The rate of production of uric acid by hepatocytes is a sensitive index of compromised cell ATP homeostasis

John L. Petrie,1* Gillian L. Patman,2* Ishita Sinha,1 Thomas D. Alexander,1 Helen L. Reeves,2 and Loranne Agius1

1Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom; and 2Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, United Kingdom

Submitted 15 April 2013; accepted in final form 10 September 2013

The rate of production of uric acid by hepatocytes is a sensitive index of compromised cell ATP homeostasis. This is supported by the finding that the rate of uric acid production (UAP) by rat hepatocytes is a potential marker of hepatic ATP homeostasis, particularly in liver disease. It is also supported by a model of rat hepatocytes, which shows that UAP by hepatocytes is a potential marker of hepatic ATP homeostasis, particularly in liver disease.

PLASMA LEVELS OF URIC ACID. The final product of purine degradation in humans (Fig. 1), is strongly associated with insulin resistance (39) and with nonalcoholic fatty liver disease (NAFLD) (2, 20, 28). Plasma uric acid reflects the balance between endogenous production by the liver and excretion by the kidney and gut (29). Genome meta-analyses identified common variants in the GCKR gene (31, 32), which encodes the urate oxidase enzyme (31). This enzyme is important in the metabolism of uric acid and its degradation to allantoin and xanthine (31). However, UAP is higher in humans than in other species (32). Accordingly, compromised hepatic Pi or ATP homeostasis is a possible link between NAFLD and hyperuricemia. In this study we optimized a rat hepatocyte model to test the hypothesis that UAP by hepatocytes is a potential marker of hepatic ATP homeostasis. Fructose-induced changes in hepatic ATP and Pi are similar in the rat and in humans (21), supporting the validity of the rat model. However, UAP is higher in humans than in other species because of evolutionary loss of functional uric acid oxidase (29), which in other species further metabolizes uric acid to allantoin (31). Our rat hepatocyte model therefore uses a uric acid oxidase inhibitor (oxonic acid) to measure UAP without its further degradation to allantoin (31).

EXPERIMENTAL PROCEDURES

Primary hepatocyte culture. Hepatocytes were isolated from male Wistar rats (obtained from Harlan) by collagenase perfusion (5). All animal procedures conformed to Home Office Regulations and were approved by Newcastle University Ethical Committee. Hepatocytes were suspended in MEM with 5% calf serum and seeded in multiwell plates. After cell attachment the medium was replaced by serum-free MEM containing 10 mM glucose, 10 nM dexamethasone, and 1 nM insulin, and the hepatocytes were cultured for 24 h (5).

Cell lines. Hep-G2 and AML12 cells were cultured in DMEM/F-12 supplemented with 10% FBS, 5 μg/ml transferrin, 5 ng/ml selenium, 10 mM insulin, and 10 nM dexamethasone. They were seeded in multiwell plates in the same medium and after attachment cultured in serum-free DMEM/F-12 for 24 h and then used for determination of UAP in 1-h incubations as described for hepatocytes.
Fructose → ATP
Fructose 1-P → ADP
ADP → AK → AMP
AMP → ADK → ADP
ADP → AK → AMP
AMP → AMPD → NH₃
NH₃ + Pi → NADH
NADH + H⁺ + ADP → ATP
ATP → OP

Hypoxanthine → Xanthine → Uric acid

Adenosine → Inosine → AMP
AMP → ADK → ADP
ADP → AK → AMP
AMP → AMPD → NH₃
NH₃ + Pi → NADH
NADH + H⁺ + ADP → ATP
ATP → OP

Allantoin → Oxonic acid

Fructose

Hepatocyte incubations for determination of UAP.

Results are expressed as means ± SE for the number of hepatocyte preparations indicated. Statistical analysis was by the paired t-test.


Enzyme activity determination.

Hypoxanthine (XHX)

Uric acid was determined on 30 µl of medium from the ferric reducing activity using a SpectraMax M5e reader (Molecular Devices), and results are expressed as micromole per gram weight of tissue.

Purine metabolite determination.

Uric acid accounted for >90% of the ferric reducing activity as determined from parallel incubations without or with allopurinol (10 µM), an inhibitor of xanthine dehydrogenase (XDH) (Fig. 1). The limit of detection of uric acid was 0.2 µM (6 pmol/assay). Rates of UAP determined from 60-min incubations are expressed as nanomoles per hour per milligram cell protein. For most experimental conditions, the rates were approximately linear during 60 min as determined from 30-, 60-, and 90-min incubations. Combined, XHX was determined by a modification of Ref. 12 by incubating the medium with an equal volume of buffer containing 100 mM KCl, 50 mM Tris, pH 7.5, 1 mM 2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium chloride, and 0.1 U/ml xanthine oxidase (X1875; Sigma) for 15 min followed by determination of uric acid as above. Inosine was determined as for XHX but with addition of 0.3 U/ml purine nucleoside phosphorylase (PNP, no. 40809; Sigma) (12). Rates of formation of inosine and XHX are expressed as nanomoles per hour per milligram protein.

Enzyme activity determination. The cells were extracted in buffer containing 50 mM Tris, pH 7.5, 1 mM sodium phosphate, and 1 mM EDTA, pH 7.5, sonicated, and centrifuged (13, 000 g, 5 min).

Adenosine deaminase (ADA) and PNP were determined enzymatically (4) in buffer supplemented with 0.5 mM oxonate, 0.5 mM iodonitrotetrazolium, and 0.1 U/ml xanthine oxidase from the formation of uric acid as determined above. XDH was determined in buffer supplemented with 0.1 mM xanthine, 0.5 mM NAD, and 0.5 mM oxonate, with or without 10 µM allopurinol and determined from the allopurinol-sensitive formation of uric acid. Activities are expressed as milliumoles per milligram, representing nanomole of uric acid formed per minute per milligram protein.

Results are expressed as means ± SE for the number of hepatocyte preparations indicated. Statistical analysis was by the paired t-test.
results of real-time PCR using a SYBR-Green assay kit (Roche) as described (5) with the following primer sets: GCK, CCAGACACCTTGACTCTGG (forward), TCACCTTCTTCAGGTCCT (reverse); GCKR, CAGATTCTGCTGCTTCTCAT (forward), CATAAAACAGAGACACAGAGAA (reverse); SLC17A3, GAAGCCCTTTCCACAGACATT (forward), CCTGCTGTGGGACTCAACT (reverse).

RESULTS

**UAP from endogenous substrates in hepatocytes.** The basal rate of hepatic UAP in humans, determined by catheterization of the hepatic vein, is 3–5 nmol min⁻¹ g⁻¹ and is increased severalfold during intravenous fructose infusion (14, 15). In rat hepatocytes, the rate of UAP from endogenous substrates determined in basal medium with oxonic acid, to inhibit uric acid degradation by uricase (5), was 6.7 ± 2.5 nmol·h⁻¹·mg⁻¹ in freshly isolated hepatocytes and 2.4 ± 0.2 nmol·h⁻¹·mg⁻¹ (Fig. 2A) after 24 h culture. These rates are approximately six- and twofold higher than the basal rate in human liver (assuming 200 mg protein/g). The decline in basal rate during 24-h culture may be because of cell recovery from the isolation procedure. During 1-h incubations in basal medium with glucose as sole substrate, UAP was decreased (~18%) by insulin and increased (~21%) by glucagon or phenylephrine (Fig. 2A). Fructose (20 mM) stimulated UAP more than twofold in 1-h incubations, whereas ethanol and acetate had no effect (Fig. 2B). Cell ATP was lowered by fructose (Fig. 2C), as expected (21). Time courses (30–120 min) for effects of fructose showed greater fractional UAP stimulation at 30 min (results not shown) and greater lowering of ATP at 30 min as shown previously (5, 21), indicating rapid effects of fructose.

**UAP from exogenous substrates in hepatocytes.** Exogenous adenosine, inosine, and xanthine, which are taken up by hepatocytes and metabolized either by salvage pathways or by degradation to uric acid (Fig. 1), can be used to measure the maximum capacity of the uric acid pathway downstream of AMP deaminase at the respective sites (Fig. 1). Adenosine, inosine, and xanthine (at high micromolar concentrations) caused a much greater stimulation of UAP than fructose (Fig. 2B). Inosine and xanthine had no significant effect on ATP, whereas adenosine, which is a substrate for adenosine kinase, caused a significant increase in ATP (Fig. 2C), as expected (21).

With adenosine and inosine, as exogenous substrates, maximum UAP occurred at ~20–50 μM and with xanthine at ~200 μM (Fig. 2D). With adenosine as substrate, the hepatocytes also produced inosine and XHX (Fig. 2E), whereas, with inosine as substrate, XHX was produced at a higher rate than from adenosine (Fig. 2F). This indicates that XDH is ratelimiting for UAP at high concentrations of adenosine and inosine. The activities of ADA, PNP, and XDH in hepatocytes (ADA, 5.7 ± 1.7; PNP, 57 ± 6; XDH 0.24 ± 0.11 mU/mg) were consistent with net metabolism of adenosine and inosine to products upstream of uric acid (Fig. 2, E and F).

**UAP in liver-derived cell lines.** HepG2 human hepatoma cells metabolized adenosine and inosine to XHX at higher rates than hepatocytes but did not produce uric acid with any of the substrates (Fig. 3, A and B) and had no detectable XDH activity but had high activities of ADA and PNP (30.6 ± 3.4 and 22.9 ± 2.3 mU/mg). Mouse AML12 cells had similar rates of metabolism of adenosine and inosine to uric acid.
ADA, PNP, and XDH were comparable to hepatocytes (ADA, inosine, or xanthine as substrate (Fig. 3), and the activities of CUA as hepatocytes in basal medium and with adenosine, AML12 cells.

![Graph A](image1)

**Fig. 3.** Production of purine metabolites by hepatocytes, Hep-G2 cells, and AML12 cells. A–C: rat hepatocytes (A), Hep-G2 cells (B), and AML12 cells (C) were incubated for 1 h in basal medium containing 0.5 mM oxonic acid (filled bars) or in medium lacking oxonic acid (open bars) for determination of formation of uric acid and XHX. The medium was supplemented with 200 μM adenosine (Aden), 200 μM inosine (Inos), or 200 μM xanthine (Xan) substrate as indicated. Results are means ± SE for 3 (A) or 4 (B and C) experiments.

UAAP as hepatocytes in basal medium and with adenosine, inosine, or xanthine as substrate (Fig. 3C), and the activities of ADA, PNP, and XDH were comparable to hepatocytes (ADA, 10.3 ± 1.3; PNP 34.9 ± 4.4; XDH, 0.2 ± 0.05 mU/mg).

Unlike in hepatocytes, in AML12 cells, UAAP was not responsive to oxonic acid (Fig. 3, A and C) or fructose (results not shown). Rat primary hepatocytes were used in the rest of this study.

**Fructose stimulation of UAAP is enhanced by elevated glucose or Pi depletion.** Intravenous fructose infusion at concentrations >5 mM lowers hepatic Pi and ATP in humans and rats (21) and raises blood uric acid in humans (15, 23, 38), but whether dietary fructose elicits effects on UAAP is uncertain because fructose levels in the portal vein after oral ingestion are thought not to exceed 2.5 mM (16, 34). We next tested the effect of fructose (2–20 mM) at either basal (5 mM) or elevated (15–25 mM) glucose to simulate glucose concentrations in the portal vein after carbohydrate-containing meals. At basal glucose (5 mM), fructose stimulated UAAP at concentrations above 5 mM (P < 0.05). However, at elevated glucose the response to fructose was shifted to lower concentrations, with significant elevation at 2 mM fructose (Fig. 4A). Cell ATP determined at 60 min (Fig. 4B) and Pi determined at 20 min (Fig. 4C) were decreased by fructose but without significant differences between low vs. high glucose. Because the fructose-induced lowering of hepatic Pi is transient (21), we determined Pi release in a Pi-free medium (Fig. 3D) as a measure of cumulative changes in cytoplasmic Pi (27). Pi release was attenuated additively (P < 0.05) by fructose and 25 mM glucose (Fig. 4D). We next tested the effects of extracellular Pi (0 vs. 1 mM) on the response to low (2–5 mM) or high (20 mM) fructose. UAAP stimulation by low fructose was enhanced by 15 mM glucose and by a Pi-free medium (Fig. 4E), whereas cell ATP was similar at low and high glucose (Fig. 4F). This shows that the fructose threshold for stimulation of UAAP is shifted to lower concentrations by either elevated glucose or by lower extracellular Pi.

**Glycerol stimulates UAAP concomitantly with depletion of Pi and ATP.** Ethanol consumption is implicated as a cause of hyperuricemia (13, 14, 24, 43), and combined administration of ethanol (0.7 mol) and glycerol (0.02 mol) caused a similar elevation of serum uric acid in humans as fructose (0.4 mol) (38). We tested the separate effects of glycerol and ethanol (Fig. 5). Glycerol (0.5–2 mM) stimulated UAAP (P < 0.05), lowered cell ATP, and increased glycerol 3-phosphate (Fig. 5, A–C). UAP correlated inversely with ATP (Fig. 5) and Pi determined at 20 min was lowered by 1–2 mM glycerol (Fig. 5D), whereas cell ATP determined at 60 min (Fig. 4D) was similar at low and high glucose (Fig. 4F). This shows that the fructose threshold for stimulation of UAAP is shifted to lower concentrations by either elevated glucose or by lower extracellular Pi.

Stimulation of UAAP by inhibition of the mitochondrial respiratory chain. The above studies show stimulation of UAAP by fructose or glycerol at concentrations associated with low-
ering of both cell ATP and Pi. We next tested the effects of inhibition of mitochondrial oxidative phosphorylation with inhibitors of the respiratory chain complexes I, III, and V using rotenone, antimycin, and oligomycin, respectively (Fig. 6). As expected (11), the inhibitors markedly raised cell Pi (Fig. 6A). Rotenone increased lactate production (Fig. 6B), lowered ATP (Fig. 6C), and raised UAP (Fig. 6D), with an inverse correlation between ATP and UAP (Fig. 6E). Likewise, antimycin and oligomycin (at 0.2–2.5 μM) raised lactate production and UAP and lowered ATP (results not shown), with an inverse correlation between UAP and ATP (Fig. 6F and G). The relation between UAP and ATP determined from the initial slope of double-log plots gave coefficients of 4.0, 4.1, and 2.8 for rotenone, antimycin, and oligomycin, respectively, compared with a coefficient of 2.6 for glycerol (Fig. 5D). This shows very high sensitivity of UAP to lowering of cell ATP in conditions of depletion (glycerol) and elevation (mitochondrial inhibitors) of cell Pi.

Effects of a high-energy diet on liver adenine nucleotides. The experiments in Figs. 4–6 show significant stimulation of UAP by metabolic perturbations that lower cell ATP content by ≥20%. We next measured liver ATP, ADP, and AMP in mice fed a high-energy diet for 24 wk. Liver ATP content (mol/g) and also the ratio of ATP to total adenine nucleotides were significantly lower by 30 and 17%, respectively, on the high-energy diet (Table 1), indicating compromised ATP homeostasis.

Roles of cell Pi and the redox state on UAP from exogenous purines. Two unexpected findings from this study were: the lack of effect of ethanol, and the similar but partial stimulation of UAP by substrates that are phosphorylated rapidly (fructose and glycerol) or by inhibitors of the respiratory chain. This corresponded to a maximum rate that is 30% of the rate with saturating concentrations of adenosine or inosine as exogenous substrates. We considered the possibility that the lack of stimulation by ethanol and the partial stimulation by other conditions could be the result of inhibition of a downstream enzyme (ADA, PNP, or XDH) in the pathway (Fig. 1). To test this possibility, we compared in parallel the effects of ethanol with stimuli that deplete Pi (fructose and glycerol) or raise Pi (rotenone) on the degradation of xanthine, inosine, and adenosine, which enter the pathway upstream of XDH, PNP, and ADA, respectively (Fig. 7). The basal rate of UAP was increased similarly by fructose, glycerol, and rotenone but was not affected by ethanol (Fig. 7A). Xanthine conversion to uric acid (Fig. 7B) and likewise inosine conversion (Fig. 7C) were partially inhibited by glycerol, ethanol, and rotenone but not by fructose. However, inosine clearance was decreased by fructose, glycerol, and ethanol (Fig. 7D), and inosine conversion to XHX showed similar trends (Fig. 7E). In contrast, adenosine clearance was decreased by rotenone but not by the other treatments (Fig. 7F). Fructose increased the fractional conversion of adenosine to inosine (Fig. 7G) and decreased conversion to XHX (Fig. 7H). Rotenone, in contrast, increased XHX formation, and glycerol and ethanol decreased fractional conversion of adenosine to uric acid (Fig. 7I).

Cumulatively, these results show: first, that fructose, glycerol, and rotenone stimulate UAP only in basal conditions and not with adenosine, inosine, or xanthine as substrate; second, that metabolic conditions that cause a more reduced NAD+/NADH redox state (glycerol, ethanol, and rotenone) inhibit
xanthine and inosine conversion to uric acid; and third, metabolic conditions that lower cell Pi (fructose, glycerol, and ethanol) inhibit inosine clearance and increase inosine release during metabolism of adenosine. This concurs with a role for the cosubstrates of PNP (Pi) and of XDH (NAD⁺/H⁺) in regulating inosine clearance and xanthine degradation, respectively (Fig. 1). It can be inferred that stimulation of UAP by fructose, glycerol, and rotenone occurs entirely at a site(s) upstream of adenosine and inosine. In addition, the lack of effect of ethanol on basal UAP cannot be explained by inhibition at downstream sites because rotenone inhibited xanthine metabolism similarly to ethanol but nonetheless stimulated basal UAP (Fig. 7, A and B). When the effects of rotenone were further studied in the presence of allopurinol (10 μM) to inhibit XDH (Fig. 6H), the release of XHX in the presence of allopurinol was only slightly higher than UAP in the absence of allopurinol, further con-

Fig. 5. Stimulation of UAP by glycerol correlates with ATP depletion. A–F: hepatocytes were incubated for 1 h in basal medium with glycerol (0.5–10 mM) for determination of UAP (A), cell ATP (B), and glycerol 3-phosphate (C), n = 6. D: inverse correlation between UAP and ATP. E: effects of glycerol on cell Pi, determined after 20 min, n = 3. F: glycerol decreases release of cell Pi in a Pi-free medium, n = 4, *P < 0.05, effect of glycerol (E and F). G–I: hepatocytes were precultured (24 h) in MEM with either 5 or 30 mM glucose to replete glycogen stores. They were then incubated for 1 h with 5 or 25 mM glucose, and without (open bars) or with (filled bars) 50 mM ethanol for determination of UAP (G) and ATP (H) and glycerol 3-phosphate (I). J: cell Pi, in hepatocytes incubated with ethanol for 20 min in basal medium. K: medium Pi in hepatocytes incubated with ethanol for 30 min in a Pi-free medium. Means ± SE, n = 6 (G–I) or 4 (J and K). *P < 0.05 effect of ethanol.
firming that inhibition of XDH cannot explain the lack of stimulation of UAP by ethanol in the basal medium. Throughout this study release of XHX by hepatocytes was only detectable either in the presence of allopurinol (e.g., Fig. 6H) or in incubations with exogenous adenosine or inosine as substrates.

Correlation between expression of sodium phosphate transporters and GCKR in human liver Genome meta-analysis (18, 19, 41, 44) identified associations between raised serum urate concentration and polymorphisms in genes encoding anion transporters that are expressed in kidney or ubiquitously and also with a common variant in the GCKR gene that is expressed in hepatocytes and is functionally linked to UAP (3, 5). We checked human liver microarray datasets for expression of anion transporter genes that are associated with uric acid (18, 41) and confirmed expression of SLC2A9, SLC17A1, and SLC17A3 in human liver. In these microarrays, expression of SLC17A1 and SLC17A3, which encode Na-Pi transporters, correlated with expression of GCKR, whereas expression of GCK, the target protein of GCKR, did not correlate with GCKR expression \( \text{ATP/ADP + AMP, \mu mol/g} \) 4.63 ± 0.22 3.86 ± 0.24 ATP/ADP 1.11 ± 0.11 0.81 ± 0.17 ATP/ADP + AMP, \mu mol/g 0.40 ± 0.02 0.33 ± 0.01

Values are means ± SE; \( n = 4 \) experiments. \( *P < 0.05, t \)-test.

Correlation between expression of sodium phosphate transporters and GCKR in human liver Genome meta-analysis (18, 19, 41, 44) identified associations between raised serum urate concentration and polymorphisms in genes encoding anion transporters that are expressed in kidney or ubiquitously and also with a common variant in the GCKR gene that is expressed in hepatocytes and is functionally linked to UAP (3, 5). We checked human liver microarray datasets for expression of anion transporter genes that are associated with uric acid (18, 41) and confirmed expression of SLC2A9, SLC17A1, and SLC17A3 in human liver. In these microarrays, expression of SLC17A1 and SLC17A3, which encode Na-Pi transporters, correlated with expression of GCKR, whereas expression of GCK, the target protein of GCKR, did not correlate with GCKR expression \( (n = 22; \text{SLC17A1, } r = 0.882, P < 0.00001; \text{SLC17A3, } r = 0.780 P < 0.00001; \text{GCK, } r = -0.02, P = 0.92) \). We next determined mRNA levels of these genes by RT-PCR in liver biopsies from an independent cohort of 12 subjects with NAFLD (Fig. 8). In this cohort, GCKR mRNA correlated with mRNA levels of SLC17A1 \( (r = 0.944, P < 0.00001) \) and SLC17A3 \( (r = 0.882, P < 0.0001) \) but not of GCK \( (r = -0.161, P = 0.618) \) (Fig. 8, A–C), indicating an association in gene expression in human liver between GCKR and phosphate transporter genes identified through genome meta-analysis in association with serum uric acid.

**DISCUSSION**

Raised serum uric acid levels correlate with NAFLD (2, 20, 28) and with insulin resistance (39). Because hyperuricemia is associated with increased UAP (42), it may be a marker of altered hepatic metabolic function. Genomewide and meta-analysis studies identified variants in anion transporters and in the GCKR gene in association with raised serum urate (18, 19, 44), and functional studies showed that forced elevation in the GCK-to-GCKR ratio in hepatocytes leads to ATP depletion and raised UAP at high glucose (5). However, the GCKR variant that is linked to raised urate and NAFLD is associated with inhibition of XDH cannot explain the lack of stimulation of UAP by ethanol in the basal medium. Throughout this study release of XHX by hepatocytes was only detectable either in the presence of allopurinol (e.g., Fig. 6H) or in incubations with exogenous adenosine or inosine as substrates.
with lower insulin resistance (3, 31). Accordingly, other mechanisms must account for the association between hyperuricemia and insulin resistance.

Diet-induced insulin resistance is a possible explanation for the association between serum urate and insulin resistance (1, 5). During consumption of high-carbohydrate diets, the liver is exposed to high portal vein concentrations of glucose and/or fructose, which cause elevation in hepatic organic phosphate esters and activation of the transcription factor ChREBP-Mlx (3, 6). A suggested function of ChREBP-Mlx is intrahepatic phosphate homeostasis (3, 5). Accordingly, its target genes include GCKR, which restrains hepatic glucose uptake, and glucose-6-phosphatase gene, which hydrolyses glucose 6-phosphate to glucose and buffers the hepatic concentration of phosphate esters (3, 6). ChREBP deficiency is associated with decreased ATP phosphorylation potential (9), consistent with a potential role for ChREBP activation in gene regulation to maintain cell ATP homeostasis (3).

Studies in humans have shown that hepatic ATP is decreased in obesity (10) and that hepatic Pi and ATP correlate negatively with hepatic insulin resistance assessed by euglycemic-hyperinsulinemic clamps (32). Here we optimized a hepatocyte model to test the hypothesis that elevated UAP is linked to compromised Pi and/or ATP homeostasis. The validity of the rodent model for human liver metabolism is well supported by noninvasive NMR imaging of the temporal changes in liver Pi and ATP after fructose challenge (21). In the rat hepatocyte model, the basal rate of UAP was comparable to the rate in human liver (14, 15), and it was stimulated more than twofold during inhibition of mitochondrial respiration or during depletion of ATP with fructose or glycerol. However, it was not affected by ethanol or acetate, which did not lower cell ATP. UAP was significantly, although marginally, inhibited by insulin and stimulated by glucagon. The underlying mechanisms for this hormonal regulation remain to be determined.

Fig. 7. Effects of substrates and rotenone on metabolism of xanthine, inosine, and adenosine. Hepatocytes were incubated with the additions indicated: fructose (20 mM), glycerol (10 mM), ethanol (20 mM), or rotenone (5 μM) either in basal medium without substrates (A) or supplemented with 200 μM xanthine (B), 100 μM inosine (C–E), or 100 μM adenosine (F–I) for determination of purine degradation products (inosine, XHX, and uric acid). For incubations with adenosine (F–I), adenosine clearance (F) represents production of UA + XHX + inosine, and the formation of inosine, XHX, and uric acid (G–I) is expressed as %adenosine clearance. Means ± SE, n = 4, *P < 0.05 relative to no additions.
The key finding from our study is that UAP is very sensitive to ATP depletion irrespective of whether cell Pi is lowered, as occurs during fructose and glycerol metabolism, or raised, as occurs during inhibition of the mitochondrial respiratory chain.

In incubations with glycerol or respiratory chain inhibitors, lowering of ATP by 23–28% was associated with approximately twofold elevation in UAP. A similar degree of liver ATP depletion was observed in the animal model on a high-energy diet. Measurement of hepatic ATP and Pi by magnetic resonance imaging in subjects with type 2 diabetes showed that ATP and Pi were lower in diabetes by about 26–28% and correlated with decreased hepatic insulin sensitivity (32). Our data show approximately twofold elevation in UAP by comparable ATP depletion. This suggests that liver ATP depletion may be the link between NAFLD and UAP.

Stimulation of UAP in humans by intravenous fructose infusion is well documented (23) and is explained by hepatic elevation in fructose 1-phosphate, causing depletion of cell Pi and adenine nucleotides (ATP, GTP) and activation of AMP deaminase, which converts AMP to IMP (34–36). AMP deaminase is allosterically inhibited by Pi and GTP, but it is stimulated by ATP (35, 36). Thus the inverse correlation between ATP and UAP implies that mechanisms consequent to ATP depletion (e.g., depletion of GTP or other effectors) must have an overriding stimulatory effect on the attenuation of AMP deaminase by ATP depletion. Whether dietary fructose intake affects serum uric acid levels remains debated (1, 34, 40) because fructose concentrations in the portal vein are thought not to exceed 2.5 mM (17, 34). We show that stimulation of UAP occurs at lower fructose concentrations (2 vs. >5 mM) at elevated glucose (15–25 mM) compared with basal glucose concentration. Thus the propensity of dietary fructose to stimulate UAP would be dependent on the accompanying glucose load. Fructose affects hepatocellular phosphate ester concentrations by two mechanisms: through elevation in fructose 1-phosphate (22, 34) and by fructose 1-phosphate-mediated activation of the GCK-GCKR protein complex, causing elevated glucose 6-phosphate formation from glucose (3). Thus the left shift in fructose-induced UAP caused by elevated glucose is consistent with an additive effect of high glucose and precursors of fructose 1-phosphate on phosphate ester concentrations as supported by Pi release in the Pi-free medium.

Ethanol consumption in humans is widely implicated as a cause of hyperuricemia (13, 43). Acute stimulation of UAP by ethanol was shown by catheterization of the hepatic vein (14). However, whether this was because of a direct effect of ethanol metabolism by hepatocytes or may have resulted from secondary endocrine or autonomic stimuli (25) induced by the ethanol infusion could not be ascertained from the in vivo study (14). In this study, we found no evidence for effects of ethanol (5–50 mM) on cell ATP or UAP irrespective of the glucose concentration. Small or negligible changes in the ATP-to-Pi ratio with ethanol in vivo or in hepatocytes have been reported (11, 37). Although ethanol inhibited the final step in the uric acid pathway determined from the conversion of xanthine to uric acid, this mechanism could not explain the lack of effect of ethanol on the basal rate of UAP because ethanol caused similar inhibition of xanthine conversion to uric acid as rotenone, consistent with reduction in the NAD+/NADH ratio in regulating flux through XDH. However, stimulation of UAP by rotenone correlated with ATP depletion. The lack of effect of ethanol on cell ATP is the most likely explanation for the lack of stimulation of UAP. It could be speculated that ethanol may stimulate UAP in humans either in conditions of compro-
mised ATP, as is observed with inhibitors of the respiratory chain, or through secondary endocrine or autonomic control of UAP (25).

The association between raised serum uric acid and polymorphisms in anion transporter genes is often attributed to defects in renal clearance of uric acid (18, 41). Whether the anion transporters have a role in hepatic UAP has not been explored. We show a correlation in human liver between gene expression of GCKR, which is functionally linked to UAP at elevated glucose (5), and expression of the sodium phosphate transporters encoded by SLC17A1 and SLC17A3. These data do not prove causality but are suggestive of a role for Pi homeostasis in the pathogenesis of NAFLD. Metabolomic studies on hepatic concentrations of purine intermediates have shown converse changes with aging between inosine (lowering) and hypoxanthine and xanthine (elevation), which could be explained by changes in redox state (30). We show in this study that changes in cell Pi, as occur during fructose challenge, can also affect the clearance of inosine and the production of inosine from adenosine. Thus carbohydrate-induced phosphate depletion can affect metabolism of exogenous purine intermediates as well as basal UAP from endogenous substrates.

In summary, current evidence to date links elevated hepatic UAP to fructose loading (22, 23, 28) and to common GCKR gene variants (18, 19, 44). The present study shows that stimulation of UAP is very sensitive to depletion of hepatocellular phosphate depletion can affect metabolism of exogenous purine intermediates as well as basal UAP from endogenous substrates.

REFERENCES


DISCLOSURES

None of the authors has any conflict of interest, financial or otherwise to declare.

AUTHOR CONTRIBUTIONS


GRANTS

This work was supported by Diabetes UK (BDA 11/0004231) and the Medical Research Council (G0501543). H. L. Reeves and G. L. Patman were supported by the European Community’s Seventh Framework Programme (FP7/2001–2013) under grant agreement HEALTH-F2-2009-241762 for the project FLIP.


