APLS is a hypercoagulable state that predisposes patients to arterial and/or venous thromboses that can result in graft failure [1]. A 2006 consensus conference defined patients with definite APLS as having at least one laboratory criterion (e.g. persistent, elevated titres of APL) and one clinical criterion (e.g. deep vein thrombosis, pregnancy complications) [2]. However, APLS encompasses a wide range of clinical symptoms [3]. Notably, a subset of patients manifest thrombotic complications without detectable antiphospholipid titres. These patients are classified as having seronegative antiphospholipid syndrome (SNAPS) [2]. This patient's history of pregnancy complications was subtle, and the absence of LAC and ACA antibodies did not raise the possibility of APLS at the time of transplantation. We therefore propose the diagnosis of SNAPS in this patient. SNAPS increases the risk of graft thrombosis and necessitates anti-coagulation before and after the transplant operation [4]. Therefore, a second transplant operation in this patient would be considered high risk and would require peri-operative anti-coagulation [5]. This case highlights the difficulty of diagnosing 'seronegative' APLS within the current diagnostic guidelines. It also identifies a need for new diagnostic tests to identify at-risk patients.

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## Severe thiamine deficiency complicated by weight loss protects against renal ischaemia-reperfusion injury in rats

Sir,

Injury due to reperfusion after prior ischaemia (IRI) is an important cause of delayed graft function after renal transplantation [1]. Studies in dogs and rats suggest that thiamine is protective against IRI in heart and brain [2–4]. It has been argued that many organs, including kidneys are deficient in thiamine at the moment of transplantation [4]. We aimed to investigate the effect of severe tissue thiamine deficiency on ischaemia-reperfusion injury in rat kidneys.

**Table 1.** Transketolase activity and thiamine and thiamine metabolites

| | Control ($n = 11$) | Thiamine deficient ($n = 8$) | $P$-value |
|---|---|---|---|
| TK activity | 13.9 (2.4) | 7.7 (1.5) | <0.001 |
| TPP | 81.2 (13.2) | 15.7 (6.5) | <0.001 |
| TMP | 26.2 (4.9) | 0.4 (0.4) | <0.001 |
| THM | 90.2 (15.2) | 2.5 (0.8) | <0.001 |

TK activity is expressed as mU/mg protein. TPP, TMP and THM are expressed as pmol/mg protein.

Male inbred Lewis rats (± 270 g) (Harlan, Zeist, The Netherlands) were fed with a thiamine-deficient diet (Arie Blok, Woerden, The Netherlands). The diet only contained trace amounts of thiamine (0.16 μg/kg, equalling ∼0.04% of the thiamine content of regular chow). Control animals were orally supplemented with 400 μg thiamine/day in a 2.5% sucrose solution, whereas the thiamine-deficient animals only received the same volume of the sucrose solution. After 4 weeks, ischaemia-reperfusion procedures were performed. Anaesthesia was induced by 5% isoflurane and maintained on 3% isoflurane. The rats were placed on a homothermic table to maintain core body temperature at 37°C and the left kidney was subjected to 45 min of ischaemia, followed by reperfusion. Nephrectomy of the contralateral right kidney was performed during ischaemia of the left kidney. Kidney tissue samples were snap-frozen and stored at −80°C in 4% formalin. Plasma and red blood cells were also stored at −80°C. Tissue transketolase activity was measured according to the kinetic method of Chamberlain *et al*. [5]. Thiamine, thiamine monophosphate and thiamine pyrophosphate were determined by HPLC with fluorimetric detection [6]. All experimental procedures were approved by the Committee for Animal Experiments of the University of Groningen and performed according to the principles of laboratory animal care.

In the third week of the experiment, growth of the thiamine-deficient rats was significantly slower than that in the control rats (12.1 ± 6.3 g versus 21.0 ± 4.7 g, respectively, $P = 0.003$). In the fourth week, the thiamine-deficient rats lost weight, whereas the control rats gained weight (−9.5 ± 8.8 g versus 15.4 ± 5.2 g, respectively, $P < 0.001$), resulting in a significant difference in body weight before ischaemia reperfusion (326 ± 13.8 g versus 355 ± 22.8 g respectively, $P = 0.006$). Induction of thiamine deficiency resulted in significant decreases in renal biochemical and functional thiamine status at the moment of ischaemia reperfusion (Table 1). There was no difference in baseline plasma creatinine concentrations prior to ischaemia reperfusion between thiamine-deficient and control rats (16.7 ± 1.8 μmol/L versus 16.9 ± 2.4 μmol/L respectively, $P = 0.88$). At the first day after

ischaemia-reperfusion, plasma creatinine concentrations were significantly lower in thiamine-deficient rats than in control rats (71.7 ± 22.2 versus 162 ± 106, respectively, $P = 0.02$).

In this study, we found that thiamine deficiency complicated by weight loss is protective against renal IRI rather than a factor that increases susceptibility. Interestingly, it is long known that in hearts prolonged fasting protects against IRI [7]. To the best of our knowledge our study is the first to suggest that a similar phenomenon may be present for the kidney. If future studies confirm that fasting/wasting protects against IRI in the kidney, this may lead to identification of new mechanisms and methods for priming of kidneys for prevention of IRI.

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## *RET* gene mutations are not a common cause of congenital solitary functioning kidney in adults

Sir,

RET (rearranged during transfection) is a transmembrane receptor tyrosine kinase. Loss-of-function *RET* mutations occur in Hirschsprung disease, while gain-of-function mutations cause multiple endocrine neoplasia type 2 (MEN2) and familial medullary thyroid carcinoma (FMTC) [1]. In mice, Ret initiates mouse kidney development by enhancing ureteric bud outgrowth [2]. Renal agenesis occurs in mice with homozygous null mutation of either *Ret*, *Gdnf* or *Gfra1*, the latter two genes, respectively, coding for a key ligand that activates Ret and a Ret co-receptor [2].

In humans, unilateral renal agenesis occurs in around 0.01% births and results in a solitary functioning kidney, whereas bilateral renal agenesis and/or severe dysplasia occurs in a similar frequency and usually leads to neonatal death [3].

A role for RET in human kidney development was suggested by a report of unilateral renal agenesis in two members of a FMTC family with *RET* mutation [4]. More recently, *RET* mutations were reported in 7 of 19 fetuses with bilateral renal agenesis, and in 2 of 10 with unilateral agenesis and contralateral dysplasia [5]. However, the prevalence of *RET* mutations in adults with congenital solitary functioning kidney is unknown.

A cohort of 14 subjects were selected for our current study. They were living adults born with a solitary functioning kidney, one of whom had two failed pregnancies with fetuses affected by malformed kidneys. We screened exons 1–20 and the conserved splice-sites of *RET* using direct sequencing. None of the cohort were known to have Hirschsprung disease or MEN2/FMTC or other recognized genetic syndrome. We did not identify any mutations in the 14 adults, and thus it is unlikely that *RET* mutations will be a common cause of solitary functioning kidney in adults.

Because mutations of *hepatocyte nuclear factor 1B* have been reported in the context of solitary functioning kidney [6], we also screened this gene by sequencing and by dosage analysis but failed to find mutations. Other genes such as *GDNF* and *GFRA1* have yet to be elucidated in our cohort.

Our study was in adults while Skinner *et al*. [5] studied fetuses. We therefore suggest that human *RET* mutations causing renal disease lead to severe renal failure incompatible with postnatal survival. Mutations of nephrogenesis genes other than *RET* should be sought to explain human congenital solitary functioning kidney.

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