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Isolated rat hepatocyte metabolism is affected by chronic renal failure

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Isolated rat hepatocyte metabolism is affected by chronic renal failure. Metabolic changes due to chronic renal failure (CRF) were studied in isolated liver cells. In 14 CRF and 14 sham-operated rats, liver cells were isolated by the Berry and Friend method and incubated with various substrates in order to study gluconeogenesis, ureagenesis, ketogenesis, oxygen consumption as well as cytosolic and mitochondrial adenine nucleotide content. CRF rat hepatocytes exhibited a 25% to 45% decrease in gluconeogenesis and ureagenesis ($P < 0.05$) from all the tested substrates (lactate plus pyruvate, fructose, glycerol, dihydroxyacetone, alanine and glutamine for gluconeogenesis and alanine, glutamine, ammonia and ammonia plus ornithine for ureagenesis), while endogenous rates were unaffected. CRF did not alter ketone body production (acetoacetate and β-hydroxybutyrate) from oleate or octanoate. In the presence of either oleate, lactate plus pyruvate or ammonia, oxygen uptake as well as cytosolic and mitochondrial total adenine nucleotides were unaffected by CRF, while the mitochondrial ATP/ADP ratio decreased ($P < 0.001$). Thus, this study of hepatocyte intermediary metabolism during CRF showed an alteration of only gluconeogenesis and ureagenesis pathways. Moreover, the association of normal oxygen uptake together with decreased mitochondrial ATP/ADP ratio suggest a possible increase in hepatocyte ATP demand during uremia.

During chronic renal failure (CRF), protein malnutrition is common and is responsible for increased morbidity and mortality [1–8]. In this condition, several abnormalities of hepatosplanchnic nutrient utilization have been described *in vivo.* In particular: (1) amino acid exchanges have been shown to be impaired both in absorptive [9, 10] and in post-absorptive phases [11]; (2) decreased ureagenesis has been reported [11, 12]; (3) protein meals are followed by an enhancement of the blood amino acid disturbances observed at basal state [13]; and (4) impairments of carbohydrate metabolism involving gluconeogenesis and glycogen storage have been described [14, 15]. Liver dysfunction is mainly responsible for these metabolic alterations. Such abnormalities of nutrient utilization and/or release by the liver could be due either to general uremia-related factors or to hepatocyte dysfunction.

Although numerous studies on urea synthesis have been performed during experimental CRF [16–22], the effect of CRF on liver metabolism has not been extensively explored at a cellular level. To our knowledge, only one study on isolated hepatocyte has been reported under these conditions [23], and it focused on ureagenesis and gluconeogenesis from alanine, glutamine and serine. The present work was undertaken to assess the main pathways of isolated-hepatocyte intermediary metabolism during CRF, that is, gluconeogenesis, ureagenesis, ketone body production and cell energy status. These investigations, performed on CRF and sham-operated control rats, showed a decrease in gluconeogenesis and ureagenesis pathways without change in ketogenesis. Morever, cellular O_2 consumption was either slightly enhanced or unaffected by CRF while the mitochondrial ATP/ADP ratio was significantly decreased. These results are in favor of an increased ATP consumption although a change in ATP synthesis efficacy cannot be entirely ruled out.

Methods

Animals

In 14 male Wistar rats weighing 300 to 350 g, CRF was obtained by a 3/4 left renal artery ligation and, after two weeks, a right nephrectomy (method modified from Avioli et al [24]). Rats were included in the study when they presented, three weeks after the nephrectomy, with a plasma creatinine higher than 1.5-fold the mean of control creatinine. The control group was comprised of 14 sham-operated animals which underwent 2 laparotomies without renal procedure at the time of uremic rat operations. Control and CRF rats were fed with stock pellets given *ad libitum.* At the time of metabolic studies, CRF rats presented with elevated plasma creatinine (112.38 ± 9.54 vs. 52.46 ± 2.23 μmol/liter, mean ± SE) and urea (19.22 ± 1.45 vs. 6.99 ± 0.32 mmol/liter). Body mass was similar in the two groups: 341 ± 9 versus 361 ± 4 g. During the week before metabolic studies, daily food intakes were 22 ± 3 g (mean ± SD) in controls and 22 ± 4 g in CRF rats.

Isolation and incubation of liver cells

Hepatocytes were isolated from 24-hour starved rats according to the method of Berry and Friend [25] as modified by Groen et al. [26]. Incubations were performed in closed vials at 37°C in a shaking water bath (60 strokes/min). Incubates were composed of Krebs-bicarbonate buffer (NaCl 120 mM, KCl 4.8 mM, KH_2PO_4 1.2 mM, $MgSO_4$ 1.2 mM, $NaHCO_3$ 24 mM, Ca^{2+}: 2.4 mM, pH 7.4) and bovine serum albumin-oleate (2% to 2 mM). For the ketogenesis study, fatty acid free bovine serum albumin was used and octanoate or oleate were separately added. The gas atmosphere was O_2/CO_2 (19:1).

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Table 1. Urea and glucose production

	Urea		Glucose	
	Cont (15)	CRF (15)	Cont (8)	CRF (8)
Endogenous rate	34 ± 4	36 ± 2	20 ± 4	18 ± 1
Lactate + pyruvate			542 ± 40	329 ± 49[a]
Fructose			770 ± 64	523 ± 74[a]
Dihydroxyacetone			811 ± 43	477 ± 65[a]
Glycerol			186 ± 5	102 ± 15[a]
Alanine	152 ± 9	97 ± 7[a]	202 ± 11	157 ± 11[a]
Glutamine	387 ± 20	256 ± 20[a]	207 ± 8	137 ± 11[a]
Ammonia	147 ± 17	85 ± 7[a]		
Ammonia+ornithine	242 ± 14	201 ± 12[a]		

Isolated hepatocytes from chronic renal failure (CRF) and control (Cont) rats were incubated for 60 minutes. The number of incubations in each condition is given in parentheses. Substrate concentrations were 20 mM, except for ammonia 5 mM, pyruvate and ornithine 2 mM. Results are expressed as μmol/g dry mass, mean ± SE.
[a] Significant differences between the 2 groups, $P < 0.05$.

For gluconeogenesis and/or ureagenesis studies, hepatocytes (final concentration of approximately 13 mg dry cell/ml) were incubated with: no addition (endogenous rate), lactate plus pyruvate (20-2 mM), fructose, dihydroxyacetone, glycerol, alanine, glutamine (20 mM), ammonia (5 mM) and ammonia plus ornithine (5 mM-2 mM). Ureagenesis was studied both with ammonia and ammonia plus ornithine in order to avoid a rate-limiting effect of low endogenous ornithine content on citrulline formation and urea synthesis [27, 28]. At the beginning of incubations (t0) and after 60 minutes (t60), 0.5 ml samples of the cell suspension were taken and quenched into perchloric acid [$HClO_4$, final concentration 5% (mass/vol)], then centrifuged at 13,500 g for five minutes. The supernatant was neutralized with KOH (2 M)-morpholinopropane sulphonic acid (0.3 M) for subsequent glucose and urea determinations.

For ketogenesis measurements, hepatocytes were incubated for 20 minutes with no addition, oleate (2 mM) and octanoate (2 mM). Samples of cell suspension (0.7 ml) were taken at t0 and t20 and treated as described above for β-hydroxybutyrate and acetoacetate measurements.

We also investigated O_2 consumption and cytosolic and mitochondrial adenine nucleotide contents. Hepatocytes were incubated for 20 minutes with no addition, oleate (2 mM), oleate plus lactate plus pyruvate (2 mM-20 mM-2 mM), oleate plus ammonia (2 mM-5 mM) and oleate plus lactate plus pyruvate plus ammonia (2 mM-20 mM-2 mM-5 mM). At t20, 0.5 ml of cell suspension were taken from the incubation vials and put into an oxygraph for O_2 uptake measurements. Simultaneously, 0.3 ml of the cell suspension were treated with digitonin to separate cytosolic and mitochondrial spaces. As described by Zuurendonk et al [29, 30] and with previously reported modifications [31], the digitonin-treated cell suspension was centrifuged through a layer of silicone oil for subsequent measurements of adenylic nucleotides.

Metabolite assays

Metabolites were measured in neutralized protein-free extracts. Glucose [32], urea [33], β-hydroxybutyrate [34] and acetoacetate [35] were determined spectrophotometrically. Adenylic nucleotides were separated by high-performance liquid chromatography as previously described [31].

Table 2. Ketone body production

	βOHB/AcAc		βOHB + AcAc	
	Cont (15)	CRF (15)	Cont (15)	CRF (15)
Endogenous	0.067 ± 0.003	0.067 ± 0.002	39.7 ± 1.3	41.5 ± 1.6
Oleate	0.549 ± 0.029	0.525 ± 0.033	84.4 ± 2.5	89.6 ± 4.8
Octanoate	0.479 ± 0.029	0.620 ± 0.044[a]	97.8 ± 2.9	114 ± 2.2[a]

Isolated hepatocytes from chronic renal failure rats (CRF) and sham-operated controls (Cont) were incubated for 20 minutes. The number of incubations in each condition is given in parentheses. Substrate concentrations were 2 mM. The sum of β-hydroxybutyrate (βOHB) and acetoacetate (AcAc) production is expressed as μmol/g dry mass, mean ± SE.
[a] Significant differences between the 2 groups.

Materials

Amino acids and lactate were purchased from Sigma Chemical Co. (Lyon, France). Pyruvate, collagenase A and enzymes were purchased from Boehringer (Meylan, France). Fructose, oleate, octanoate, dihydroxyacetone and digitonin were obtained from Merck (Lyon, France) and rhodorsil silicone oil from Rhône-Poulenc (Lyon, France). Substrates were of the highest grade of purification available.

Statistics

Results are given as mean ± SE. For gluconeogenesis, ureagenesis and ketogenesis, differences between groups in each incubation condition were tested by the Student's *t*-test. Hepatocyte respiration and adenine nucleotide concentrations were analyzed by two-factor repeated-measure ANOVA to compare data from uremic and control animals as well as to study incubation condition-related variations. When significant substrate-related differences were demonstrated by repeated-measure ANOVA, each parameter variation was studied by one-factor ANOVA. Differences were considered as significant at the level of $P < 0.05$.

Results

Gluconeogenesis and ureagenesis

Table 1 shows the endogenous and substrate-induced rates of gluconeogenesis and ureagenesis. The endogenous rates of both pathways were unaffected by CRF. Hepatocytes from CRF rats, when compared with controls, exhibited a significant decrease in glucose production whatever the tested substrate ($P < 0.05$). This decrease in gluconeogenesis ranged from 25% with alanine to 45% with glycerol. Ureagenesis from alanine, glutamine, ammonia and ammonia plus ornithine was also significantly reduced by uremia ($P < 0.05$). The reduction of urea synthesis was of the same order of magnitude as the decrease of gluconeogenesis. It should be noted that the decrease in urea production when ornithine was added to ammonia was smaller than with ammonia alone (17% vs. 41%). Indeed, ornithine addition stimulated ureagenesis from ammonia by 60% in controls and 136% in CRF rats.

Ketone body production

Table 2 shows total ketone body production values. Endogenous ketogenesis (sum of β-hydroxybutyrate and acetoacetate) was similar in the two groups. As expected, fatty acid addition

Table 3. Oxygen uptake and cytosolic and mitochondrial ATP/ADP ratios

	O_2 uptake	ATP/ADP	
		Cytosol	Mitochondria
Controls			
Endogenous	12.5 ± 0.8	14.87 ± 0.85	1.30 ± 0.06
Oleate	16.5 ± 0.3[a]	15.13 ± 0.78	1.68 ± 0.07[a]
O + ammonia	20 ± 0.1[a]	16.70 ± 1.34	1.57 ± 0.06[a]
O + L + P	28.1 ± 1.1[a]	20.74 ± 1.17[a]	1.61 ± 0.09[a]
O + L + P + A	27.3 ± 1.2[a]	19.20 ± 0.86[a]	1.70 ± 0.02[a]
CRF			
Endogenous	13.2 ± 0.38	13.57 ± 1.29	1.12 ± 0.04
Oleate	20.1 ± 1.3[a]	14.08 ± 2.02	1.26 ± 0.10
O + ammonia	21.5 ± 1.2[a]	20.18 ± 1.34[a]	1.32 ± 0.07[a]
O + L + P	32.1 ± 1.9[a]	18.05 ± 1.92	1.44 ± 0.07[a]
O + L + P + A	29.5 ± 1.9[a]	21.32 ± 1.48[a]	1.59 ± 0.11[a]

Isolated hepatocytes from chronic renal failure rats (CRF, $N = 9$) and sham-operated controls ($N = 8$) were incubated for 20 minutes with oleate (O), 2 mM, O plus ammonia (A), 5 mM, O plus lactate (L) plus pyruvate (P), 20-2 mM, and O plus L plus P plus A. Oxygen uptake is expressed as μmol/g dry mass/min, mean ± SE. Comparisons between CRF and control groups and according to the incubation conditions were studied by using a 2-factor repeated-measure ANOVA. For the O_2 uptake and cytosolic ATP/ADP ratio, we found a significant difference depending on the incubation conditions, but not between the two groups. For mitochondrial ATP/ADP ratio, we found significant differences both between groups and incubation conditions.

[a] Significant differences between endogenous and substrate-related data.

increased ketogenesis in both groups and this effect was significantly more pronounced with octanoate than in presence of oleate. In addition, ketogenesis was slightly but significantly higher in CRF rats than in controls ($P < 0.05$) when octanoate was added. These effects on total ketone body production were accompanied by changes in the mitochondrial redox potential as estimated by the β-hydroxybutyrate/acetoacetate ratio [36]: this ratio was identical in the 2 groups at basal state and increased in both groups after fatty acid addition. Furthermore, the β-hydroxybutyrate/acetoacetate ratio was significantly higher in CRF rats as compared with controls in presence of octanoate ($P < 0.05$).

Cellular energy metabolism

Energy metabolism was studied under several conditions from basal state (that is, the lowest respiratory rates) to the maximal respiratory rates (stimulation of both ureagenesis and gluconeogenesis in presence of oleate).

Table 3 shows the O_2 uptake results. In control cells, as usually reported, oleate addition induced a significant increase in O_2 uptake. This respiratory rate was further increased when ammonia, lactate plus pyruvate, or lactate plus pyruvate and ammonia were added to oleate. These modifications in O_2 uptake related to the incubation conditions were significant. CRF rat hepatocytes exhibited similar results. It should be stressed that the highest respiratory rate was obtained with lactate plus pyruvate and oleate and that ammonia addition did not lead to further increase. Thus, in these conditions, the increase in O_2 uptake either related to maximal gluconeogenesis or ureagenesis was not cumulative. The comparison of O_2 consumption in the two groups showed that in every condition uremic hepatocyte respiration tended to be higher than in controls, although the difference was not significant.

Simultaneously to changes in O_2 consumption, cytosolic and mitochondrial ATP/ADP ratios were affected by incubation conditions (Table 3). In control cells, the cytosolic ATP/ADP ratio was significantly higher when maximal respiratory rate occurred, that is, when gluconeogenesis was activated (addition of oleate plus lactate plus pyruvate and oleate plus lactate plus pyruvate plus ammonia). Concomitantly, the mitochondrial ATP/ADP ratio was significantly increased with every tested substrate compared with basal conditions. In hepatocytes from CRF rats, the cytosolic ATP/ADP ratio was also increased by substrate addition. This increment was significant only when ureagenesis was stimulated (addition of oleate plus ammonia and oleate plus lactate plus pyruvate plus ammonia). Mitochondrial ATP/ADP ratio elevation, observed with every substrate, was significant when gluconeogenesis and/or ureagenesis were stimulated.

The comparison between the two groups did not show any difference in cytosolic ATP/ADP ratios. On the other hand, the mitochondrial ATP/ADP ratio was lower in every condition in CRF rat cells with a significant difference at basal state and with oleate and oleate plus ammonia as substrates ($P < 0.05$).

The changes in cytosolic and mitochondrial ATP/ADP ratios described above could be due to variations of either ATP or ADP or both. Moreover, the AMP and total nucleotide content also could be affected by incubates and uremia. Cytosolic and mitochondrial ATP, ADP and AMP concentrations are given in Table 4. The sum ATP plus ADP plus AMP was identical in control and uremic rat hepatocytes both in cytosol and in mitochondria. Among cytosolic nucleotides, only ADP was significantly affected by the incubation conditions. In controls, ADP was lower when gluconeogenesis was enhanced whereas in the CRF group, ADP was reduced in the conditions stimulating ureagenesis. The comparison of the two groups revealed a significant increase in ATP and in the sum ATP plus ADP in uremic hepatocytes ($P < 0.05$).

In the mitochondrial space, substrate additions significantly affected ATP, ADP and AMP. ATP was reduced by the addition of oleate and oleate plus ammonia in both control and CRF cells, these variations reaching significance in controls only. ADP concentrations were significantly decreased by all substrates in the two groups. AMP concentrations were significantly increased, both in control and CRF rat hepatocytes, in the presence of oleate. The comparison of the two groups showed a significant ADP increase in uremic rat mitochondrial space ($P < 0.05$).

Several conclusions can be drawn from these data on cell energy metabolism: (1) oxygen uptake was significantly enhanced by substrate addition both in control and CRF cells; (2) the increase of cell respiration was associated with an increase in ATP/ADP ratios in cytosol and mitochondria mainly due to an ADP decrease; (3) the main changes in cytosolic ADP concentration and ATP/ADP ratio were associated with the stimulation of gluconeogenesis in control hepatocytes and ureagenesis in uremia; (4) CRF was characterized by an increase in cytosolic ATP and mitochondrial ADP, a decrease in mitochondrial ATP/ADP ratio and a slight but not significant increase in O_2 uptake.

Discussion

Liver dysfunction seems to play a key role in the pathogenesis of undernutrition in end-stage renal insufficient patients. The aim of this work was to investigate the effect of CRF on the intermediary metabolism of the isolated hepatocyte.

In the present study, CRF animals presented moderate renal insufficiency as previously reported in similar models [37]. Although several studies of CRF in rats show a significant loss of

Table 4. Cytosolic and mitochondrial adenine nucleotides

	ATP	ADP	AMP	ATP+ADP	ATP+ADP +AMP
Cytosol					
Controls ($N = 8$)					
Endogenous	9.92 ± 0.39	0.68 ± 0.04	2.03 ± 0.45	10.60 ± 0.41	12.63 ± 0.66
Oleate	9.12 ± 0.29	0.62 ± 0.05	1.59 ± 0.38	9.74 ± 0.32	11.33 ± 0.58
O+L+P	10.19 ± 0.34	0.51 ± 0.04[a]	1.60 ± 0.34	10.70 ± 0.36	12.29 ± 0.36
O+A	9.79 ± 0.24	0.61 ± 0.04	1.94 ± 0.29	10.40 ± 0.23	12.34 ± 0.34
O+L+P+A	10.04 ± 0.25	0.53 ± 0.03[a]	1.55 ± 0.34	10.58 ± 0.27	12.13 ± 0.32
CRF ($N = 9$)					
Endogenous	10.41 ± 0.25	0.80 ± 0.07	1.38 ± 0.20	11.21 ± 0.29	12.60 ± 0.29
Oleate	10.78 ± 0.81	0.97 ± 0.20	1.23 ± 0.12	11.75 ± 0.72	12.98 ± 0.81
O+L+P	10.84 ± 0.33	0.65 ± 0.07	0.99 ± 0.05	11.49 ± 0.34	12.48 ± 0.34
O+A	10.36 ± 0.31	0.53 ± 0.04[a]	1.33 ± 0.07	10.89 ± 0.32	12.22 ± 0.31
O+L+P+A	10.60 ± 0.30	0.52 ± 0.04[a]	1.01 ± 0.03	11.11 ± 0.33	12.12 ± 0.36
Mitochondria					
Controls ($N = 8$)					
Endogenous	2.83 ± 0.16	2.19 ± 0.11	1.21 ± 0.15	5.03 ± 0.25	6.23 ± 0.43
Oleate	2.34 ± 0.13[a]	1.39 ± 0.06[a]	1.65 ± 0.17[a]	3.73 ± 0.18[a]	5.38 ± 0.23
O+A	2.41 ± 0.09[a]	1.54 ± 0.04[a]	1.60 ± 0.17	3.96 ± 0.12[a]	5.56 ± 0.10
O+L+P	2.64 ± 0.12	1.66 ± 0.06[a]	0.89 ± 0.16	4.30 ± 0.13	5.19 ± 0.12
O+L+P+A	2.74 ± 0.08	1.61 ± 0.05[a]	0.97 ± 0.16	4.35 ± 0.12	5.32 ± 0.11
CRF ($N = 9$)					
Endogenous	2.55 ± 0.08	2.29 ± 0.10	1.08 ± 0.15	4.84 ± 0.16	5.92 ± 0.11
Oleate	2.17 ± 0.18	1.75 ± 0.1[a]	1.76 ± 0.10[a]	3.92 ± 0.24[a]	5.68 ± 0.30
O+A	2.27 ± 0.12	1.73 ± 0.06[a]	1.55 ± 0.13	3.99 ± 0.17[a]	5.55 ± 0.12
O+L+P	2.48 ± 0.12	1.74 ± 0.06[a]	0.83 ± 0.13	4.22 ± 0.14[a]	5.05 ± 0.13[a]
O+L+P+A	2.42 ± 0.07	1.55 ± 0.07[a]	0.73 ± 0.10	3.98 ± 0.08[a]	4.71 ± 0.1[a]

Incubation conditions and abbreviations are as indicated in Table 3. Comparisons between CRF and control groups and according to the incubation conditions were studied by using a two-factor repeated-measure ANOVA. Significant differences between control and CRF groups were found for cytosolic ATP, mitochondrial ADP and the sum of cytosolic ATP and ADP, whereas incubate-related differences were found to be significant for cytosolic AMP, cytosolic ADP and mitochondrial ATP, ADP, AMP, ATP plus ADP, and ATP plus ADP plus AMP.

[a] Significant differences between endogenous and substrate-related data.

weight [23, 37], this was not the case in our group. This can be explained by two factors. First, we used adult rats weighing 300 g. As reported in a chronic acidosis model [38, 39], the body mass of animals of this size seems to be only slightly affected by CRF. In the present model, food intakes were similar in CRF rats and controls during the week before hepatocyte isolation. Secondly, the experimental procedure used (partial left renal artery ligation 2 weeks before nephrectomy) was chosen to decrease the severity of insult. This point is of great importance since the nutritional status and the severity of stress are well known to alter hepatocyte metabolism [20, 23, 40].

The decrease of gluconeogenesis in CRF animals reported here was significant and ranged from 25% to 45% according to the tested substrate. These results were in accordance with a previous study with alanine, glutamine and serine [23]. In the present work, several steps of gluconeogenic pathway were studied by using different gluconeogenic precursors: pyruvate-phosphoenolpyruvate cycle with lactate plus pyruvate [41], amino acid transport across plasma membrane by alanine [26], glutaminase activity by glutamine [42, 43], cytosolic redox potential by glycerol [44], pyruvate kinase activity by dihydroxyacetone [45], and fructose 1-6 diphosphate cycle by fructose [46]. These results suggested a general effect of CRF on gluconeogenesis since all pathways were affected.

Ureagenesis was significantly diminished with all substrates used in the same proportion as gluconeogenesis. In particular with alanine and glutamine, the effect of uremia was the same in the two pathways for each substrate. These findings are in accordance with classic reports on gluconeogenesis and ureagenesis interrelationships [27, 28]. Studying a model of isolated hepatocytes similar to ours, Klim et al [23] reported an increased ureagenesis together with a decreased gluconeogenesis from alanine and glutamine. The discrepancy between the two studies may be explained by two differences in the experimental design. First, the methods for obtaining renal insufficiency (partial nephrectomy instead of arterial ligation) and the delay between surgery and hepatocyte isolation were different and may have induced a different level of stress. Indeed, the effect of the procedure used by Klim et al [23] resulted in significant changes in nutritional status leading these authors to study a pair-fed group (reducing by ⅓ the oral intake of controls). Secondly, these authors measured total ammonia accumulation (ammonia plus urea) and not only urea production. This could be of importance because, as reported here, ureagenesis from ammonia was impaired during CRF. Therefore, the act of considering the accumulation of ammonia and urea together may induce an overestimation of ureagenesis flux. Moreover, as pointed out by Meijer et al [27, 28] in *in vitro* conditions, cellular concentrations of urea cycle intermediates can be flux-limiting. Indeed, in the present study, the addition of ornithine increased ureagenesis from ammonia and this effect was more pronounced in uremic rat hepatocytes. In addition, it has been shown that the availability of carbon precursor for aspartate formation limits urea synthesis in hepatocytes incubated with ammonia and ornithine [47]. This may account for the fact that synthesis of urea under these conditions was lower than that observed in the presence of glutamine (Table 1). Thus,

decreased aspartate formation is a possible cause of the reduction of urea synthesis observed in hepatocytes from CRF rats in the presence of ammonia and ornithine.

Taken together, the present data on gluconeogenesis and ureagenesis suggested a general effect of CRF on isolated hepatocyte metabolism rather than specific alterations of these pathways. Following this hypothesis, one could expect that other pathways would be affected in a similar manner. Although we did not directly study fatty acid consumption, the measurement of ketone body production and O_2 uptake in the presence of fatty acids provided an insight into their metabolism. CRF was associated with either no change or a slight increase in ketone body production and fatty acid-induced respiratory rate. Thus, β-oxydation and ketogenesis were not altered by CRF. It is of interest to note that octanoate addition resulted in a slight but significantly higher ketone body production together with a higher β-hydrobutyrate/acetoacetate ratio in hepatocytes from CRF rats compared with controls. As the major difference between oleate and octanoate metabolism in liver cell is represented by the location of their activation [48, 49], these data suggest an increased intramitochondrial activation of medium-chain fatty acid in CRF.

Since oxidative phosphorylation accounts for most ATP production, O_2 uptake can be considered as a reliable reflection of ATP turnover. When compared with controls, CRF rat hepatocytes exhibited a slight increase in O_2 consumption which never reached significance. Thus, ATP turnover in CRF hepatocyte was probably as high as in control cells. This contrasts with the 30% decrease in both glucose and urea production when ureagenesis and/or gluconeogenesis were maximally stimulated. This discrepancy suggests that other energy consuming processes may be involved in CRF rat hepatocyte. The existence of such a waste of energy may also be suggested by the fact that the mitochondrial ATP/ADP ratio was significantly lower in CRF rat hepatocyte while the total adenine nucleotide content was not modified. The diminution of the mitochondrial ATP/ADP ratio in CRF rat hepatocyte due to ADP increase was not accompanied by a decrease in the cytosolic ATP/ADP ratio. This can be explained by the adenylate kinase activity which tends to buffer the cytosolic ATP/ADP ratio [50].

At a steady state of ATP turnover (stable O_2 consumption), a decrease in the mitochondrial ATP/ADP ratio can be essentially explained either by an increased ATP demand or by a decrease in oxydative phosphorylation efficiency. Our data do not make it possible to definitely rule out either of these hypotheses. However, in isolated mitochondria from control and CRF rat hepatocytes, we were not able to find any change in state 4 and state 3 oxygen uptakes, uncoupled maximal respiratory rate or in state 4 ATP/ADP and ATP/O ratios (data not shown). These data in isolated mitochondria strongly argue against a diminished mitochondrial efficiency.

Thus, these data on hepatocyte intermediary metabolism in CRF rats essentially showed (1) an alteration of stimulated gluconeogenesis and ureagenesis, and (2) a decrease in mitochondrial ATP/ADP ratio associated with normal O_2 uptake. The role of the insulin/glucagon ratio in the alteration of gluconeogenesis has been evoked [23]. The decrease in ureagenesis is consistent with the diminution of carbamoyl phosphate synthetase and ornithine transcarbamoylase activities reported in a similar rat model [17]. Among the metabolic disturbances linked to CRF, acidosis may explain the decrease of both gluconeogenesis and ureagenesis [51–54]. In this experimental model, extracellular pH (incubation medium) was identical in both groups, making it possible to exclude a direct effect of this factor. However, a chronic effect of acidosis on enzyme activities can be evoked. As a matter of fact, in isolated hepatocytes from chronically acidotic rats, a reduction of gluconeogenesis and ureagenesis from glutamine has been reported [55]. One could also speculate on the role of acidosis on cell respiration and energy status. However, although the pH of the incubation medium can influence O_2 consumption and ATP production by isolated hepatocytes (unpublished personal data), the effect of chronic acidosis on cell respiration and energy status is poorly known.

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