

Is Hepatic Uric Acid Production a Potential Biomarker of Compromised Mitochondrial Function?

Background

Raised plasma uric acid levels are associated with cardiovascular disease, renal disease and metabolic syndrome. Uric acid is produced by the liver during purine metabolism and is excreted by the kidneys. Therefore raised plasma uric acid can result from either decreased renal clearance or increased production by the liver. Recent studies have shown an association between raised serum uric acid levels and hepatic steatosis in Non-Alcoholic Liver Disease (NAFLD) after adjustment for various features of metabolic syndrome (1-3) and proposed a role for uric acid in the pathogenesis of liver disease through its pro-inflammatory effects (3).

Uric acid production by human liver is stimulated by fructose (> 5mM) which causes transient depletion of hepatic inorganic phosphate (Pi) and ATP (4). This mechanism is explained by increased conversion of AMP to IMP by activation of AMP-deaminase (Fig. 1).

Hypothesis

The hypothesis that was the basis of this study was that lowering of hepatic ATP without lowering of Pi as occurs during impaired mitochondrial function stimulates uric acid production. If ATP depletion stimulates uric acid production, then raised serum uric acid levels may be a marker of compromised liver mitochondrial function.

Aims

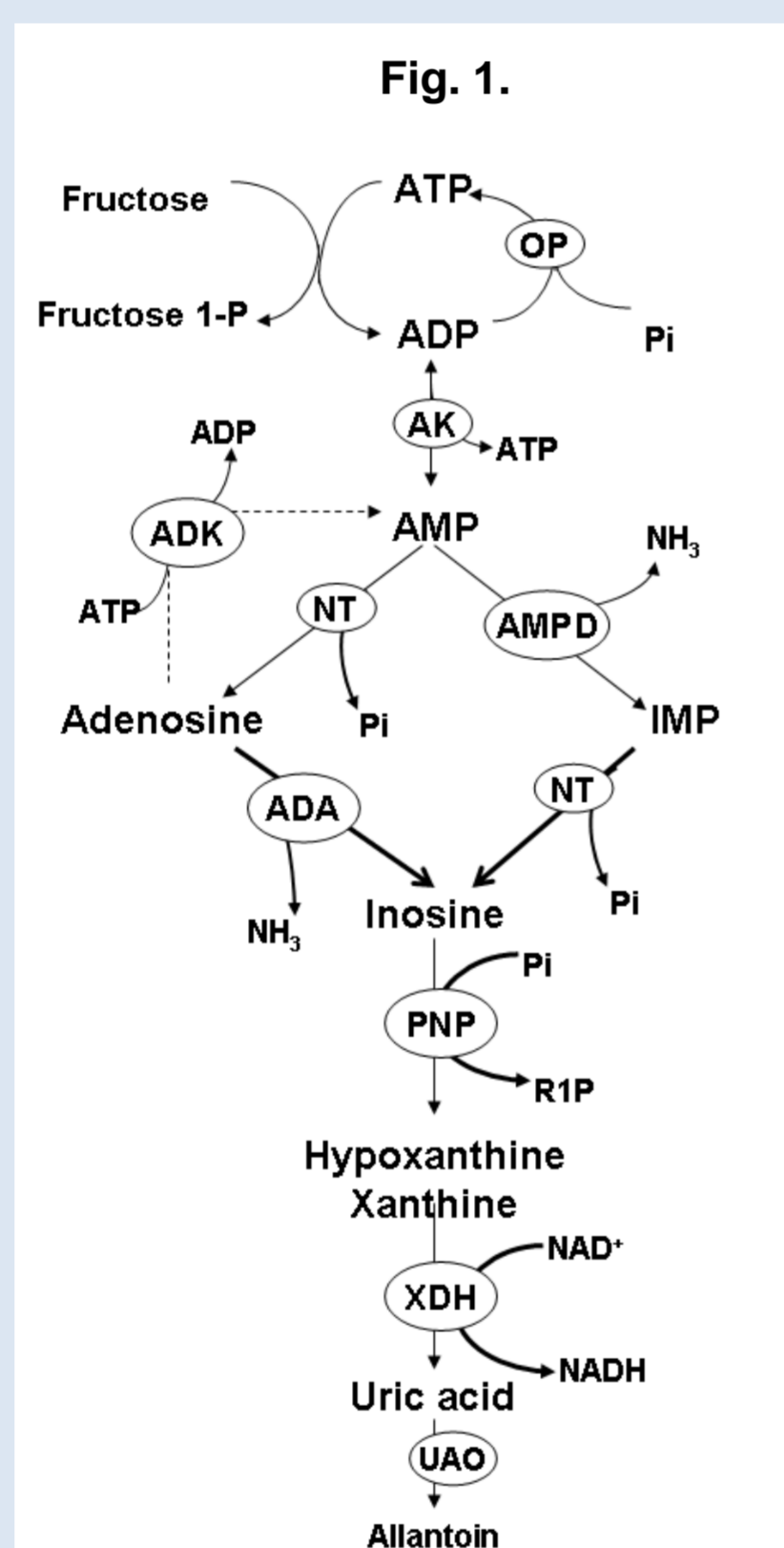
❖ To test whether substrates (e.g. fructose, glycerol, ethanol) that deplete Pi or ATP stimulate uric acid production (UAP).

❖ To test whether inhibitors of the mitochondrial respiratory chain (rotenone, antimycin, oligomycin) stimulate UAP in conditions of ATP depletion but raised Pi.

❖ To determine the relation between cell ATP and UAP and accordingly whether raised serum uric acid levels could be a potential marker of liver ATP depletion.

Fig. 1. Degradation of AMP to uric acid is stimulated by phosphate depletion

During phosphorylation of fructose, ATP consumption is coupled to mitochondrial oxidative phosphorylation (OP). This results in depletion of inorganic phosphate (Pi) at [fructose] > 5mM [5]. Degradation of AMP to IMP by AMP-deaminase (AMPD) is inhibited by physiological levels of Pi and activated by Pi depletion. IMP is further metabolized to inosine by nucleotidase (NT) and then to hypoxanthine by purine nucleoside phosphorylase (PNP). AMP can also be degraded to inosine by dephosphorylation (NT) and deamination by adenosine deaminase (ADA). Xanthine dehydrogenase (XDH) converts hypoxanthine to xanthine and then to uric acid which is the final product in man but is further metabolized in other species by uric acid oxidase (UAO, uricase) to allantoin.



Methods

Principle: In human liver uric acid is the final product of purine degradation because of loss of function of uricase (UAO) (Fig. 1) in primate evolution. In other species plasma uric acid levels are much lower than in man and rates of UAP by hepatocytes are also lower because of further metabolism of uric acid to allantoin. An in vitro method that uses oxonic acid to inhibit uric acid oxidase was previously developed to study UAP in rat hepatocytes (5).

Hepatocyte Culture: Hepatocytes were isolated from Wistar rats by collagenase perfusion of the liver and cultured in monolayer in Minimum Essential Medium for 24h as in (5)

Uric acid production (UAP): was determined during 1 h incubations in balanced salts medium with oxonic acid and 5mM glucose (5) and substrates (fructose, glycerol, ethanol) or inhibitors of the respiratory chain: rotenone (complex I), antimycin (complex III), oligomycin (complex V). The medium was collected for determination of uric acid and lactate (5). Cell ATP, glycerol 3-P and Pi were determined colorimetrically or enzymically (5). Results are expressed as Means \pm SEM, for n preparations. Statistical analysis was by the student's t-test: * P < 0.05.

References

[1] Li Y et al. (2009) J Hepatol. 50:1029-34; [2] Afzali A et al. (2010) Hepatology 52:578-89; [3] Sirota JC et al (2012) Metabolism in press; [4] Mayes PA (1993) Am. J. Clin. Nutr. 58:754S-765S ; [5] Arden et al (2011) Diabetes 60:3110-3120.

Fig. 2. Stimulation of UAP by glycerol is associated with lowering of cell Pi and ATP

A. UAP in hepatocytes incubated with the substrates indicated: fructose (20 mM), glycerol (5mM), ethanol (20mM), adenosine (100 μ M), inosine (100 μ M) or xanthine (200 μ M). B. Cell inorganic phosphate (Pi) in hepatocytes incubated for 30min with substrates: 20mM fructose; 5mM glycerol, 20mM ethanol or inhibitors (2 μ M): rotenone, antimycin or oligomycin. C-F. Hepatocytes incubated with 0.5 to 5 mM glycerol for determination of: glycerol 3-P (C), ATP (D) and UAP (E). F. Correlation between UAP and ATP. Means SEM for 4-6.

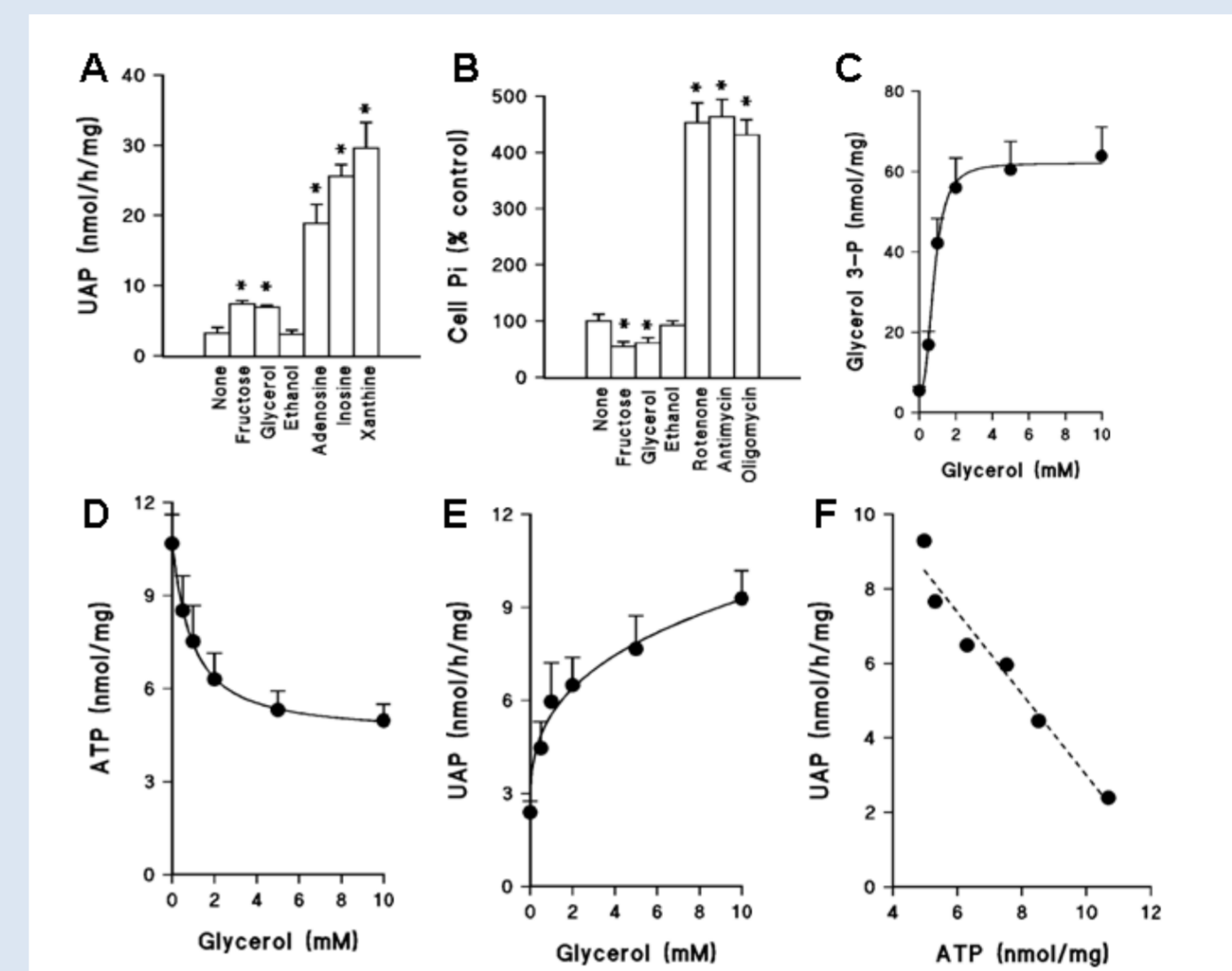
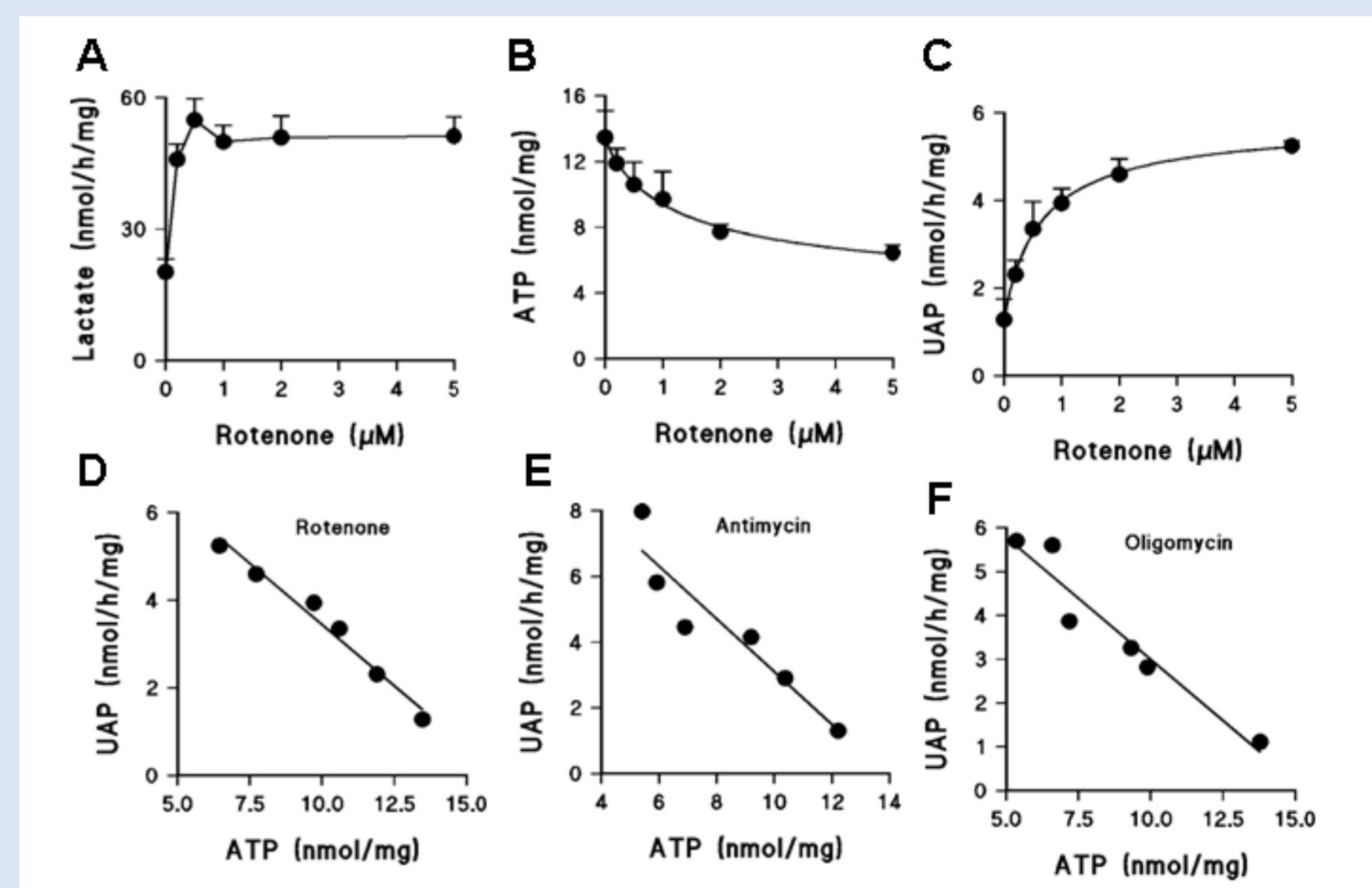


Fig. 3. UAP correlates with ATP depletion during inhibition of the respiratory chain with rotenone, antimycin or oligomycin

Hepatocytes were incubated with varying concentration or rotenone, antimycin or oligomycin and medium lactate, cell ATP and UAP were determined. A-C. Effects of rotenone on lactate production (A), cell ATP (B) and UAP (C). D-F. Correlation between ATP and UAP for incubations with rotenone (D), antimycin (E) and oligomycin (F). Means \pm SEM for 4-6.



Summary of Results

Fig. 2A: Uric acid production (UAP) was stimulated > 2-fold by fructose (20mM) and by 5mM glycerol but was not affected by ethanol (20mM). Adenosine, inosine and purine which are degraded to uric acid (Fig. 1) caused a much larger stimulation of UAP than fructose and glycerol.

Fig. 2B: Cell Pi was lowered by fructose and glycerol but raised by rotenone, antimycin and oligomycin which inhibit complexes I, III and V of the respiratory chain.

Fig. 2D-F: Stimulation of UAP by glycerol was associated with elevation in glycerol 3-P and depletion of ATP and correlated inversely with cell ATP.

Fig. 3A-C: Rotenone lowered cell ATP and stimulated lactate and UAP.

Fig. 3D-F: During inhibition of mitochondrial respiratory chain complex I (rotenone), complex III (antimycin) or complex (V), UAP correlated inversely with ATP depletion.

Conclusion

Uric Acid Production is stimulated by substrate-induced depletion of Pi and ATP as occurs with glycerol and fructose and also in conditions of decreased mitochondrial activity which is associated with depletion of ATP but elevation of Pi.

Therefore raised plasma concentrations of uric acid may be a marker of decreased hepatic mitochondrial function.