



Fatty acids uptake and oxidation are increased in the liver of rats with adjuvant-induced arthritis



Mariana M.N. Wendt^a, Monique Cristine de Oliveira^a, Gabriela B. Franco-Salla^a, Lorena Santos Castro^a, Ângela V. Parizotto^a, Francielli M. Souza Silva^b, Maria R.M. Natali^c, Ciomar A. Bersani-Amado^b, Adelar Bracht^a, Jurandir F. Comar^{a,*}

^a Department of Biochemistry, State University of Maringá, PR, Brazil

^b Department of Pharmacology and Therapeutics, State University of Maringá, PR, Brazil

^c Department of Morphological Sciences, State University of Maringá, PR, Brazil

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ABSTRACT

Severe rheumatoid cachexia is associated with pronounced loss of muscle and fat mass in patients with advanced rheumatoid arthritis. This condition is associated with dyslipidemia and predisposition to cardiovascular diseases. Circulating levels of triglycerides (TG) and free fatty acids (FFA) have not yet been consistently defined in severe arthritis. Similarly, the metabolism of these lipids in the arthritic liver has not yet been clarified. Aiming at filling these gaps this study presents a characterization of the circulating lipid profile and of the fatty acids uptake and metabolism in perfused livers of rats with adjuvant-induced arthritis. The levels of TG and total cholesterol were reduced in both serum (10–20%) and liver (20–35%) of arthritic rats. The levels of circulating FFA were 40% higher in arthritic rats, possibly in consequence of cytokine-induced adipose tissue lipolysis. Hepatic uptake and oxidation of palmitic and oleic acids was higher in arthritic livers. The phenomenon results possibly from a more oxidized state of the arthritic liver. Indeed, NADPH/NADP⁺ and NADH/NAD⁺ ratios were 30% lower in arthritic livers, which additionally presented higher activities of the citric acid cycle driven by both endogenous and exogenous FFA. The lower levels of circulating and hepatic TG possibly are caused by an increased oxidation associated to a reduced synthesis of fatty acids in arthritic livers. These results reveal that the lipid hepatic metabolism in arthritic rats presents a strong catabolic tendency, a condition that should contribute to the marked cachexia described for arthritic rats and possibly for the severe rheumatoid arthritis.

1. Introduction

Rheumatoid arthritis is an autoimmune and chronic inflammatory disease that leads to progressive joint destruction. In addition to the joints it affects other organs, such as brain, liver and muscles [1,2]. Rheumatoid arthritis affects 1.0% of the adult population worldwide and is associated with progressive disability and premature mortality, mainly due to cardiovascular complications [3]. Metabolic alterations are prominent in rheumatoid arthritis, particularly the muscle wasting condition known as rheumatoid cachexia, which occurs in approximately two-thirds of all patients and is related to dyslipidemia and

cardiovascular disease [3,4]. In rheumatoid cachexia, muscle wasting normally occurs with unaltered body weight, however, nearly 10% of the patients experience a stage of chronic wasting disease, with simultaneous muscle and fat wasting and reduced body weight [5,6]. The latter occurs in severe rheumatoid arthritis and is associated with an even greater occurrence of cardiovascular illness and mortality [5]. Arthritic cachexia is mediated by cytokines, especially tumor necrosis factor- α (TNF- α) and interleukin 1 (IL-1), which stimulate the whole-body protein breakdown and lipolysis [7].

Increased risk for cardiovascular diseases in rheumatoid arthritis seems also related to altered lipid metabolism, however, this

Abbreviations: FFA, free fatty acids; FA, fatty acids; TG, triglycerides; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; ROS, reactive oxygen species; GSH, reduced glutathione; GSSG, oxidized glutathione; TNF- α , tumoral necrosis factor alpha; IL-1 and 6, interleukin 1 and 6; NFkB, nuclear factor kappa B; FAS, fatty acid synthase; ACC, acetyl-CoA carboxylase; MDH, malate dehydrogenase; CS, citrate synthase; CAT I and II, carnitine acyltransferase I and II; CoA, coenzyme A; (FAT)/CD36, fatty acids translocase/CD36; MCT1, monocarboxylate transporter 1; PGC-1 α , peroxisome proliferator-activated receptor gamma (PPAR- γ) coactivator-1 alpha; HPLC, high-performance liquid chromatography; LBM, loss of body mass

* Corresponding author at: Department of Biochemistry, University of Maringá, 87020900 Maringá, Brazil.

E-mail address: jurandircomar@yahoo.com.br (J.F. Comar).

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relationship has been not yet fully clarified [7,8]. Hyperlipidemia is not common, but dyslipidemia is frequent and patients present reduced levels of plasma HDL cholesterol and normal or even diminished total cholesterol [9,10]. The latter is further reduced in severe arthritis and related to an even higher incidence of cardiovascular diseases [11]. The plasma cholesterol profile in severe rheumatoid arthritis is similar to that in other chronic wasting diseases. However, the reports about the levels of triglycerides (TG) and free fatty acids (FFA; non esterified fatty acids) present inconsistencies as reductions, no alterations and even increases have been reported [12–14].

High levels of plasma FFA occur as the result of increased adipose lipolysis associated with reduced FFA uptake in muscle and liver [15]. Hypertriglyceridemia results from increased hepatic synthesis of fatty acids (FA) and TG associated to reduced peripheral uptake [16]. It has been proposed that in rheumatoid arthritis proinflammatory cytokines stimulate adipose tissue lipolysis, hepatic synthesis of FA and TG and inhibit peripheral lipoprotein lipase, what together culminates in high plasma levels of TG, and perhaps FFA [14,16]. However, it has been also claimed that severe inflammation promotes consumption and reduces the synthesis of lipoproteins, resulting in lower levels of TG in advanced rheumatoid arthritis [3,8]. These assumptions seem contradictory and in fact they lack experimental confirmation as they were extrapolated from data relating cachexia to accelerated atherogenesis in chronic wasting diseases [17,18]. It is known that lipolysis is increased in severe arthritis, however, high levels of plasma FFA do only occur if their uptake is reduced, especially in the liver, which plays a central role in this respect [5].

The hepatic lipid metabolism has been proposed to be similarly modified for most chronic wasting diseases and can be summarized as follows: increased FFA uptake and synthesis associated to increased TG synthesis and release [17]. However, it is not known if this also applies to rheumatoid arthritis. Aiming at filling these gaps this study presents a characterization of the circulating lipid profile and of fatty acids uptake and metabolism in perfused livers of Holtzman rats with adjuvant-induced arthritis. Several metabolic modifications have been found in perfused livers of rats with adjuvant-induced arthritis. They consist in accelerated oxidative metabolism, reduced gluconeogenesis and increased glycolysis [19,20]. This indicates that, at least for glucose, hepatic metabolism in arthritis seems to be characterized by a prevalence of catabolic over anabolic pathways, in disagreement to what has been reported for other chronic diseases [17,21].

The accelerated oxidative metabolism in the liver of arthritic rats has been proposed to be caused by a more oxidized cellular environment which, in turn, is associated to an increased oxidative stress in the organ [22]. Under such conditions one can also expect stimulation of the FA catabolism, a phenomenon that should, in turn, accentuate cachexia and diminish the plasma levels of TG in severe arthritis. Moreover, the NADH/NAD⁺ and NADPH/NADP⁺ ratios, which regulate directly FA metabolism, were not yet measured in the arthritic liver. Therefore, in the present study, the investigations of FA metabolism were complemented by evaluations of the hepatic redox status in terms of the NADH/NAD⁺ and NADPH/NADP⁺ ratios [23,24]. The results of the present study should allow extrapolations to patients with severe rheumatoid arthritis.

2. Materials and methods

2.1. Materials

Free Fatty Acid Quantitation Kit, fatty acid-free bovine serum albumin, enzymes and coenzymes were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Labelled [1-¹⁴C]octanoic acid, [1-¹⁴C]palmitic acid, [1-¹⁴C]oleic acid and L-[methyl-³H]carnitine were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and New England Nuclear (Boston, MA, USA). All other chemicals were of analytical grade.

2.2. Animals and induction of arthritis

Male Holtzman rats weighting 180–210 g (50 days old) were obtained from the Center of Animal Breeding of the State University of Maringá (UEM) and maintained under standard laboratory conditions at a temperature of 24 ± 3 °C under a regulated 12 h light/dark cycle. The animals were housed in conventional steel cages (3 rats/cage) and fed ad libitum with a standard laboratory diet (Nuvilab®, Colombo, Brazil; see dietary composition in Table S1). Eighty animals were used in eight different experimental protocols followed throughout the study and, for each one, the animals were randomly distributed into control and arthritic groups. For arthritis induction, animals were injected subcutaneously in the left hind paw with 0.1 mL (500 µg) of Freund's adjuvant (heat inactivated *Mycobacterium tuberculosis*, derived from the human strain H37Rv), suspended in mineral oil [25]. Injection of the adjuvant was performed under analgesic action (sodium dipyrone 100 mg·kg⁻¹). Animals were used for experiments at day 18th after adjuvant injection. Rats of similar ages were injected with mineral oil and served as controls. All procedures followed the guidelines of the Brazilian Council for the Control of Animal Experimentation (CONCEA) and the European Directive 2010/63/EU. The animal facilities and protocols were previously approved by the Ethics Committee for Animal Experimentation of the State University of Maringá (Protocol number 094/2013-CEAE).

2.3. Cachexia and arthritis characterization

The body weight of animals was monitored daily and the loss of body mass (LBM) was calculated as follows $LBM = 100 \times (BM_i - BM_f + GBM)/(BM_i + GBM)$, where BM_i and BM_f are, respectively, initial and final body mass of the arthritic rats (g). GBM is the body mass gain of the control rats (g). Rats are considered cachectic when the LBM was higher than 10% (18). The volume of both hind paws up to the tibiotarsal joint was measured daily by plethysmography [5] and severity of secondary lesions were assessed from the 10th to the 18th day according previously described [19]. Food intake was monitored daily and expressed as g per rat per 100 g body weight.

2.4. Plasma and liver metabolites

Fed and 12 h fasted rats were intraperitoneally anesthetized with sodium thiopental (100 mg·kg⁻¹) plus lidocaine (10 mg·kg⁻¹). The peritoneal cavity was surgically exposed and blood was collected from the cava vein to obtain the plasma. The liver was removed, freeze-clamped and stored in liquid nitrogen for adenine nucleotides and lipids assessment. Epididymal and mesenteric adipose tissue and gastrocnemius and soleus muscles were removed and weighted. Total lipids were extracted from the freeze-clamped livers with chloroform and methanol as previously described [26]. Total lipids were measured by gravimetry and thereafter dissolved in a mixture of chloroform and isopropanol (1:2) for TG and cholesterol. Plasma and hepatic glucose, TG, total cholesterol and HDL cholesterol were assayed using commercial kits (Gold Analisa®). LDL and VLDL cholesterol were calculated according to Friedewald's equation. FFA was measured using a FFA Quantification Kit (CN MAK044; Sigma-Aldrich). Acetoacetate and β-hydroxybutyrate were assayed by spectrophotometry (340 nm) using β-hydroxybutyrate dehydrogenase [27]. The levels of glycerol in the plasma were measured by spectrophotometry as previously described [27]. Briefly, 50 µL of deproteinized plasma was added to 1.0 mL of a mixture containing 0.137 M glycine, 0.69 M hydrazine, 137 mM magnesium chloride, 3-glycerophosphate dehydrogenase (4 U·mL⁻¹), 1.23 mM ATP and 0.49 mM NAD⁺. After reading the absorbance (E1) at 340 nm, glycerokinase (8.5 U·mL⁻¹) was added and the absorbance (E2) was read again at 340 nm. The increment in the absorbance (E2–E1) was used to calculate the glycerol concentration. The activity of myeloperoxidase (MPO) in the plasma was measured by the o-

dianisidine method [19].

2.5. Liver perfusion

Hemoglobin-free non-recirculating liver perfusion was performed as previously described [28]. After cannulation of the portal and cava veins, the liver was removed and positioned in a plexiglass chamber. The perfusion fluid was Krebs/Henseleit-bicarbonate buffer (pH 7.4) containing 25 mg% bovine serum albumin and saturated with a mixture of oxygen and carbon dioxide (95:5) by means of a membrane oxygenator with simultaneous temperature adjustment at 37 °C. The flow was maintained constant by a peristaltic pump (Minipuls 3, Gilson, France). Oxygen concentration in the venous perfusate was monitored by a teflon-shielded platinum electrode. Samples of the effluent perfusion fluid were collected at two minutes intervals and analyzed for the following metabolite content: lactate, pyruvate, acetoacetate and β -hydroxybutyrate [27].

2.6. Activity of citric acid cycle

Livers from fed and 12 h fasted rats were perfused with Krebs/Henseleit-bicarbonate buffer. After stabilization of oxygen consumption, the livers were perfused with trace amounts of [^{14}C]octanoic acid ($0.01 \mu\text{Ci}\cdot\text{mL}^{-1}$) during 20 min. This procedure effectively measures the citric acid cycle via labelling of the acetyl-CoA pool [29]. The perfusate effluent was collected in Erlenmeyer flasks and carbon dioxide was measured by trapping $^{14}\text{CO}_2$ in phenylethylamine [30]. Radioactivity was measured by liquid scintillation spectrometry (Tri-Carb 2810 TR counter, Perkin Elmer) and the results expressed as fraction of the inflowing radioactivity. The following scintillation solution was used: toluene/ethanol (2:1) containing $5 \text{ g}\cdot\text{L}^{-1}$ 2,5-diphenyloxazole and $0.15 \text{ g}\cdot\text{L}^{-1}$ 2,2-*p*-phenylenebis(5-phenyloxazole).

2.7. Fatty acids uptake and oxidation to $^{14}\text{CO}_2$

Livers were perfused with Krebs/Henseleit-bicarbonate buffer and 0.3 mM palmitic acid or oleic acid during 10 min. The FA concentrations were determined in the effluent and inflowing perfusate using the FFA Quantification Kit (CN MAK044). The rates of FFA uptake were calculated from the portal-venous concentration differences.

For measuring the oxidation of FA to $^{14}\text{CO}_2$, 0.3 mM [^{14}C]palmitic acid or 0.3 mM [^{14}C]oleic acid ($0.2 \mu\text{Ci}\cdot\text{mL}^{-1}$), complexed with free-fatty acid bovine-serum albumin (0.15 mM), were infused during 20 min. The effluent perfusate was collected in Erlenmeyer flasks, $^{14}\text{CO}_2$ trapped in phenylethylamine and measured as described for the citric acid cycle.

2.8. Hepatic contents of adenine nucleotides

The hepatic contents of adenine nucleotides were determined by high-performance liquid chromatography (HPLC) analysis (Shimadzu, Japan). The acid-stable adenine nucleotides (ATP, ADP, AMP, NADP^+ and NAD^+) were extracted with 0.6 M perchloric acid [31] and the acid-labile NADH and NADPH were extracted with ethanol plus chloroform [32]. A reversed-phase C18 CLC-ODS column ($5 \mu\text{m}$, $250 \times 4.6 \text{ mm}$ i.d.; Shimadzu) protected with a CLC-ODS precolumn ($5 \mu\text{m}$, $4 \times 3 \text{ mm}$ i.d.; Phenomenex), was used with a gradient from reversed-phase $0.044 \text{ M}\cdot\text{L}^{-1}$ phosphate buffer solution (PBS; pH 6.0) to $0.044 \text{ M}\cdot\text{L}^{-1}$ PBS plus methanol (1:1; pH 7.0). For acid-stable nucleotides, in percent methanol, the gradient was the following: at 0 min, 0%; at 2.5 min, 0.5%; at 5 min, 3%; at 7 min, 5%; at 8 min, 12%; at 10 min, 15%; at 12 min, 20%; at 20 min, 30%. For NADH and NADPH, the gradient was linear from 0 to 20 min ranging from 0 to 30% of methanol. Monitoring was performed spectrophotometrically at 254 nm for acid-stable nucleotides and at 340 nm for NADH and NADPH.

2.9. Activity of carnitine acyltransferases I and II (CAT I and II)

Firstly, rat liver mitochondria were isolated by differential centrifugation and frozen in liquid nitrogen [31]. The extraction of the CAT I and II fractions was performed as previously described [33]. Both enzymes were assayed in the direction of palmitoyl-carnitine synthesis. The substrates were 0.5 mM ($5 \mu\text{Ci}/\mu\text{mol}$) [^3H]carnitine and 50 μM palmitoyl-CoA. The incubation system contained 50 mM manitol, 75 mM KCl, 5% fatty acid-free bovine-serum albumin, 2 mM KCN, 25 mM EGTA, 50 μM palmitoyl-CoA and approximately 0.3 mg protein- mL^{-1} of either the CAT I or the CAT II preparation. The reaction (at 37 °C) was started by the addition of 0.5 mM carnitine plus $5 \mu\text{Ci}/\mu\text{mol}$ [^3H]carnitine and stopped at various times (1–6 min) by the addition of perchloric acid. The reaction product, [^3H]palmitoyl-carnitine, was extracted with butanol and the radioactivity was measured by liquid scintillation spectrometry.

2.10. Activity of acetyl coenzyme A carboxylase (ACC)

The acetyl-CoA carboxylase (ACC) activity was determined by [^{14}C] sodium bicarbonate incorporation leading to the formation of [^{14}C] malonyl-CoA as previously described [34]. Briefly, the freeze-clamped liver were macerated in liquid nitrogen and eight volumes of a medium containing 0.1 M manitol, 50 mM sodium fluoride, 10 mM Tris, 1 mM EDTA, $5 \text{ mg}\cdot\text{L}^{-1}$ aproptin and $5 \text{ mg}\cdot\text{L}^{-1}$ leupeptin. The suspension was homogenized in a van Potter-Elvehjem homogenizer, centrifuged ($48,000\text{g}/20 \text{ min}$), the enzyme was precipitated with $144 \text{ mg}\cdot\text{mL}^{-1}$ ammonium sulfate and centrifuged again. The pellet was resuspended in the homogenization medium and the reaction was initiated by adding an aliquot (200 μL ; 0.1–0.2 mg protein) of the enzyme to 0.8 mL of the assay solution containing 50 mM Tris-HCl (pH 7.5), potassium citrate up to 20 mM, 10 mM MgCl_2 , 3.75 mM ATP, 0.125 mM acetyl-CoA, 3.75 mM GSH, $0.75 \text{ mg}\cdot\text{mL}^{-1}$ fatty acid-free bovine-serum albumin and 12.5 mM [^{14}C]sodium bicarbonate ($4.5 \text{ nCi}\cdot\text{mL}^{-1}$). After centrifugation, an aliquot of the supernatant was deposited in the scintillation flask and the remaining $^{14}\text{CO}_2$ removed in a drying oven. The Bray's scintillator solution was then added to the flask and ^{14}C measured by liquid scintillation spectrometry.

2.11. Statistical and numerical analyses

The error parameters presented in graphs and tables are standard errors of the means. Statistical analysis was done by means of the GraphPad Prism Software (version 5.0). The Student's *t*-test was applied and the 5% level ($p < 0.05$) was adopted as a criterion of significance. Least-squares fitting of Eq. (1) to the experimental data (acetyl-CoA carboxylase activity) was done by means of the non-linear iterative procedure of the Scientist® program from MicroMath Scientific Software. The same program was also used for fitting a polynomial of the third degree to the time courses of the experimental carnitine acyltransferases activities. The initial slopes of the fitted curves were taken as the enzyme activities.

3. Results

3.1. Cachexia and arthritis characterization

Adjuvant-induced arthritis has been already characterized in rats [19,23,24,35–37]. For this reason, the results of cachexia and arthritis characterization in the present study are shown as supplementary material (Figs. S1 and S2). Temporal evolution of paw edema and arthritic score is shown in Fig. S1. At day 18 the volume of the injected and contralateral paws had considerably increased in arthritic rats (Fig. S1A and B). Secondary lesions appeared at day 10 and reached the highest scores at day 18 (Fig. S1C). A five-fold higher activity of MPO was found in the plasma of arthritic rats (Fig. S1D). In the liver, arthritis

Table 1

Lipid and ketone bodies profiles in the plasma and liver of control and arthritic rats. The levels of lipids and glycerol were assessed in fed and 12 h fasted rats. The levels of ketone bodies were assessed only in the fasted rats. The values are the mean \pm standard error of five animals for each experimental condition. Pairs of values in the same line labelled with superscripts are statistically different: [#]p < 0.05, *p < 0.01; **p < 0.001.

	Fasted		Fed	
	Control	Arthritis	Control	Arthritis
Plasma				
Glycemia (mg·dL ⁻¹)	69.5 \pm 2.4	73.5 \pm 5.9	106.2 \pm 6.7	111.0 \pm 5.6
Triglycerides (TG; mg·dL ⁻¹)	75.1 \pm 3.7	59.3 \pm 2.0*	73.9 \pm 3.8	78.1 \pm 1.5
Total cholesterol (mg·dL ⁻¹)	95.8 \pm 4.3	73.8 \pm 3.8*	97.2 \pm 1.1	89.3 \pm 1.1**
HDL cholesterol (mg·dL ⁻¹)	14.1 \pm 0.9	11.3 \pm 0.6 [#]	17.0 \pm 0.9	13.8 \pm 0.4*
LDL cholesterol (mg·dL ⁻¹)	67.6 \pm 3.2	51.0 \pm 3.3 [#]	–	–
VLDL cholesterol (mg·dL ⁻¹)	15.0 \pm 0.7	11.9 \pm 0.4*	–	–
Free fatty acids (FFA; μ M)	89.5 \pm 3.5	123.4 \pm 5.2*	40.0 \pm 2.3	57.3 \pm 2.8*
Glycerol (μ M)	44.8 \pm 3.4	74.2 \pm 4.2*	26.0 \pm 3.1	36.2 \pm 2.2 [#]
β -Hydroxybutyrate (μ M)	314.8 \pm 9.9	105.8 \pm 4.8**	–	–
Acetoacetate (μ M)	20.8 \pm 2.8	80.6 \pm 7.8*	–	–
Total ketone bodies (μ M)	335.6 \pm 8.9	186.4 \pm 3.9*	–	–
β -Hydroxybutyrate/acetoacetate	16.2 \pm 2.9	1.4 \pm 0.2**	–	–
Liver				
Total lipids (g·100 g liver ⁻¹)	4.0 \pm 0.2	3.0 \pm 0.1 [#]	2.9 \pm 0.1	2.4 \pm 0.1 [#]
Triglycerides (mg·g total lipids ⁻¹)	55.3 \pm 0.6	35.8 \pm 2.1**	60.5 \pm 2.5	38.9 \pm 2.2**
Total cholesterol (mg·g total lipids ⁻¹)	106.6 \pm 5.8	80.4 \pm 3.5 [#]	72.0 \pm 2.4	58.5 \pm 2.4 [#]

increased the MPO activity by 37%.

Temporal evolution of body weight gain and food intake is shown in Fig. S2. In practical terms, arthritic rats did not gain body weight during this period and compared to the healthy condition, thus, arthritis prevented a body mass gain of 32.8% (Fig. S2A). Food intake of arthritic rats was lower during the whole time interval, except on day 12 (Fig. S2B). At day 18 the weight of epididymal and mesenteric adipose tissue and gastrocnemius and soleus muscles were considerably lower in arthritic rats (Fig. S2C).

3.2. Lipid and ketone bodies profiles

Plasma and hepatic lipid profiles of control and arthritic rats are shown in Table 1. For the fasted state, the levels of TG and total, HDL, LDL and VLDL cholesterol were approximately 20% lower and the levels of FFA and glycerol were 38% and 66% higher in the plasma of arthritic rats. The levels of acetoacetate were 280% higher and the levels of β -hydroxybutyrate and total ketone bodies were 67 and 45% lower, respectively, in the plasma of arthritic rats. These opposite effects on the acetoacetate and β -hydroxybutyrate levels produced a 11.6-fold diminution of the β -hydroxybutyrate/acetoacetate ratio. For the fed state, the levels of total, HDL and LDL cholesterol were 8, 19 and 13% lower in the plasma of arthritic rats. The levels of plasma TG were not affected by arthritis in the fed state, but those of FFA and glycerol were 43 and 38% higher in the arthritic condition.

Hepatic levels of total lipids, TG and total cholesterol in the fasted state were 25, 35 and 25% lower in arthritic rats. For fed state, hepatic levels of total lipids, TG and total cholesterol were 17, 36 and 19% lower in arthritic rats. Fed and fasted glycemia were not modified by arthritis. Glucose tolerance test (GTT) was also performed and no difference was observed between control and arthritic rats (not shown).

3.3. Citric acid cycle

Fig. 1A illustrates the time courses of ¹⁴CO₂ production of the experiments in which trace amounts of [¹⁴C]octanoate were infused in livers from fed rats in order to label the acetyl-CoA pools. The ¹⁴CO₂ production under these conditions reflects the citric acid cycle activity [38]. The infusion of trace amounts of [1-¹⁴C]octanoate produces no net alterations in oxygen uptake, does not affect the glycolytic flux, but effectively labels the acetyl-CoA pool [29]. The numerical values of each parameter at 30 min perfusion time in Fig. 1A (steady state) were

displayed in Table 2. In livers from arthritic rats the rates of ¹⁴CO₂ production were 23% higher and those of lactate and glycolysis 50% higher. The lactate/pyruvate ratio was 1.7-fold higher in the arthritic liver, indicating a higher cytosolic NADH/NAD⁺ ratio.

Fig. 1B illustrates the time courses of ¹⁴CO₂, acetoacetate and β -hydroxybutyrate productions in the perfused livers of fasted rats upon the infusion of trace amounts of [¹⁴C]octanoate. Under this condition, perfused livers survive at the expense of endogenous FA oxidation and may additionally produce ketone bodies. The numerical steady state values are shown in Table 2. ¹⁴CO₂ and β -hydroxybutyrate productions were not different, but oxygen consumption and acetoacetate production were 23 and 190% higher in the perfused livers of arthritic rats. The β -hydroxybutyrate/acetoacetate ratio was 4.8-fold lower in the arthritic condition, what indicates a very low mitochondrial NADH/NAD⁺ ratio.

3.4. Hepatic uptake and oxidation of fatty acids (FA)

Fig. 2 shows the net uptake rate of palmitic and oleic acids in perfused livers. The uptake of palmitic acid was 40 and 53% higher in perfused livers of fed and fasted arthritic rats, respectively. For oleic acid the differences were similar, 48 and 67%, respectively.

Fig. 3 illustrates the time courses of palmitic (Panel A) and oleic (Panel B) acids catabolism in perfused livers. The numerical values of each parameter at 20 minute perfusion time in Fig. 3A and B were subtracted from those values at 10 minute perfusion time (basal rates) and displayed in Table 3. The production of ¹⁴CO₂ was 46 and 80% higher in livers from arthritic rats when palmitic and oleic acids were the substrates, respectively. The increments of oxygen consumption caused by palmitic and oleic acids were 114 and 90% higher in the arthritic condition, respectively. The acetoacetate production was also higher in the arthritic condition, 150 and 450%, respectively, with palmitic and oleic acids. The β -hydroxybutyrate, however, was lower in the arthritic condition, -46 and -58%, respectively, with palmitic and oleic acids. The total ketone bodies were not modified by arthritis, but the β -hydroxybutyrate/acetoacetate ratio was decreased 11-fold with both fatty acids.

3.5. Hepatic content of adenine nucleotides

Table 4 shows the contents of adenine nucleotides in μ mol per gram liver. The hepatic levels of NADH and NADPH were approximately 35%

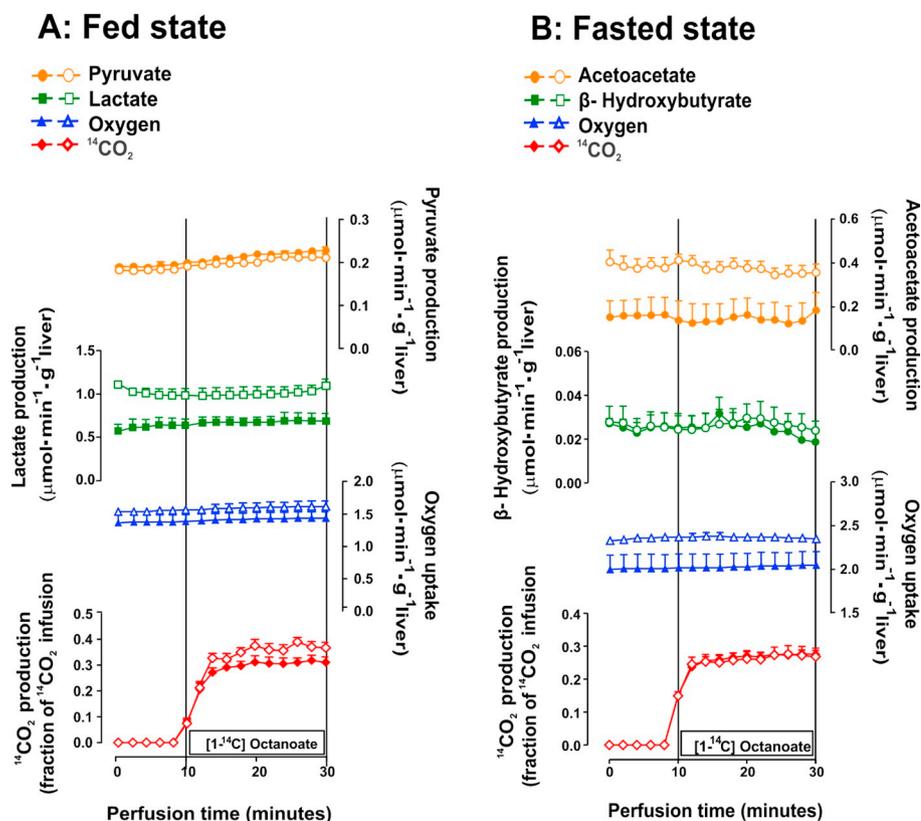


Fig. 1. The activity of citric acid cycle, glycolysis and ketogenesis in perfused livers from control and arthritic rats. A: Citric acid cycle and glycolysis in the perfused livers from fed rats. B: Citric acid cycle and ketogenesis in the perfused livers from fasted rats. Livers were perfused with Krebs-Henseleit bicarbonate buffer and trace amounts of $[1-^{14}\text{C}]$ octanoate as shown by the horizontal bars. Data are the mean \pm standard error of four liver perfusion experiments for each condition. Full symbols (\bullet , \blacktriangle , \blacklozenge) represent control rats and empty symbols (\circ , \triangle , \lozenge) represent arthritic rats.

Table 2

Citric acid cycle, glycolysis and ketogenesis from endogenous substrates in perfused rat livers. The livers of fed and fasted rats were perfused with Krebs/Henseleit bicarbonate buffer and trace amounts of $[^{14}\text{C}]$ octanoate ($0.01 \mu\text{Ci mL}^{-1}$) as shown in Fig. 1. Values are the rates of each parameter at 30 min perfusion time in Fig. 1 (steady-state). The activity of citric acid cycle was inferred from the $^{14}\text{CO}_2$ production; glycolysis was expressed as pyruvate + lactate production (fed rats); and ketogenesis was expressed as acetoacetate + β -hydroxybutyrate production (fasted rats). Data are the mean \pm standard error of four animals for each experimental condition. Pairs of values in the same line labelled with superscripts are statistically different: $^{\#}p < 0.05$, $^*p < 0.01$, $^{**}p < 0.001$.

Parameter	Fasted		Fed	
	Control	Arthritis	Control	Arthritis
$^{14}\text{CO}_2$ production (fraction of inflow)	0.27 ± 0.01	0.27 ± 0.03	$0.30 \pm 0.01^{\#}$	$0.37 \pm 0.01^{\#}$
Oxygen consumption ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$)	1.92 ± 0.16	$2.36 \pm 0.04^{\#}$	1.44 ± 0.01	1.61 ± 0.08
Lactate production ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$)	–	–	0.73 ± 0.13	$1.12 \pm 0.14^{\#}$
Pyruvate production ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$)	–	–	0.20 ± 0.03	0.17 ± 0.04
Glycolysis ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$)	–	–	$0.82 \pm 0.05^*$	$1.28 \pm 0.20^*$
Lactate/pyruvate	–	–	$3.96 \pm 0.43^*$	$6.69 \pm 0.95^*$
Acetoacetate production ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$)	0.13 ± 0.02	$0.38 \pm 0.01^{\#}$	–	–
β -Hydroxybutyrate production ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$)	0.03 ± 0.01	0.02 ± 0.01	–	–
Ketogenesis ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$)	0.16 ± 0.02	$0.40 \pm 0.01^{**}$	–	–
β -Hydroxybutyrate/acetoacetate	0.24 ± 0.06	$0.05 \pm 0.01^{\#}$	–	–

lower in the arthritic condition (fasted and fed rats). Hepatic levels of NAD^+ and NADP^+ were 14 and 38% lower in fasted arthritic rats, but not different in fed rats. The NADH/NAD^+ and $\text{NADPH}/\text{NADP}^+$ ratios were lower in the arthritic conditions in both fasted and fed rats by factors ranging from 1.30 to 1.45-fold. Regarding the adenine mononucleotides, only the hepatic levels of ATP in the fed state were different in arthritic rats (+33%). However, irrespective of being control or arthritic rats, the hepatic levels of ATP were lower and those of AMP higher in the fasted when compared to the fed state. The latter

phenomenon has been associated to a reduced hepatic energy state that occurs due to metabolic stressors, such as fasting [39]. Figs. S3 and S4 show an overview of the chromatographic profile of hepatic adenine nucleotides.

3.6. Enzyme activities

Entry of fatty acyl-CoA into the mitochondria precedes oxidation and for this reason the activities of CAT I and II were evaluated in livers

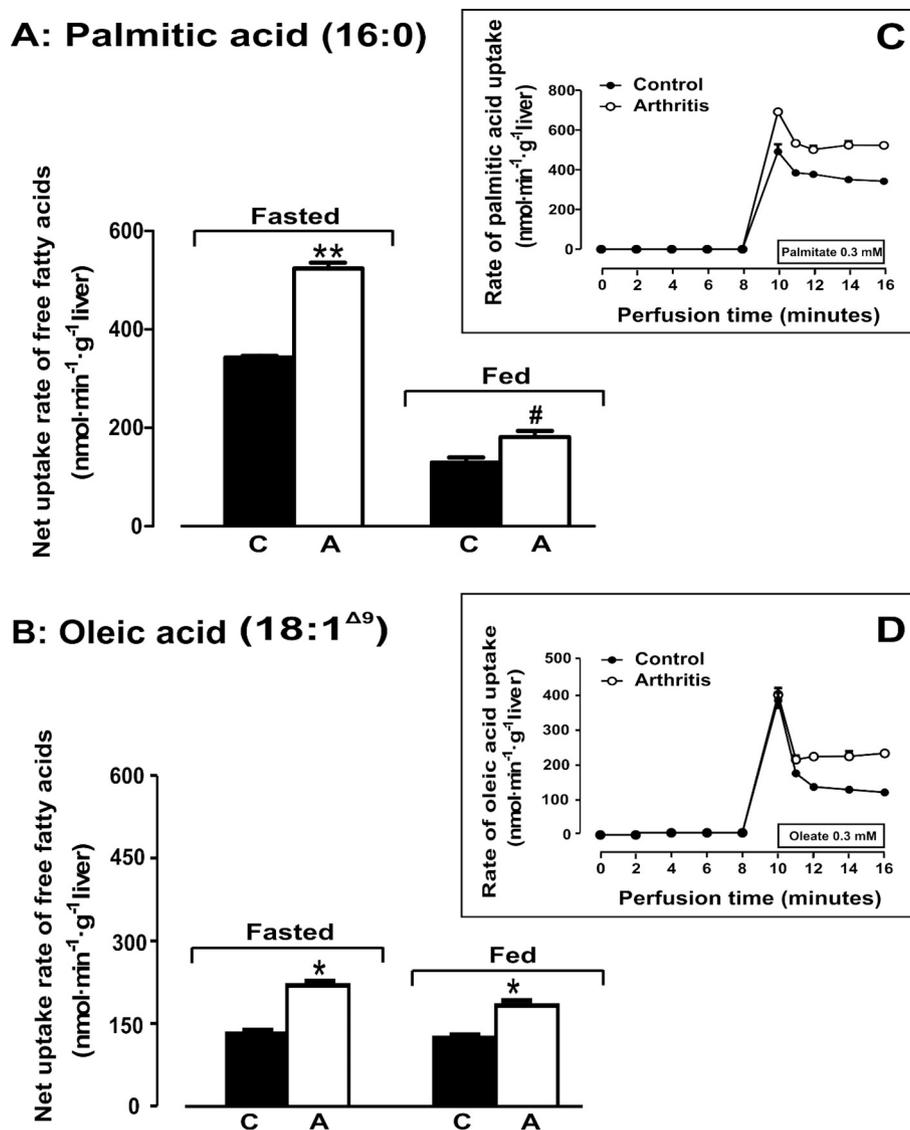


Fig. 2. Net uptake rate of free fatty acids (FFA) by perfused livers of control and arthritic rats. Net uptake rate of palmitic acid (A) and oleic acid (B). The time courses of palmitic acid and oleic acid uptake shown in the insets (C and D, respectively) were measured in livers from fasted rats. The uptake rates in panels A and B represent steady-state values attained between 12 and 16 minute perfusion time. Rates were calculated from the portal-venous concentration differences. C, controls and A, arthritis. Data are the mean \pm standard error of four perfused livers for each experimental condition. # $p < 0.05$, * $p < 0.01$, ** $p < 0.001$.

from fasted rats. The time courses of palmitoyl-carnitine formation in the range up to 6 min are shown in Fig. 4A (CAT I) and 4B (CAT II). No difference was found for the CAT I activity (24.1 ± 1.3 and 23.6 ± 3.3 nmol·min⁻¹·mg protein⁻¹, respectively, for control and arthritic rats). The initial rate of the CAT II activity, however, was 40% lower in the arthritic condition (33.2 ± 4.4 and 19.5 ± 0.3 nmol·min⁻¹·mg⁻¹, respectively, for control and arthritic rats; $p = 0.036$).

The activity of acetyl-CoA carboxylase (ACC) was measured as an attempt of inferring about the production of malonyl-CoA, a negative modulator of CAT I. Experiments were done by varying the concentration of citrate, which is an allosteric activator of the ACC [40,41]. The results are shown in Fig. 5. In fasted rats and in the absence of citrate the activity of ACC was 27.4% lower in the arthritic state. In fed rats the ACC activity was 14.7% lower in the arthritic state ($p = 0.001$). These activities correspond to the non-phosphorylated ACC, as phosphorylation inactivates the enzyme [40,41]. It should be noted that in the absence of citrate the activity of ACC in fed rats considerably exceeds that of fasted rats. Citrate reverses inactivation by phosphorylation, a known phenomenon that was more pronounced in the fed state. Activation by citrate follows Michaelian saturation kinetics [40,41],

and in order to obtain quantitative parameters a Michaelis-Menten relationship was fitted to the experimental rates (v):

$$v = \frac{V_{\max}[\text{Citrate}]}{[\text{Citrate}] + K_{\text{act}}} + v_0 \quad (1)$$

K_{act} is the activation constant of citrate, V_{\max} corresponds to the maximal stimulation caused by citrate above the basal rate, which is represented by v_0 . The continuous lines in Fig. 5 were calculated after substituting the optimized parameters into Eq. (1). The optimized values are given in the legend to Fig. 5, together with their standard deviations obtained in the fitting procedure. The latter were relatively small in the fed state but quite pronounced for the data obtained in the fasted state. This occurred because there was practically no activation in the fasted state. For the control condition there was also practically no saturation, so that the determination of K_{act} by the least-squares procedure is extremely uncertain, a characteristic that is reflected in the standard deviations of the estimate. Arthritis did not change significantly the activation constant. It diminished, however, the maximal activation in the fed state by 19.5% ($p < 0.001$). No such change was found in the fasted state.

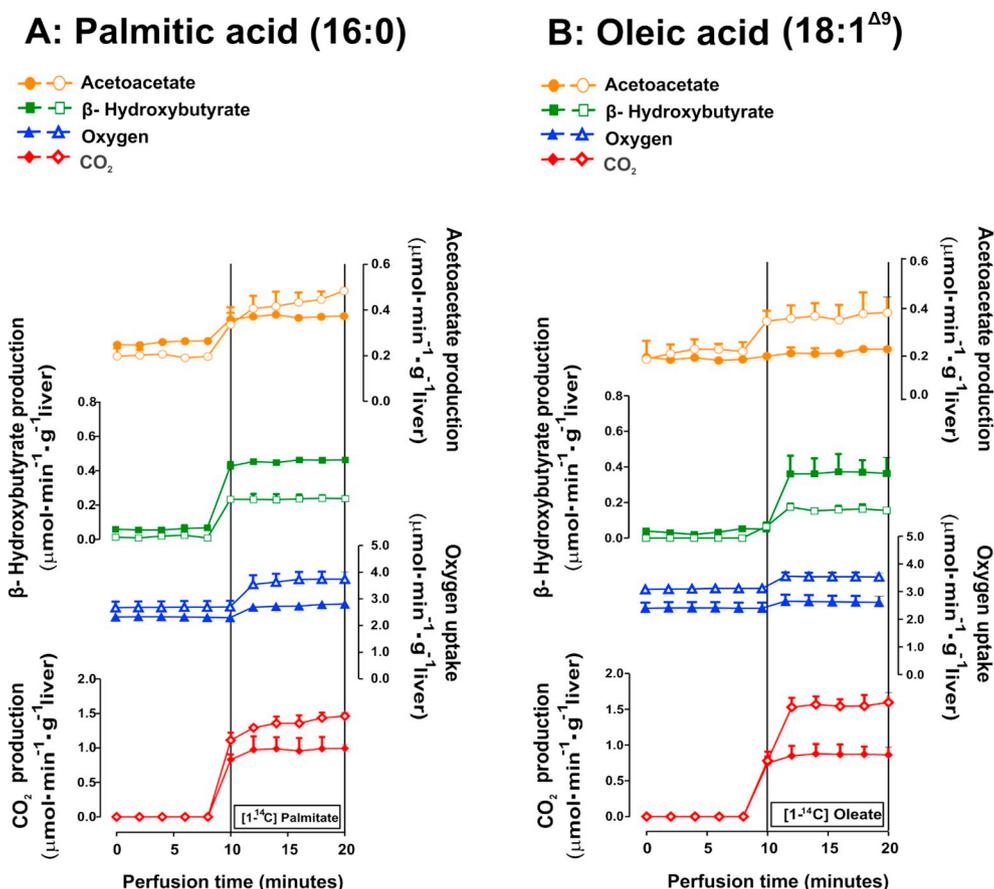


Fig. 3. Time courses of fatty acids (FA) catabolism in perfused livers of control and arthritic rats. A: Catabolism of palmitic acid. B: Catabolism of oleic acid. Livers from 12 h fasted rats were perfused with Krebs/Henseleit-bicarbonate buffer. Palmitic acid (0.3 mM) plus [^{14}C]palmitic acid ($0.2 \mu\text{Ci}\cdot\text{mL}^{-1}$) or oleic acid (0.3 mM) plus [^{14}C]oleic acid ($0.2 \mu\text{Ci}\cdot\text{mL}^{-1}$) were infused during 20 min (horizontal bars). Data are the mean \pm standard error of the mean of four liver perfusion experiments for each condition. Full symbols (\bullet , \blacksquare , \blacktriangle , \blacklozenge) represent control rats and empty symbols (\circ , \square , \triangle , \lozenge) represent arthritic rats.

4. Discussion

4.1. General aspects

Rats with adjuvant-induced arthritis present generalized inflammatory manifestations, particularly between the 14th and 21st days after arthritis induction, when an intense inflammatory response to the adjuvant is observed in all paws (polyarthritis) in addition to leukocytosis, high levels of systemic proinflammatory cytokines and inflammatory cell infiltration in the liver [19,23,24,35–37]. Severe cachexia also occurs and it resembles that one occurring in chronic wasting diseases, in which lean body wasting is associated to adipose

tissue wasting and body weight reduction [23,24,35]. Cachexia is characterized by a loss of body mass > 10% associated with an underlying illness and severe cachexia is considered to occur when the loss of body mass is higher than 15% [42]. In this study, arthritic rats did not gain body mass whereas healthy animals gained 30%, a phenomenon that can be regarded as an indication of severe cachexia [35,43,44]. Cachexia in arthritic rats has been reported to be due not only to anorexia, but also to metabolic alterations in consequence of the systemic inflammation [35,44].

In general terms it is apparent from the results obtained in the present work that severe arthritis in rats influences the hepatic metabolism of fatty acids in several ways. The most important events are

Table 3

Fatty acids catabolism in perfused livers from control and arthritic rats. Livers of 12 h fasted rats were perfused with 0.3 mM fatty acids (palmitic and oleic acids) plus trace amounts of the corresponding [^{14}C]fatty acid ($0.2 \mu\text{Ci}\cdot\text{mL}^{-1}$). The values are the increment (Δ) in the rate of each parameter due to fatty acid infusion (values at 20 min – values at 10 min perfusion time in Fig. 3). Ketogenesis is the sum of β -hydroxybutyrate and acetoacetate productions. Data are the mean \pm standard error of four animals for each experimental condition. Pairs of values in the same line labelled with superscripts are statistically different: $^{\#}p < 0.05$, $^*p < 0.01$, $^{**}p < 0.001$.

Parameter	Palmitic acid		Oleic acid	
	Control	Arthritis	Control	Arthritis
$^{14}\text{CO}_2$ production ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$)	0.99 ± 0.11	$1.45 \pm 0.06^{\#}$	0.87 ± 0.11	$1.57 \pm 0.14^{\#}$
Δ Oxygen consumption ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$)	0.49 ± 0.14	$1.05 \pm 0.11^{\#}$	0.22 ± 0.04	$0.42 \pm 0.06^{\#}$
$\Delta\beta$ -Hydroxybutyrate production ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$)	0.41 ± 0.01	$0.22 \pm 0.01^*$	0.33 ± 0.06	$0.14 \pm 0.01^{\#}$
Δ Acetoacetate production ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$)	0.11 ± 0.01	$0.27 \pm 0.03^*$	0.04 ± 0.01	$0.23 \pm 0.05^{\#}$
Δ Ketogenesis ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$)	0.52 ± 0.02	0.49 ± 0.02	0.38 ± 0.05	0.37 ± 0.02
β -Hydroxybutyrate/acetoacetate	3.85 ± 0.33	$0.83 \pm 0.09^{**}$	8.63 ± 1.60	$0.78 \pm 0.21^{**}$

Table 4

The levels of adenine mono- and dinucleotides in the liver of control and arthritic rats. Acid-stable and acid-labile adenine nucleotides were extracted from freeze-clamped livers of fed and fasted rats and measured by HPLC (see Section 2.8). The values are expressed as $\mu\text{mol} \cdot (\text{g fresh liver})^{-1}$ and data are the mean \pm standard error of five animals for each experimental condition. Pairs of values in the same line labelled with superscripts are statistically different: $p < 0.05$, $^*p < 0.01$, $^{**}p < 0.001$.

Parameter	Fasted		Fed	
	Control	Arthritis	Control	Arthritis
ATP	1.22 \pm 0.06	1.36 \pm 0.08	2.03 \pm 0.10	2.71 \pm 0.10 [#]
ADP	1.17 \pm 0.03	1.16 \pm 0.04	0.81 \pm 0.04	0.83 \pm 0.04
AMP	1.11 \pm 0.10	1.02 \pm 0.04	0.39 \pm 0.03	0.37 \pm 0.02
NADP ⁺	0.13 \pm 0.01	0.08 \pm 0.01 [*]	0.13 \pm 0.02	0.11 \pm 0.01
NAD ⁺	0.44 \pm 0.02	0.38 \pm 0.01 [#]	0.47 \pm 0.02	0.45 \pm 0.01
NADPH	0.38 \pm 0.02	0.24 \pm 0.01 ^{**}	0.37 \pm 0.01	0.23 \pm 0.01 ^{**}
NADH	0.11 \pm 0.01	0.07 \pm 0.01 [#]	0.09 \pm 0.004	0.06 \pm 0.003 [#]
	Ratios			
ATP/ADP	1.05 \pm 0.04	1.17 \pm 0.04	2.71 \pm 0.21	3.40 \pm 0.05 [*]
ATP/AMP	1.85 \pm 0.07	1.34 \pm 0.07	5.99 \pm 0.67	7.74 \pm 0.37 [#]
NADPH/NADP ⁺	3.48 \pm 0.25	2.53 \pm 0.21 [#]	3.26 \pm 0.27	2.25 \pm 0.13 [#]
NADH/NAD ⁺	0.25 \pm 0.02	0.19 \pm 0.02 [#]	0.19 \pm 0.02	0.14 \pm 0.01 [#]

illustrated by Fig. 6, which also utilizes previously published data to represent in a schematic way the integrated lipid metabolism occurring in the adipose tissue and liver of arthritic rats. These events will be discussed in the following paragraphs.

4.2. Lipid and ketone bodies levels in plasma and liver

It is known that proinflammatory cytokines, especially those released from the rheumatic joints, stimulate the adipose tissue to release FFA and glycerol. This explains the reduced adipose mass and decreased expression of fatty acid synthase (FAS) and adiponectin associated with a higher expression of TNF- α found in rats with adjuvant-induced arthritis [35,45]. Lipogenesis is in fact decreased in the adipose tissue of arthritic rats, however, the adipose lipolysis has been reported as non-increased in these animals [35]. In the present study, the proposed increased lipolysis in arthritic rats is corroborated by the increased levels of circulating glycerol and FFA that were found, observations that are consistent with the previously reported elevated levels of serum corticosterone and TNF α [17,36]. The increased lipolysis in the adipose tissue seems to occur even in the fed state as indicated by the higher levels of FFA and glycerol in the plasma of fed arthritic rats. It should be remarked that the plasma levels of FFA were elevated in spite of their enhanced uptake by the liver. This can only occur if uptake by other tissues, such as skeletal and heart muscles, for example, is diminished. In this respect it should be mentioned that inflammatory stress, TNF- α and IL-1 were reported to downregulate the fatty acids translocase (FAT/CD36) expression in heart and skeletal muscles whereas upregulation was found in the liver [46–48]. Such a combination, increased hepatic uptake of FFA and diminished uptake in peripheral tissues, has already been observed in animals lacking FAT/CD36 [49,50].

High levels of circulating FFA are considered by itself proatherogenic and when further associated to high degrees of inflammation can contribute to the greater occurrence of cardiovascular disease in severe rheumatoid arthritis [5,14]. Moreover, FFA dose-dependently enhances the secretion of proinflammatory cytokines and matrix-degrading enzymes in rheumatoid arthritis synovial fibroblasts [51]. The lower levels of both hepatic and circulating TG in arthritic rats seem to occur as consequence of the increased FA oxidation in the liver, especially when it is associated to decreased hepatic FA synthesis and esterification. The latter condition is the opposite of what has been observed in cachexia induced by chronic wasting diseases, in which hepatic lipogenesis and circulating TG are increased [17,21]. The lower hepatic lipogenesis may additionally explain the reduced circulating levels of TG that occur in patients with severe rheumatoid arthritis [3,8,9,11]. The enzyme

acetyl-CoA carboxylase (ACC) catalyzes the carboxylation of acetyl-CoA to produce malonyl-CoA and it is an important regulatory step of FA synthesis. Although only a relatively small diminution in the activity of ACC was found in the present study in the liver of fed arthritic rats, it notwithstanding represents an observation that is consistent with a diminished hepatic FA synthesis in these animals.

4.3. The hepatic metabolism of endogenous fatty acids in arthritic rats

The $^{14}\text{CO}_2$ productions from endogenous substrates in the substrate-free perfused liver of fasted arthritic and control rats were not different, meaning also no difference in the citric acid cycle activity. It should be remarked that perfused livers under these conditions survive mainly at the expense of the oxidation of endogenous FA. Oxygen consumption and ketogenesis, however, were higher in livers from fasted arthritic rats. Both phenomena can be regarded as evidence of an enhanced endogenous FA oxidation. In fact, breaking down of FA into acetyl-CoA is coupled to the production of reducing equivalents that are oxidized in the respiratory chain, what justifies the increased oxygen uptake. No increment in the citric acid cycle activity will occur, however, if the resulting extra acetyl-CoA units are deviated into ketogenesis. It is true that ketone bodies are produced not only from acetyl-CoA derived from endogenous FA, but also from acetoacetyl-CoA derived from the oxidation of ketogenic amino acids. The latter happens mainly under conditions in which skeletal muscle wasting is occurring in consequence of cachexia, for example [52]. Thus, in substrate-free perfused livers from fasted rats the extra ketogenesis in the arthritic condition can be regarded as representing increased oxidation of FA with a substantial contribution of amino acid oxidation.

In substrate-free perfused livers from fed rats, on the other hand, the citric acid cycle activity was somewhat higher in the arthritic condition (+23%) and oxygen uptake presented merely a tendency toward higher values. These relatively modest differences reflect most likely the differences in metabolic state. Substrate-free perfused livers from fed rats do not depend solely on the oxidation of endogenous FA. Actually, the glycolytic activity, that is enhanced in the arthritic state [19,20], leads by itself to the production of reducing equivalents and acetyl-CoA from pyruvate. Glycolysis is one of the reasons why substrate-free perfused livers from fed rats produce much less ketone bodies [53]. It is thus likely that the contribution of endogenous FA oxidation to the 23% increased citric acid cycle activity was relatively modest. Moreover, the increased flux of glucose through glycolysis in the arthritic condition, may explain at least in part the higher ATP content in the liver of fed arthritic rats.

At this point it is important to note that the higher rates of

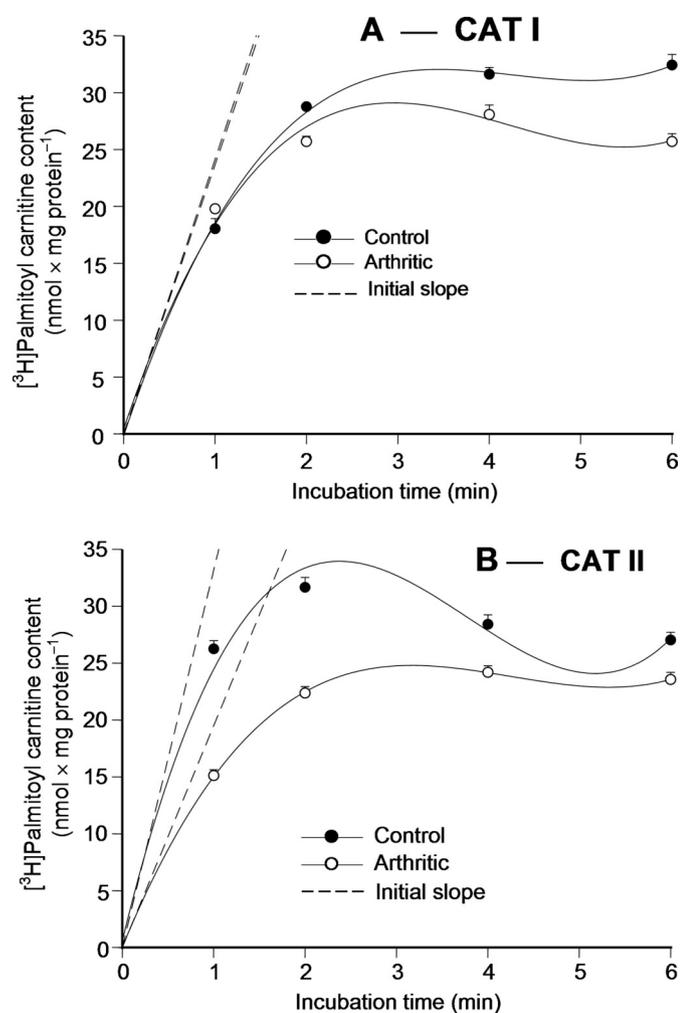


Fig. 4. Time courses of [³H]palmitoyl-carnitine formation in the incubation media containing substrates and CAT I (A) and CAT II (B). Fractions containing CAT I and CAT II activities were isolated from hepatic mitochondria from control and arthritic rats by differential centrifugation. The incubation system was: 50 mM manitol, 75 mM KCl, 5% fatty acid-free bovine-serum albumin, 2 mM KCN, 25 mM EGTA, 50 μM palmitoyl-CoA, 0.5 mM carnitine plus 5 μCi/μmol [³H]carnitine and approximately 0.3 mg protein·mL⁻¹ of either CAT I or CAT II preparation. The [³H]palmitoyl-carnitine was extracted with butanol. Data are the mean ± standard error of the mean of four independent preparations. Each of set data was fitted to a polynomial of the third degree by means of least-square procedure. The continuous lines represent the fitted curves. The dotted lines were calculated with first term of the polynomial. The slopes of these lines were taken as the initial rates of each enzyme, as given in the text.

ketogenesis described here for livers from fasted arthritic rats are in apparent contrast to the lower levels of circulating ketone bodies in the arthritic condition. However, there is evidence indicating an increased uptake by peripheral tissues in arthritis. Ketone bodies are avidly absorbed from blood in the peripheral tissues by the monocarboxylate transporter 1 (MCT1), which is expressed in virtually every cell [52]. Moreover, TNF-α and IL-1 stimulate PPAR-γ coactivator-1 alpha (PGC-1α), which upregulates MCT1 in skeletal muscle [54,55].

4.4. The hepatic metabolism of exogenously supplied fatty acids in arthritic rats

The higher rates of exogenous FFA uptake in the perfused livers of arthritic rats occurred in both fed and fasted states and it probably reflects their higher rates of oxidation in the organ. It is generally

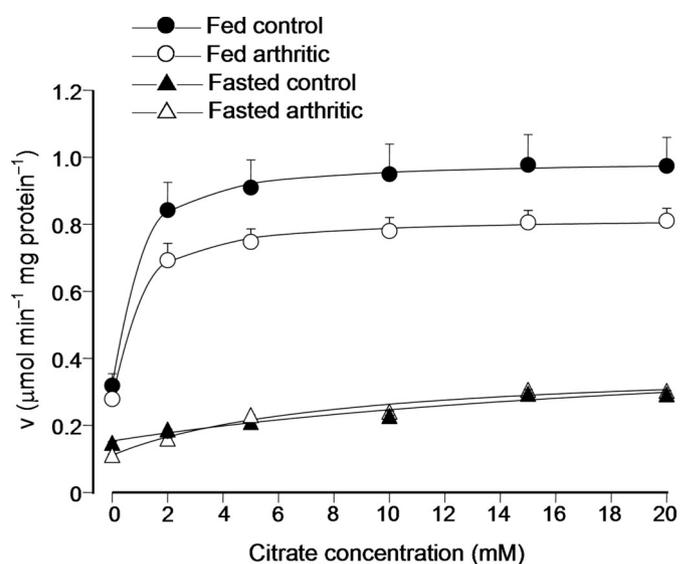


Fig. 5. Reaction rate of hepatic acetyl-coenzyme A carboxylase (ACC) from control and arthritic rats in the fed and fasted states as a function of citrate concentration. The enzymatic activity was measured at 37 °C as described in [Materials and methods section](#). The experimental points are the means ± standard error of four animals in each experimental condition. The lines were calculated by means of Eq. (1) with the optimized parameters obtained by fitting the experimental data to the equation. Control/arthritic fed rats: $v_o = 0.319 \pm 0.010 \mu\text{mol min}^{-1} \text{mg}^{-1} / 0.278 \pm 0.010 \mu\text{mol min}^{-1} \text{mg}^{-1}$ ($p = 0.001$); $V_{\max} = 0.673 \pm 0.012 \mu\text{mol min}^{-1} \text{mg}^{-1} / 0.543 \pm 0.013 \mu\text{mol min}^{-1} \text{mg}^{-1}$ ($p = 0.001$); $K_{\text{act}} = 0.602 \pm 0.065 \text{mM} / 0.661 \pm 0.087 \text{mM}$ ($p = 0.32$). Control/arthritic fasted rats: $v_o = 0.153 \pm 0.015 \mu\text{mol min}^{-1} \text{mg}^{-1} / 0.111 \pm 0.017 \mu\text{mol min}^{-1} \text{mg}^{-1}$ ($p = 0.009$); $V_{\max} = 0.329 \pm 0.215 \mu\text{mol min}^{-1} \text{mg}^{-1} / 0.274 \pm 0.051 \mu\text{mol min}^{-1} \text{mg}^{-1}$ ($p = 0.64$); $K_{\text{act}} = 25.40 \pm 29.14 \text{mM} / 8.06 \pm 4.47 \text{mM}$ ($p = 0.58$). The error parameters are standard deviations of the estimate (obtained from the co-variance matrix of the least-squares fitting procedure) and the p values were obtained by applying Student's t-test to each pair or data.

accepted that the metabolic demand for FFA, i.e., the conversion of FA into acyl-CoA and downstream metabolic intermediates, increases cellular uptake [56]. In vivo, however, the hepatic uptake of FFA is possibly even more increased than in the perfused liver due to their higher circulating levels in arthritis and because the liver extracts these substrates in proportion to their concentration in blood [57,58].

The oxygen consumption increments and the ¹⁴CO₂ productions from exogenous FA were more elevated in the liver of arthritic rats, but the increments in ketogenesis were not modified (Table 3). However, the β-hydroxybutyrate/acetoacetate ratios were lower in arthritic rats. This occurs when the near-equilibrium of the 3-hydroxybutyrate dehydrogenase reaction is shifted toward acetoacetate, a condition that reflects a lower NADH/NAD⁺ ratio [59]. The latter was actually decreased in the liver of arthritic rats (Table 4). Moreover, the same phenomenon also stimulates the citric acid cycle by shifting the near-equilibrium of the malate dehydrogenase reaction in the direction of oxaloacetate, the acceptor of acetyl-CoA in the cycle [33]. The lower β-hydroxybutyrate/acetoacetate ratios in the mitochondria of arthritic rats correlates well with the same phenomenon in the plasma. In this respect it should be noted that monocarboxylates are rapidly transported into and out of the cells by MCT1 so that their extracellular concentration ratio in blood matches their intracellular concentration ratio [60].

The more oxidized state found in the arthritic liver deserves a few additional comments. In the present study, the hepatic contents of adenine nucleotides were directly measured and they reflect their mean concentrations over the total tissue without informing about their distribution in the various cell compartments. However, the lower β-

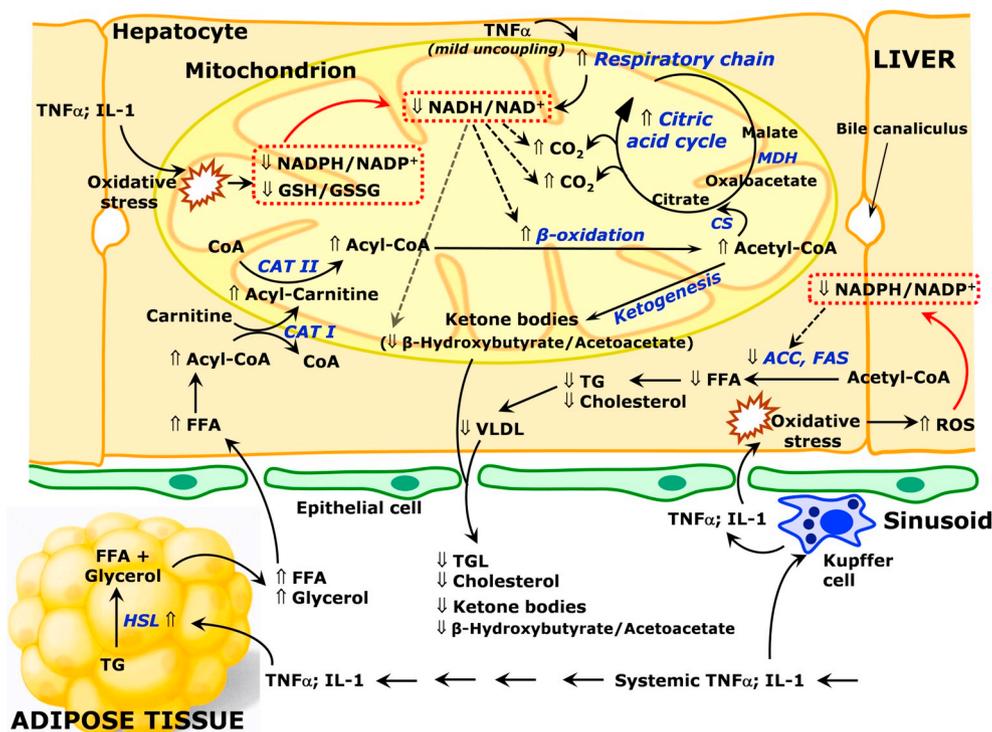


Fig. 6. Schematic representation of integrated lipid metabolism occurring in the adipose tissue and liver of arthritic rats with severe cachexia. The scheme is discussed in the text and is based on the results of the current work and on previously published data [1,14,17,22,35,62–65]. The symbol \uparrow means up-regulation and \downarrow down-regulation. Red arrows indicate effects of oxidative stress. Abbreviations: TNF- α , tumor necrosis factor alpha; IL-1, interleukin 1; FFA, free fat acids; TG, triglycerides; VLDL, very low density lipoprotein; HSL, hormone sensible lipase; FAS, fatty acid synthase; ACC, acetyl-CoA carboxylase; MDH, malate dehydrogenase; CS, citrate synthase; CAT I and II, carnitine acyltransferase I and II; CoA, coenzyme A; ROS, reactive oxygen species; GSH, reduced glutathione; GSSG, oxidized glutathione.

hydroxybutyrate/acetoacetate ratio observed in the perfused livers from arthritic rats indicates a lower mitochondrial NADH/NAD⁺ ratio. The same was found previously for the hepatocyte cytosol of fasted arthritic rats [20]. In the fed state, however, the higher lactate/pyruvate ratio indicates a higher cytosolic NADH/NAD⁺ ratio in the arthritic liver. The latter was found even under net oxidizing conditions in the total hepatic tissue and it reflects probably the higher contribution of mitochondrial oxidizing equivalents. The cytosolic redox state, however, depends not only on the NADH/NAD⁺ couple, but also on the NADPH/NADP⁺ couple. The latter is apparently decreased in the cytosol of arthritic rats due to the pronounced oxidative stress in the organ [22].

The increased oxidation of FA in the liver of arthritic rats found in this work was not paralleled by an increased activity of CAT I and CAT II, the enzymes responsible for shuttling the FA moieties into the mitochondria. For CAT II even the opposite was found, i.e., its activity measured under standard conditions was higher in healthy rats. There is evidence, however, that the ACC catalysed production of malonyl-CoA, the negative modulator of CAT I, could be diminished in arthritic fed rats, especially in the presence of citrate, so that one cannot exclude the possibility that the actual CAT I activity was increased in the liver cells of arthritic fed rats. Support this view is the general notion that, in the fasted state, the mitochondrial oxidation of FA is greatly favoured because the formation of malonyl-CoA is very low [61]. Irrespective of the participation or not of the CAT I activity in the regulation of the increased rates of hepatic FA oxidation in arthritic rats, the crucial role of the more oxidized state of the mitochondrial NADH-NAD⁺ couple can hardly be overemphasized because higher NAD⁺ concentrations are an important factor in determining the reaction rate of dehydrogenases in general. Furthermore, the lower cytosolic NADPH/NADP⁺ ratio in arthritic livers is also likely to contribute for decreasing the FA synthesis. Similarly, the energetic deficit due to anorexia should be also contributing to an accelerated fatty acid catabolism in the liver of arthritic rats.

The hepatic metabolism of FA in arthritic rats is, thus, similar to that of glucose in that it presents a predominance of catabolic over anabolic pathways [19,20]. The opposite occurs in the liver of rats with Walker 256 tumor-induced cachexia. In the latter, hepatic FA oxidation and

ketogenesis are both diminished, with a decreased activity of CAT I and an increased derivation of fatty acyl-CoA into the esterification pathway [33]. It is important to stress that, corroborating previous study [36], the glycemic homeostasis of arthritic rats was not found to be modified in this study.

4.5. The NADH-NAD⁺ and NADPH-NADP⁺ couples and oxidative stress

The reason by which the NADH-NAD⁺ and NADPH-NADP⁺ couples are shifted toward an oxidized state in the arthritic liver has not yet been completely clarified, but it has been attributed to the pronounced oxidative stress associated to the accelerated oxidative metabolism in the organ [22]. The altered oxidative stress is consequence of an impaired antioxidant system associated to an increased production of ROS, both mediated by inflammatory cytokines derived from the rheumatic joints and even from resident liver macrophages (Kupffer cells). The pronounced oxidative stress consumes an excessive amount of reducing equivalents to unsuccessfully neutralize the more intense ROS production and, therefore, to decrease the NADPH/NADP⁺ and GSH/GSSG ratios in the liver of arthritic animals [22]. In stressed hepatic mitochondria, electrons from NADH are not only directed to the respiratory chain but also to the deficient scavenging systems, for example, via the transhydrogenase activity to NADPH, a condition that strongly decreases the NADH/NAD⁺ ratio in the arthritic liver [64]. In fact, under conditions of pronounced oxidative stress, the mitochondrial ROS production may be increased in an oxidized environment even in depolarized mitochondria [65]. The higher oxygen consumption in arthritic livers (Table 2 and Fig. 2) corroborates the accelerated oxidative metabolism. Although strong uncoupling in mitochondria is known to diminish ROS production, TNF- α , which causes mild uncoupling, has been reported to increase ROS production in parallel with relatively modest increases in oxygen uptake and decreases in the membrane potential [62]. Therefore, proinflammatory cytokines may be increasing the FA oxidation mainly by modifying, via oxidative stress, the redox status of the arthritic liver and, to a lesser extent, accelerating mitochondrial oxygen consumption.

5. Conclusion

The results of the present study reveal that the levels of circulating free fatty acids (FFA) are increased in arthritic rats, possibly a consequence of cytokine-induced adipose tissue lipolysis. Uptake and oxidation of FFA are increased in the liver of arthritic rats and seem to be consequence of a more oxidized state associated with an accelerated mitochondrial oxidative metabolism accompanied by a pronounced oxidative stress. This increased oxidation of fatty acids is likely to contribute to the lower levels of circulating and hepatic triglycerides (TG) a phenomenon that may also be enhanced by a diminished biosynthesis. These results reveal that, as opposed to other chronic wasting diseases, the lipid hepatic metabolism in arthritic rats is shifted toward a catabolic state. Such a condition certainly contributes to the marked cachexia verified in rats with adjuvant-induced arthritis and possibly in patients with severe rheumatoid arthritis. Thus, the hepatic metabolism of lipids should be taken into account in the therapeutic and nutritional interventions aiming at reducing the severe arthritic cachexia. This includes a reduction in the circulating levels of free fatty acids, which are associated to increased rates of cardiovascular events and worsening of the arthritic conditions at the joint level.

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Transparency document

The [Transparency document](#) associated with this article can be found, in online version.

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Competing interests

The authors declare that no competing interest exists and that all approved the final manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbadis.2018.12.019>.

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