'U' test for independent groups. The data management and analyses were performed by using the statistical software SPSS version 15.0.

Results

Severity of CIA in Wistar rats—Clinical signs of arthritis (mild periarticular erythema and edema) accompanied by the redness and swelling of the joints first appeared in hind paws approximately 10 days post challenge. The disease progression increased in frequency and severity in a time-dependent manner and maintained a plateau of the peak of CIA response from day 15 to 30 (mean AI of 125.01) (Fig. 1a). Control rats showed no increase in hind paw diameter over time. Hind paw swelling and erythema also showed a time-dependent increase in severity with maximum arthritis scores of ~8 observed between days 12 and 30 in rats immunized with CII (Fig. 1b). There was no macroscopic evidence of either hind paw erythema in the control rats.

Change in body weight—Body weight was recorded every 3rd day during the experimental period of 30 days. The onset of arthritis was associated with a drastic body weight loss that continued till the end of the experimental period (Fig. 1c). Rats in the control group showed a gradual increase in their body weight with time.

Histopathological and radiographic analyses—On day 30, histological evaluation of the joints in the CIA animals revealed marked characteristic features of severe arthritis like synovial hyperplasia, massive mixed (neutrophil, macrophage, and lymphocyte) infiltration along with articular cartilage and bone erosion. No such arthritic features of inflammation and tissue destruction were evident in the joints of healthy control groups (Fig. 2a). On day 30, the

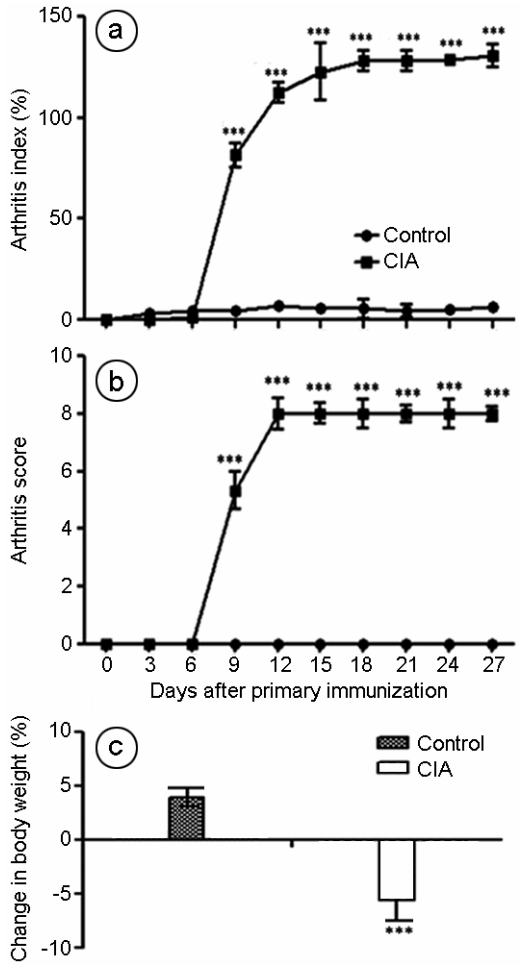

Fig. 1—Representative photographs of control and CIA rat hind paws, effect of immunization with type II collagen on arthritis progression as measured through, (a) arthritis index, (b) arthritic score and (c) change in body weight [Values are as mean ± SD of 10 animals per group. *P* value: ***< 0.0001].

radiographic analysis of hind paws from CIA rats revealed soft tissue swelling, bone matrix resorption and the joint space narrowing (Fig. 2b). There was no evidence of pathology in the control rats.

Lipid peroxidation—The lipid peroxide levels were significantly increased 2.8- ($P < 0.0001$) and 2-fold ($P < 0.0001$) in joints and plasma of CIA rats, respectively (Fig. 3a).

Antioxidant enzymes status of the joint tissue and plasma—The activities of the antioxidant enzymes, total superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were higher in the joints and plasma of CIA rats compared to control. Catalase showed 2.87- fold increase in the arthritic rat joints followed by 2.22- and 1.65-fold increases in the activity of SOD and GPx respectively. The joint tissue showed higher levels of antioxidant enzymes than the plasma (Fig. 3b-d).

Qualitative analysis of lipid components in joint tissue and plasma of rats—Lipid components were assigned in the lipid extract of joint tissue and plasma of rats. The assigned lipid components were fatty

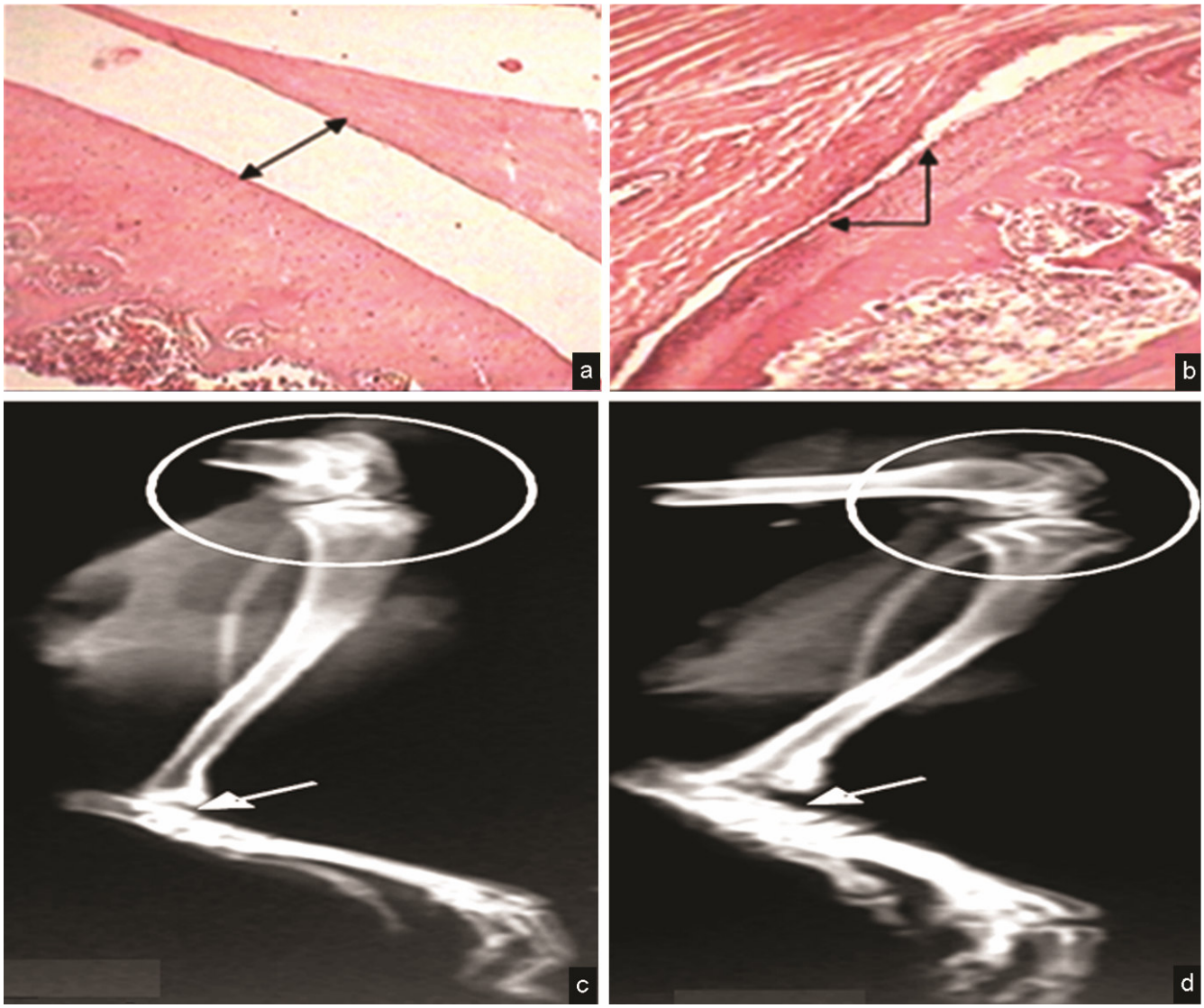

Fig. 2—(a) Histopathological features of (a) control rat showing the smooth and monolayer synovial cells lining (indication with arrow), and (b) CIA rats showing the hyperplasic synovial cell, erosion and disruption of synovial lining, Representative radiographs of the hind limbs showing the tibiotarsal and tibiofemoral joints of rats from (c) control and (d) CIA rats. Arrows indicate the bone damage and space narrowing in both tibiotarsal and tibiofemoral joints. The encircled areas grossly show the severe pathological conditions of the hind limbs, such as swollen joints and edema.

acids; saturated and unsaturated fatty acids (FA), triglycerides (TG), choline-containing phospholipids (PLc) and total cholesterol (T. Chol) (Fig. 4 a and b).

Quantitative analysis of lipid components in joint tissue and plasma rats—Quantative analysis of lipid components in the lipid extract of the joint tissue was performed. Triglyceride levels were unaltered in the joint tissue of CIA rats. There was a 1.6 times significant increase in the T. Chol and PLc in CIA rats ($P< 0.05$). The SFA/USFA ratio were 1.5 times significantly higher in the joint tissue (Table 1 and Fig. 5). Quantative analysis of lipid components in the lipid extract of plasma showed a 1.7 times decrease in TG level ($P<0.05$). There was a 4.0 times decrease in T. Chol as well as a 6.2 times decrease in PLc in CIA rats ($P< 0.05$). The ratios of SFA/USFA were 2.1 times higher in plasma of CIA rats (Table 1 and Fig. 6).

Discussion

The results of the present study demonstrated a highly significant elevation in lipid peroxidation in the joint tissue as well as in the plasma of CIA rats indicating increased oxidative stress in arthritis. The

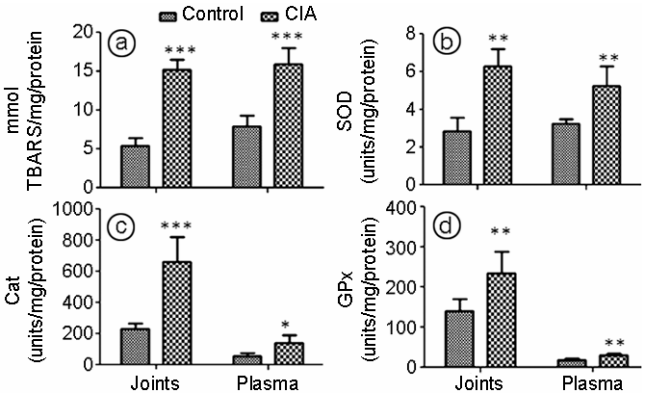

Fig. 3—Comparison of (a) lipid peroxidation, antioxidant enzyme activities (b) SOD, (c) CAT, (d) GPx in joints and plasma of control and CIA rats [Values are as mean ± SD of 10 animals per group. *P* values: *<0.05, **<0.01, ***<0.0001].

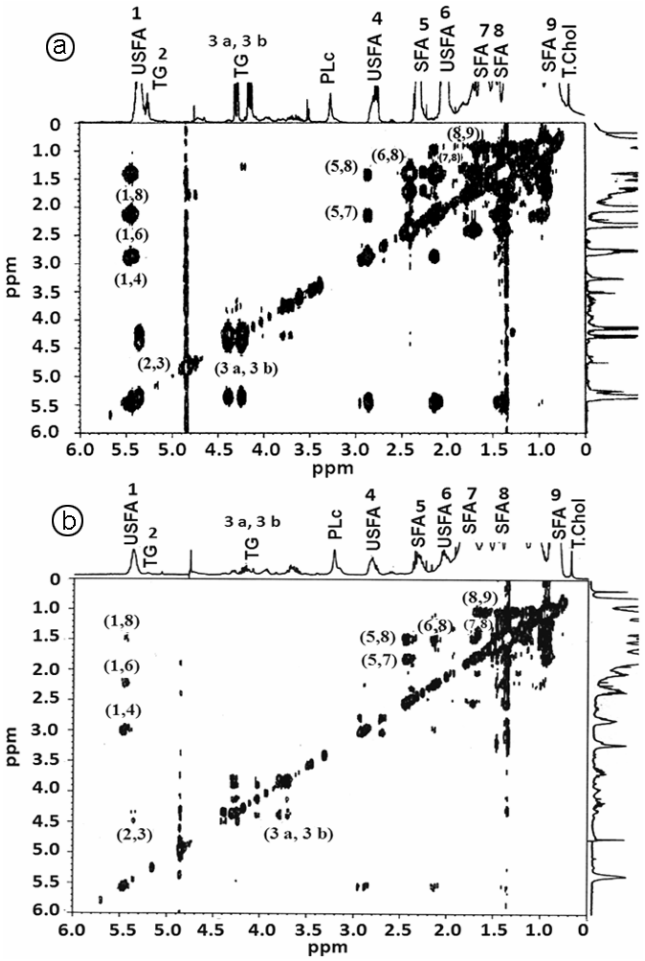

Fig. 4—Two-dimensional (2D) total correlation spectroscopy (TOCSY) of (a) joint tissue lipid extract and (b) plasma lipid extract of rat with assigned lipid components (TG = triglycerides, PLc = choline-containing phospholipids, SFA = saturated fatty acids, USFA = unsaturated fatty acids, Chol = cholesterol).

Table 1—Quantitative comparison of lipid components in lipid extract of the joint tissue and plasma of CIA and control rats [Values are mean ± SD from 10 animals in each group]

Female Wistar rats	TG (Triglycerides) (μM/g)	PLc (Choline-containing phospholipids) (μM/g)	T.Chol (Total cholesterol) (μM/g)	Ratio of SFA / USFA (Unsaturated fatty acids to saturated fatty acids)
		Joint tissue		
Control rats	163.14+84.81	15.48+1.84	10.14+0.69	7.05+ 0.73
CIA rats	149.71+69.54*	24.59+4.26 **	16.42+ 2.56 **	10.61+ 3.36***
P values: *> 0.05; **<0.05; ***<0.001	Student 't' test			Mann-Whitney U test
		Plasma		
Control rats	25.03+ 2.23	1.36+0.59	9.76+2.00	9.68+ 1.50
CIA rats	15.00+3.41**	0.22+0.07 **	2.53+ 0.74**	20.51+ 10.46 ***
P values: *> 0.05; **<0.05; ***<0.001		Student 't' test		Mann-Whitney U test

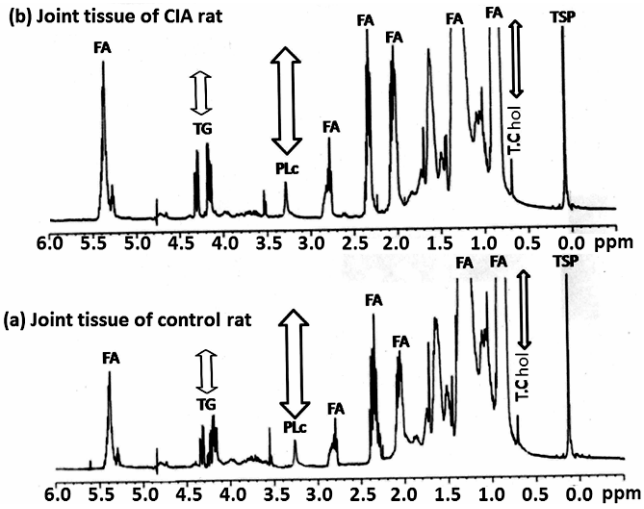

Fig. 5—One-dimensional proton NMR spectra of the joint tissue lipid extract of control rat as compared to CIA rat (FA = fatty acids).

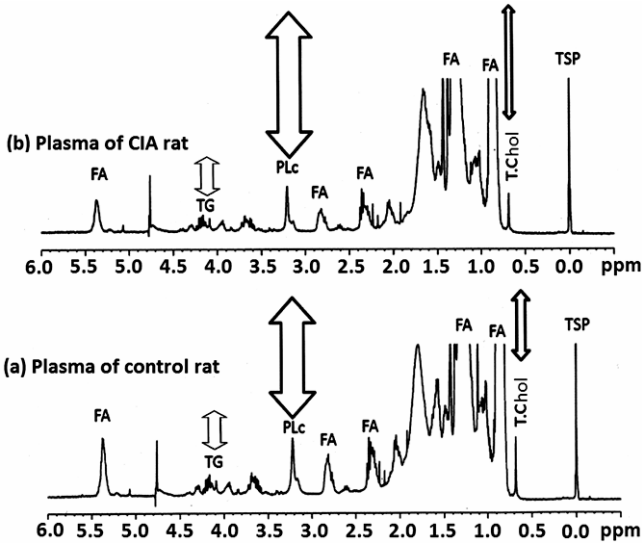

Fig. 6—One-dimensional proton NMR spectra of the plasma lipid extract of (a) control rat as compare to (b) CIA rat (FA = fatty acids).

antioxidant enzymes activities were, however, found elevated and this would obviously seem to be a compensatory response insufficient to counter the oxidative stress-causing processes. Increased activity of SOD with decreased activities of CAT and GPx has been observed in arthritic patients[34]. Even the present data showed that the increase in the activity of GPx was much lower compared with the increase in that of SOD and CAT.

The major finding of the present study emerging from the ^{1}H NMR-based analysis of the lipid components in the joint tissue and plasma of CIA rats is that there is a quantitative alteration in the lipid components (TG, T.Chol, PLc and SFA/ USFA) of the blood plasma and the joint tissue.

TG slightly decreased in the joint tissue of CIA rats as compared to control, but the decrease was not statistically significant. A significant decrease was observed in the plasma of CIA rats as compared to control. Ratio of SFA/USFA showed a marked elevation in both plasma and joints of CIA rats as compared to control. This again indicates that unsaturated fatty acids are reduced in both plasma and joint tissue of CIA rats. Synthesis of prostaglandin is known to be enhanced during inflammation, and this may be responsible for the higher consumption of unsaturated fatty acids[35,36] and these unsaturated fatty acids may come from the degradation of the triglycerides in the joint as well as in the blood. Therefore, elevated synthesis of prostaglandin may cause reduction in the levels of triglycerides and unsaturated fatty acids in the blood.

The present data showed that T.Chol and PLc were significantly reduced in the plasma of arthritic rats whereas they were elevated in their joint tissue. Weljie *et al*.[6] showed the occurrence of abnormal lipolysis in the serum of arthritic K/BxN mice and Lazarevic *et al*.[4] and other authors[34,37-41] reported that RA patients had significantly reduced level of total lipids and cholesterol in the serum. Results of these studies are thus consistent with the findings of the present study from CIA rats. Reduction in T. Chol level in the plasma of CIA rats may be due to a reduction of cholesterol in LDL and HDL as reported earlier[37-41].

Oxidative stress-associated decrease in phospholipid fraction has been reported in children with juvenile chronic arthritis[42]. It is known that there are differences between the metabolic profile of the rheumatoid joint and the serum of arthritic patients. Oxidative stress associated changes were reported in animal models of arthritis also[6]. A previous study on NMR-detectable differences between the synovial fluid and serum of rheumatoid arthritis patients showed the presence of very high levels of fatty acids and their increased utilization in the intra-articular tissue compared with that in the serum[3]. However, there is no report about alterations in choline-containing phospholipids and total cholesterol in rheumatoid arthritis. Weljie *et al*.[6] showed abnormal generation of reactive oxygen species in the serum of arthritic K/BxN mice and this may be responsible for decreased lipid levels. Since phospholipids are more susceptible to lipid peroxidation, decreased Plc in CIA rats may result from increased rate of lipid peroxidation[5].

Reduction in T. Chol in the plasma of CIA rat as observed in the present study can also be a consequence of elevated inflammation and enhanced lipid peroxidation[5]. Mature RBCs exchange the cholesterol and phospholipids from plasma as they lack capability for the *de novo* synthesis of cholesterol and phospholipids[43]. In a biochemical study[5], decreased levels of cholesterol and phospholipids were observed in the erythrocyte membranes of patients with RA. This is consistent with the NMR-based data on T.Chol and PLc levels seen in the present study on CIA rat.

Inflammation is associated with enhanced activity of the phospholipids-degrading or -hydrolyzing enzymes such as phospholipase A2 (EC 3.1.1.4), phospholipase C (EC 3.1.1.4), phospholipase D (EC 3.1.4.4), and sphingomyelinase (ceramide)[35]. Prostaglandin synthesis from unsaturated fatty acids is enhanced during inflammation[36]. Local and systemic elevation of inflammatory cytokines promotes lipolysis, and the consequent systemic release of free fatty acids contributes to the dyslipidemia in RA[44,45]. This may be a reason for the lowering of the PLc in the plasma of CIA rats observed in the present study. Higher consumption (lipolysis) of phospholipids may also be responsible for their lower levels in the blood. According to the P-31 NMR based study[15], the ratio of phosphatidylcholine to lysophosphosphatidylcholine detected in the plasma may serve as an indicator of the severity of RA in early stages. The lower value of the ratio of phosphatidylcholine/ lysophosphosphatidylcholine indicates the severity of RA i.e. more conversion of phosphatidylcholine into lysophosphatidylcholine through the two enzymes; lecithin cholesterol acyltransferase and PLA2 (phospholipase A2), which may be further responsible for the reduced value of phospholipids in plasma of CIA rats[15].

The present data showed that the quantity of T.Chol and PLc were significantly elevated in the joint tissue of CIA rats. In RA, the inflammation may cause hyperplasia of synoviocytes, infiltration of mononuclear cells, neoangiogenesis, pannus formation and finally joint destruction[46]. All of these abnormalities are also seen histopathologically in the joint tissue of CIA rats in the present study. The three most abundant cell populations in RA synovium are the monocyte/macrophage (type A) synoviocytes, the fibroblast-like (type B) synoviocytes, and -lymphocytes (which are strikingly heterogeneous). Other critically important cells of the RA synovium include B lymphocytes, plasma cells, dendritic cells, mast cells, endothelial cells, osteoclasts and adjacent chondrocytes[47]. All these cell populations are aggregated around the synovial joints and thus may lead to the elevation of cholesterol and phospholipids. Increased lipid levels may contribute to synovitis in rheumatoid arthritis through participation in the arachidonic acid pathway within the joint space[5, 48].

Synovium regeneration and limited cartilage self-regeneration occur in RA[49]. During regeneration or cell proliferation process, free cholesterol is required[50]. Phospholipids and cholesterol are the major structural components of the cell membrane[51] and the cell proliferation process uses phospholipids. In this regard, cholesterol and phospholipids are also transported from the liver via blood to the joint[50,51]. So the cell proliferation process may be further responsible for lowering the amount of total cholesterol and phospholipids in the blood and may increase their amount in the joint tissue of CIA rats.

In conclusion, the present study describes NMR-detectable abnormality in lipid metabolism in the joint tissue and plasma of CIA rats and provides novel data on these parameters. NMR spectroscopy has been extensively applied for clinical purposes such as in inherited disorders of lipid metabolism: Smith–lemli–opitz syndrome, cerebrotendinous xanthomatosis, sitosterolemia and refsum disease through the analysis of whole or lipid extract of blood and plasma[13]. NMR spectroscopy based analysis of lipid components in serum of Duchenne muscular dystrophy and brain tumor patients may be of possible diagnostic significance[28, 30]. Therefore, in the light of all these studies and the present results from collagen-induced arthritis indicate that NMR spectroscopic based results may be of diagnostic significance in clinical cases.

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References

1 Feldmann M, Brennan FM & Maini RN, Rheumatoid arthritis, *Cell*, 85 (1996) 307.

2 Kannan K, Ortmann RA & Kimpel D, Animal models of rheumatoid arthritis and their relevance to human disease, *Pathophysiology*, 12 (2005) 167.

3 Naughton D, Whelan M, Smith EC, Williams R, Blake DR & Grootveld M, An investigation of the abnormal metabolic status of synovial fluid from patients with rheumatoid arthritis by high field proton nuclear magnetic resonance spectroscopy, *FEBS*, 317 (1993) 135.

4 Lazarevic MB, Vitic J, Mladenovic V, Myones BL, Skosey JL & Swedler WI, Dyslipoproteinemia in the course of active rheumatoid arthritis, *Semin Arthritis Rheum*, 22 (1992) 172.

5 Vijayakumar D, Suresh K & Manoharan S, Altered pattern of lipids in plasma and erythrocytes membranes of rheumatoid arthritis patients, *Indian J Clin Biochem*, 20 (2005) 52.

6 Weljie AM, Dowlatabadi R, Miller JB, Vogel HJ & Jirik FR, An inflammatory arthritis-associated metabolite biomarker pattern revealed by ^{1}H NMR spectroscopy, *J Proteome Res*, 6 (2007) 3456.

7 Pasupathi P, Deepa M, Rani P & Sankar RR, Circulating lipid peroxidation, plasma and erythrocyte antioxidant status in patients with rheumatoid arthritis, *Bangladesh Med Res Counc Bull*, 35 (2009) 57.

8 Park YB, Lee SK, Lee WK, Suh CH, Lee CW, Lee CH, Song CH & Lee J, Lipid profiles in untreated patients with rheumatoid arthritis, *J Rheumatol*, 26 (1999) 1701.

9 Sattar N, McCarey DW, Capell H & McInnes IB, Explaning how "high-grade "systemic inflammation accelerates vascular risk in rheumatoid arthritis, *Circulation*, 108 (2003) 2957.

10 Hadda V, Handa R, Aggarwal P, Lakshmy R, Kumar U & Pandey RM, Disease activity and lipids in the rheumatoid arthritis: a prospective study, *Indian J Rheumatol*, 2 (2007) 137.

11 Yoo WH, Dyslipoproteinemia in patients with active rheumatoid arthritis: effect of disease activity, sex, and menopausal status on lipid profiles, *J Rheumatol*, 31 (2004) 1746.

12 Yamaguchi Y, Tanaka Y, Yamada K, Bandô Y & Kunitomo M, Abnormal Lipid Metabolism in Adjuvant Arthritic Rats, *Japan J Pharmacol*, 50 (1989) 377.

13 Oostendorp M, Engelke UF, Willemsen MA & Wevers RA, Diagnosing inborn errors of lipid metabolism with proton nuclear magnetic resonance spectroscopy, *Clin Chem*, 52 (2006) 1395.

14 Jukarainen N, NMR metabolomics techniques and mathematical tools as an aid in neurological diagnosis, *Kuopio Univ Pub Nat and Environ Sci*, 267 (2009) 18.

15 Fuchs B, Wagner U, Häntzschel H & Arnold K, The phosphatidylcholine/lyso-phosphatidylcholine ratio in human plasma is an indicator of the severity of rhe rheumatoid arthritis: investigation by 31P NMR and MALDI-TOF MS, Clin Biochem, 38 (2005) 925.

16 Myers LK, Rosloniec EF, Cremer MA & Kang AH, Collagen-induced arthritis, an animal model of autoimmunity, *Life Sci*, 61 (1997) 1861.

17 Salvemini D, Mazzon E, Dugo L, Serraino I, De Sarro A, Caputi AP & Cuzzocrea S, Amelioration of joint disease in a rat model of collagen-induced arthritis by M40403, a superoxide dismutase mimetic, *Arthritis Rheum*, 44 (2001) 2909.

18 Kumar N, Singh S, Patro N & Patro I, Evaluation of protective efficacy of *Spirulina platensis* against collagen-induced arthritis in rats, *Inflammopharmacology*, 17 (2009) 181.

19 Patro N, Sharma A, Kariaya K & Patro I, *Spirulina platensis* protects neurons via suppression of glial activation and peripheral sensitization leading to restoration of motor function in collagen-induced arthritic rats, *Indian J Exp Biol*, 49 (2011) 739.

20 Chen Z, Tu S, Hu Y, Wang Y, Xia Y & Jiang Y, Prediction of response of collagen-induced arthritis rats to methotrexate: an (1) H-NMR-based urine metabolomics analysis. *J Huazhong Univ Sci Technolog Med Sci*, 32 (2012) 438.

21 Sharma S, Sahu D, Das HR, Sharma D, Amelioration of collagen-induced arthritis by Salix nigra bark extract via suppression of pro-inflammatory cytokines and oxidative stress, Food Chem Toxicol, 49 (2011), 3395-3406.

22 Coelho MGP**,** Reis PA**,** Gava VB**,** Marques PR**,** Gayer CR**,** Laranja GAT**,** Felzenswalb I & Sabino KCC, Anti-arthritic effect and sub acute toxicological evaluation of Baccharis genistelloides aqueous extract, *Toxicol Lett,* 154 (2004) 69.

23 Cuzzocrea S, McDonald MC, Mota-Filipe H, Mazzon E, Costantino G, Britti D, Mazzullo G, Caputi AP & Thiemermann C, Beneficial effects of tempol, a membrane permeable radical scavenger, in a rodent model of collagen induced arthritis, *Arthritis Rheum*, 43 (2000) 320.

24 Ohkawa H, Ohishi N & Yagi K, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, *Anal Biochem*, 95 (1979) 351.

25 Singh RP, Padmavathi B & Rao AR, Modulatory influence of *Adhatoda vesica (Justicia adhatoda)* leaf extract on the enzymes of xenobiotic metabolism, antioxidant status and lipid peroxidation in mice, *Mol Cell Biochem*, 213 (2000) 99.

26 Srivastava NK, Pradhan S, Kumar R & Gowda GAN, An improved, single step standardized method of lipid extraction from human skeletal muscle tissue, *Anal Lett*, 39 (2006) 297.

27 Srivastava NK, Pradhan S, Mittal B, Kumar R, Pandey CM & Gowda GAN, Novel corrective equations for complete estimation of human tissue lipids after their partial destruction by perchloric acid pre-treatment: high-resolution 1H-NMR-based study, *NMR Biomed*, 21 (2008) 89.

28 Srivastava NK, Pradhan S, Gowda GAN & Kumar R, In vitro, high-resolution 1H and 31P NMR based analysis of the lipid components in the tissue, serum, and CSF of the patients with primary brain tumors, *NMR Biomed*, 23 (2010) 113.

29 Srivastava M, Jadegoud Y, Gowda GAN, Sharma A, Kapoor VK & Khetrapal CL, An accurate method for cholesterol analysis in bile, *Anal Lett,* 38 (2005) 2135.

30 Srivastava NK, Pradhan S, Kumar R & Gowda GAN, High resolution NMR based analysis of serum lipids in Duchenne muscular dystrophy patients and its possible diagnostic significance, *NMR Biomed*, 23(2010)13.

31 Ijare OB, Somashekar BS, Jadegoud Y, Gowda GAN, Sharma A, Kapoor VK & Khetrapal CL, Quantification of glycine and taurine conjugated bile acids in human bile using 1H NMR spectroscopy, *Magn Reson Med*, 53 (2005) 1441.

32 Gowda GAN, Ijare OB, Somashekar BS, Sharma A, Kapoor VK & Khetrapal CL, Single step analysis of individual conjugated bile acids in human bile using 1H NMR Spectroscopy, *Lipids*, 41 (2006) 591.

33 Gowda GAN, Somashekar BS, Ijare OB, Sharma A, Kapoor VK & Khetrapal CL, One step analysis of major bile

metabolites in human bile using 1H NMR spectroscopy, *Lipids*, 41 (2006) 577.

34 Taysi S, Polat F, Gul M, Sari RA & Bakan E, Lipid peroxidation, some extracellular antioxidants, and antioxidant enzymes in serum of patients with rheumatoid arthritis, *Rheumatol Int*, 21 (2002) 200.

35 Grimble RF, Dietary lipids and the inflammatory response, *Proc Nutr Soc,* 57 (1998) 535.

36 Calder PC, Long- chain polyunsaturated fatty acids and inflammation, *Scand J Food* Nutr, 50 (2006) 54.

37 Lorber M, Aviram M, Linn S, Scharf Y & Brook JG, Hypercholesterolemia and abnormal high-density lipoprotein in rheumatoid arthritis, *Br J Rheumatol*, 24 (1985) 250.

38 Boers M, Nurmohamed MT, Doelman CJ, Lard LR, Verhoeven AC, Voskuyl AE, Huizinga TW, van der Stadt RJ, Dijkmans BA & van der Linden S, Influence of glucocorticoid and disease activity on total and high density lipoprotein cholesterol in patients with rheumatoid arthritis, *Ann Rheum Dis*, 62 (2003) 842.

39 Situnayake R & Kitas G, Dyslipidemia & rheumatoid arthritis, *Ann Rheum Dis*, 56 (1997) 341.

40 Park YB, Lee SK, Lee WK, Suh CH, Lee CW, Lee CH, Song CH & Lee J, Lipid profiles in untreated patients with rheumatoid arthritis, *J Rheumatol*, 26 (1999) 1701.

41 Frati E, Castagna ML, Bacarelli MR, Fioravanti A, Giordano N, Taddeo A & Marcolongo R, Plasma levels of apolipoprotein and HDL cholesterol in patients with rheumatoid arthritis, *Boll Soc Ital Biol Sper*, 60 (1984) 1791.

42 Gorska A, Nawrocki A, Urban M & Florys B, Composition of phospholipid fatty acids in erythrocyte membranes of children with chronic juvenile arthritis: clinical and biochemical correlations, *Med Sci Monit*, 6 (2000) 30.

43 Van Deenen *LLM* & De Gier J, Chemical composition *and* metabolism of lipids in red cells of various animal species, in: *The* r*ed blood cell. A comprehensive treatise* (Bishop, C. Surgenor, D.M. eds.) Academic Press, New York, (1964) 243.

44 Dai L, Lamb DJ, Leake DS, Kus ML, Jones HW, Morris CJ & Winyard PG, Evidence of oxidized low density lipoprotein in synovial fluid from rheumatoid arthritis patients, *Free Radic Res*, 32 (2000) 479.

45 Winyard PG, Tatzber F, Esterbauer H, Kus ML, Blake DR & Morris CJ, Presence of foam cells containing oxidized lipoprotein in the synovial membrane from patients with rheumatoid arthritis, *Ann Rheum Dis*, 52 (1993) 677.

46 Kim HJ & Berek C B, Cells in rheumatoid arthritis, *Arthritis Res*, 2 (2000)113.

47 Fox DA, Cell-cell Interactions in Rheumatoid Arthritis Synovium, *Rheum Dis Clin North Am*, 36 (2010) 311.

48 Prete PE & Gurakar-Osborne A, The contribution of synovial fluid lipoprotein to the chronic synovitis of rheumatoid arthritis, *Prostaglandins*, 54 (1997) 689.

49 Ringe J, Burmester GR & Sittinger M, Regenerative medicine in rheumatic disease-progress in tissue engineering, *Nat Rev Rheumatol*, 8 (2012) 493.

50 Brown MS, Ho YK & Goldstein JL, The cholesteryl ester cycle in macrophage foam cells continual hydrolysis and re-esterification of cytoplasmic cholesteryl esters, *J Biol Chem*, 255 (1990) 9344.

51 David LN & Michael MC, Lehninger principle of biochemistry, 3rd edn (Worth publishers: New York) 2002, 368.