Grado en Medicina 2009-2015

TRABAJO DE FIN DE GRADO

Changes in urinary citrate and lactate levels during the first months of life. The citrate/lactate ratio as a potential marker of mitochondrial function in renal tubular cells.

Cambios en el citrato y lactato urinarios durante los primeros meses de vida. El ratio citrato/lactato como un potencial marcador de función mitocondrial en las células tubulares renales.

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Abstract

Background Urinary citrate and lactate have been previously studied for a better understanding of renal tubule development. Never before had been related in a ratio.

Methods Urine samples from twenty five healthy infants were analyzed with GCMS, comparing lactate and citrate excretion depending on age and nutrition.

Results An increase in the citrate/lactate excretion ratio related to age was found. We also observed a marked influence according to the kind of feeding on this ratio, very influenced with creatinine levels. Breastfed infants showed a reduced pattern of excretion compared to the ones fed with formula, but when adjusted for creatinine this association appears to be inverted.

Keywords lactate, citrate, citrate/lactate, tubular function development, renal maturation.

Introduction

Renal structure and function are incompletely developed at birth in a number of mammals, including humans. Functional immaturity manifests itself as an inability on the part of the kidney to vary the volume and concentration of the urine.

Maturation of glomerular and tubular function is a well-described process of development during early life[1] which can be augmented nonspecifically by dietary manipulation[2].

Renal tubular function becomes essential in maintaining electrolytes and fluids homeostasis, and thus, allowing a normal development during the first weeks-months of life[10].

Tubular cells are characterized by their high metabolic rate and energy demand, playing a key role the energy metabolism pathways. The tubules are partially and variably differentiated at birth. Proximal tubular cells are very rich in mitochondria, and at least in rats, during the first 2 weeks after birth the mitochondria increase in number and size, accumulate homogeneous matrix, and acquire small, very dense granules.

In this scenario, certain critical metabolic molecules such as lactate and citrate should play crucial roles, and therefore, become potential biomarkers of tubular function and maturation. Lactate is an anaerobic glycolysis product, originating from pyruvate by lactate dehydrogenase action. Concurrently, citrate, a tricarboxylic acid (TCA), is synthesized in mitochondria from oxaloacetate and acetyl-CoA by the enzyme citrate synthase. Through its metabolism in the TCA cycle, citrate generates a significant amount of energy.

Urinary levels of both, lactate and citrate, depend on their plasmatic levels (consistently low and constant in the infant) and their uptake/production/release by the tubular cell. In most circumstances, urinary citrate and lactate are influenced relatively little by their plasmatic levels. Subsequently, urinary lactate and citrate might reveal a picture of tubular cells metabolism[9,11].

In the present study we examined if physiological changes in renal tubular function during the first months of life (1 to 6 months) could be expressed by differences on urinary excretion of citrate and lactate. We collected urines of 25 healthy infants exclusively breast-fed or bottle-fed in order to analyze urinary citrate and lactate. We propose that within tubular maturation related with age and possibly influenced with the kind of diet, the ratio citrate/lactate should increase as results of an increase of mitochondrial activity or maturation. As glomerular filtration also increases with age, all results should be adjusted by urinary creatinine.

Methods

Study population Twenty five healthy infants aged 3-24 weeks (14 male, 11 female) were enrolled from the Pediatric Nephrology Unit of Hospital Universitario Marqués de Valdecilla (Santander, Spain). All individuals were full-term, normal weights (10^{th}- 90^{th} percentiles) and with normal weight gain from neonatal period. Infants had been approached while visiting the Unit; any baby suffering from nephro-urological diseases, inborn metabolism errors or feeding problems was excluded. All the participants were exclusively milk fed (human or formula), having passed an interval of 0-2 hours since last intake. All parents read and signed an informed consent explaining the methods and purpose of the study. Participants' samples were classified according to age and kind of nutrition:

-First group (3-10 weeks); ten infants: five formula fed and five breastfed infants.

-Second group (11-17 weeks); ten infants: five formula fed and five breastfed infants.

-Third group* (18-24 weeks); five formula fed infants.

** Some infants were excluded from the third group because were fed with both forms of lactation and/or complementary foods such as fruits, cereals and vegetables were initiated; therefore, only five exclusively formula fed infants were suitable for comparison with the other groups.*

Urinary sample collection: Urine samples were collected in the Pediatric Nephrology Unit by an experienced nurse from spontaneous micturition in a urine collection bag. Urine was then collected in a urine filter paper in order to be processed for the study. This procedure was included in the protocol of study of all recruited infants.

Urine analysis by GCMS: Urine filter paper samples were extracted using suitable protocol and pretreated with urease at 37 ºC to get rid of urea followed by deproteinisation with ethanol containing Heptadecanoic acid as internal standard. Further, sample was vacuum dried and residue was subjected to derivatisation by adding N, O,-bistrimethylsilyl) trifluroacetamide (BSTFA) and Trimethylchlorosilane (TMCS). One microlitre aliquots of derivatized sample were injected into Shimadzu QP-2010 Plus GC/MS using auto sampler in split mode. The metabolites were chromatographically analyzed as trimethylsilyl compounds. The data analyzed with computer-assisted program and NIST library. Urinary creatinine was measured enzymatically using Microlab ARX-235® semi auto analyzer. The markers were expressed as mmol/mol creatinine.

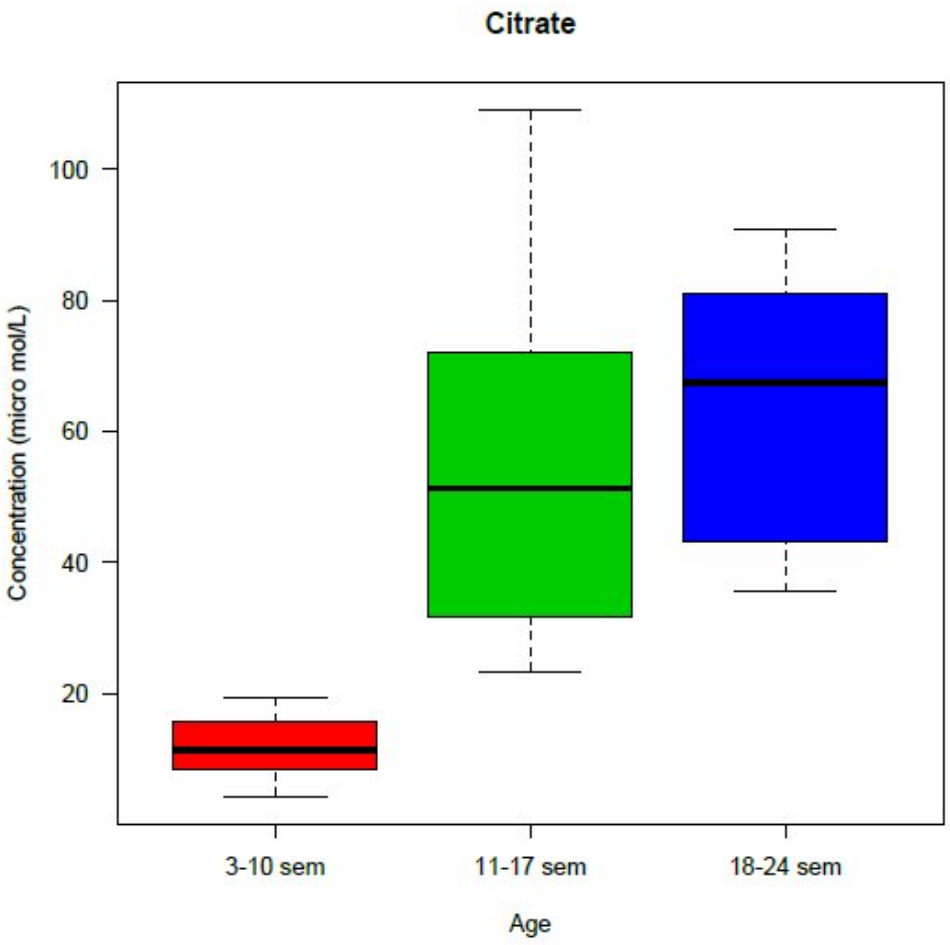
Statistical analyses Data were analyzed with the software "R" Statistical Computing®. The Kruskal-Wallis and Mann-Whitney tests were used for comparisons between independent parameters. A p value of <0,05 was considered to be statistically significant.

Results

Urinary citrate excretion and age

As infants grew older, and increase in urinary citrate was seen, with a mean excretion of 20 µmol/l at 2 months that was tripled at 6 months age (figure 1). Described rise was statistically significant (Kruskal-Wallis analysis: χ^2=17,51; p=0,0002).

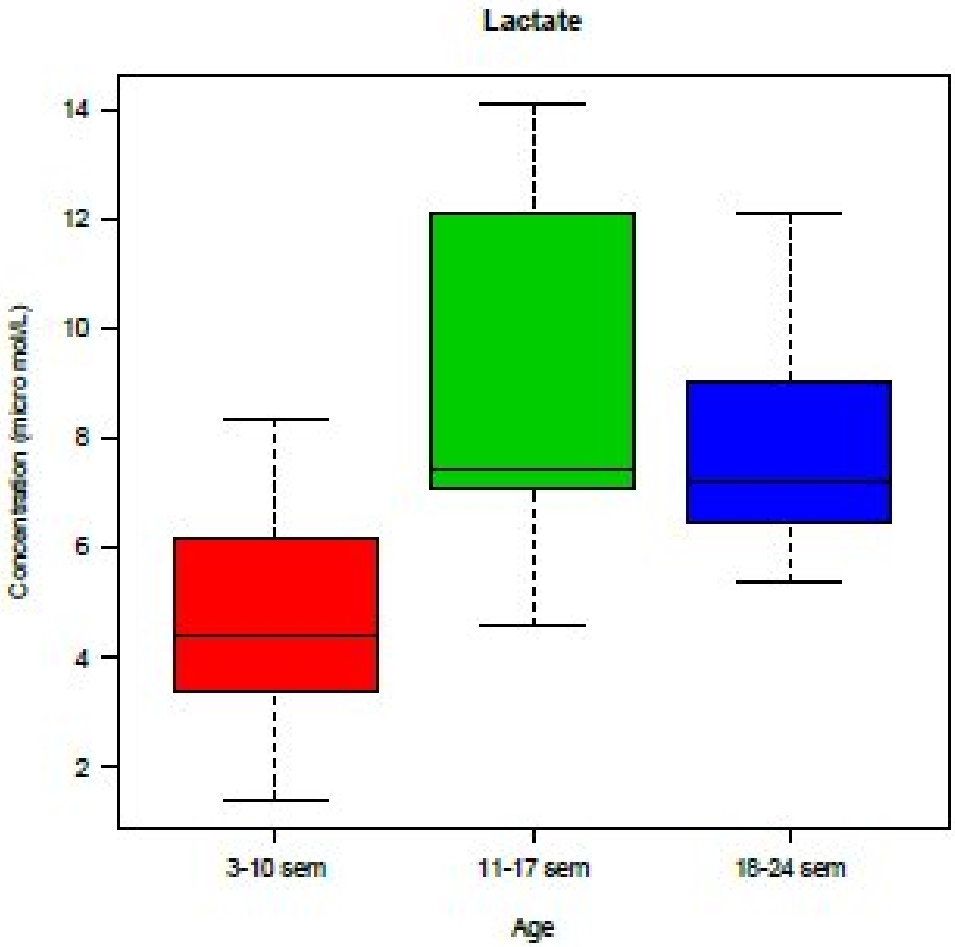
Figure 1. Urinary citrate excretion (µmol/l) and age (weeks)



Urinary lactate excretion and age

As infants grew older, and increase in urinary lactate was seen, doubling its value from 3 months on (figure 2). Described rise was statistically significant (Kruskal-Wallis analysis: χ^2=8,79; p=0,012).

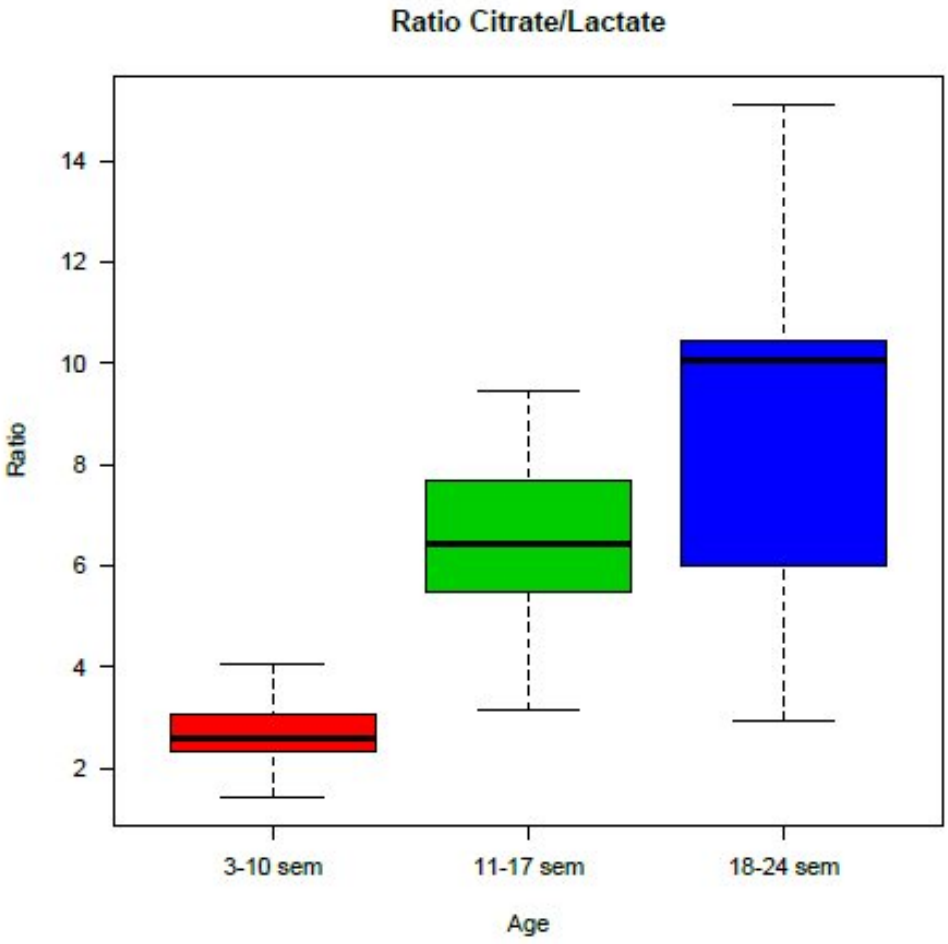
Figure 1. Urinary lactate excretion (µmol/l) and age (weeks)



Urinary citrate/lactate ratio and age

An increase in urinary citrate/lactate ratio associated to age was found (figure 3). This association was statistically significant (Kruskal-Wallis analysis: χ^2=14,61; p=0,0007).

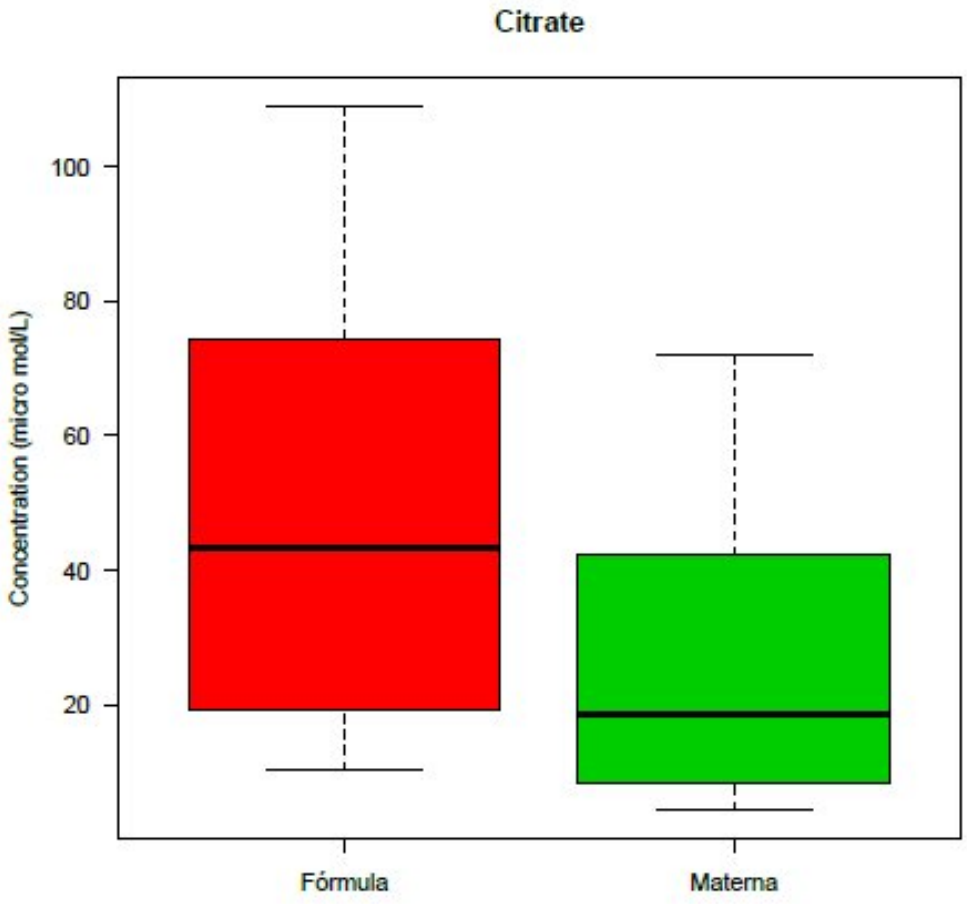
Figure 3. Urinary citrate/lactate ratio excretion and age (weeks)



Urinary citrate excretion and nutrition

Urinary citrate was lower in the breastfed infants group (figure 4). This difference was close to be statistically significant (Mann-Whitney analysis: W=110; p=0,055).

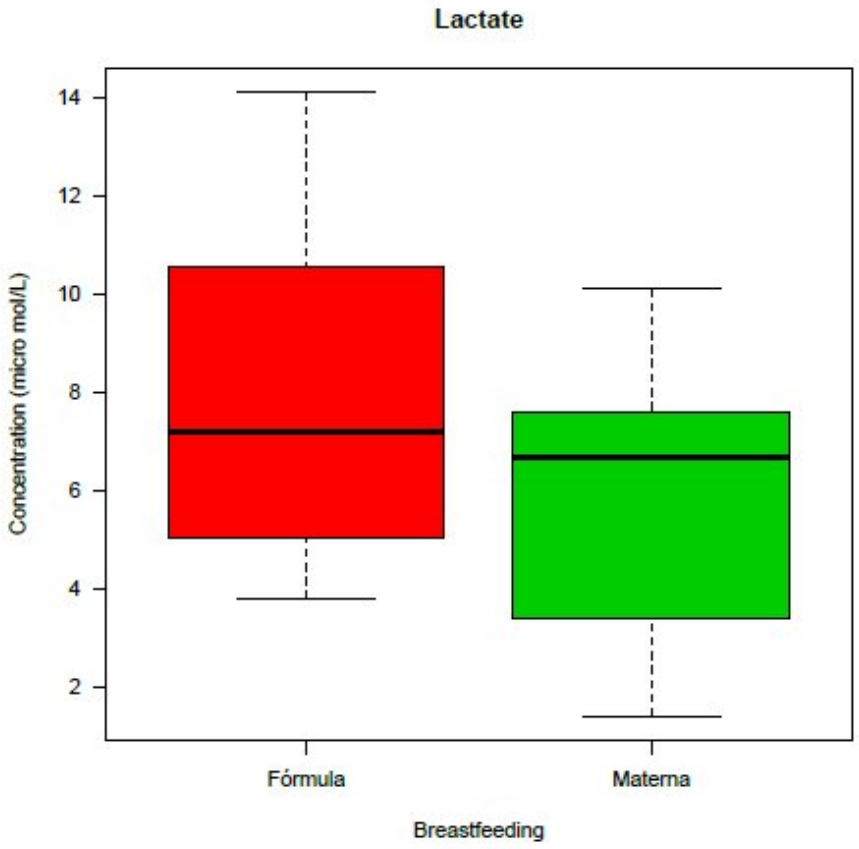
Figure 4. Urinary citrate excretion (µmol/l) and nutrition (breastfed vs. formula).



Urinary lactate excretion and nutrition

Urinary lactate was lower in the breastfed infants group (figure 5), but his difference was not statistically significant (Mann-Whitney analysis: W=98; p=0,212).

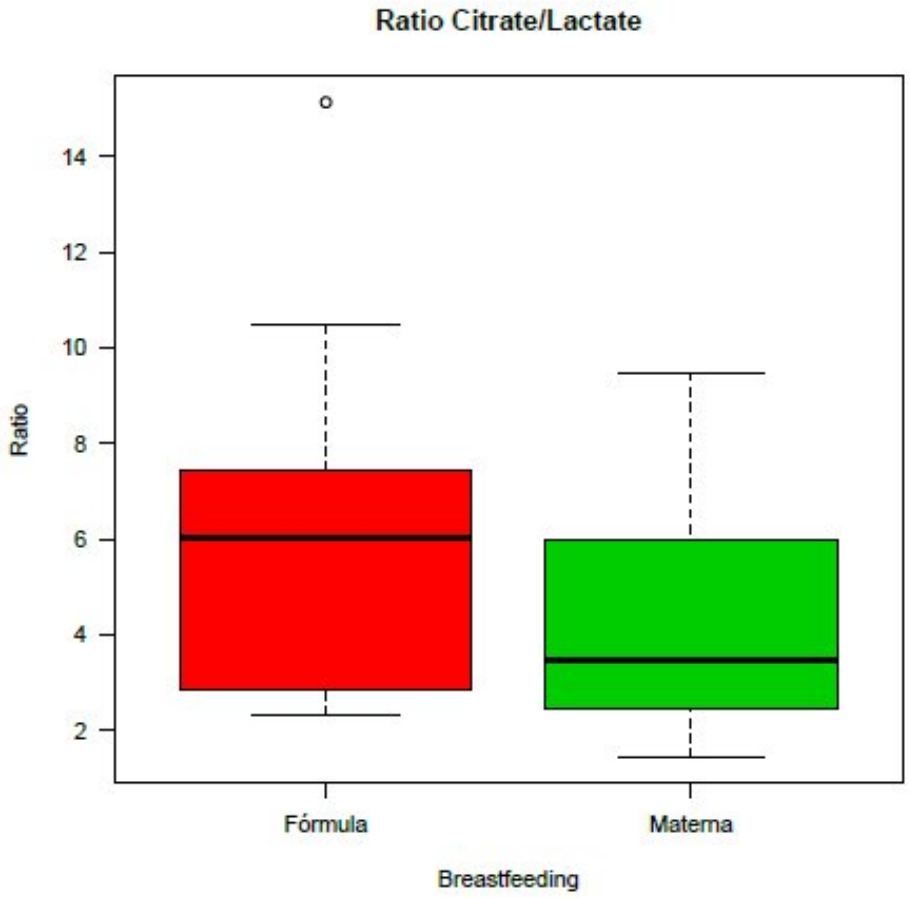
Figure 5. Urinary lactate excretion (µmol/l) and nutrition (breastfed vs. formula).



Urinary citrate/lactate ratio and nutrition

Urinary citrate/lactate was lower in the breastfed infants group (figure 5), with mean values of 4,27 for the breastfed and 6,15 for the formula fed. This difference was not statistically significant (Mann-Whitney analysis: W=99; p=0,196).

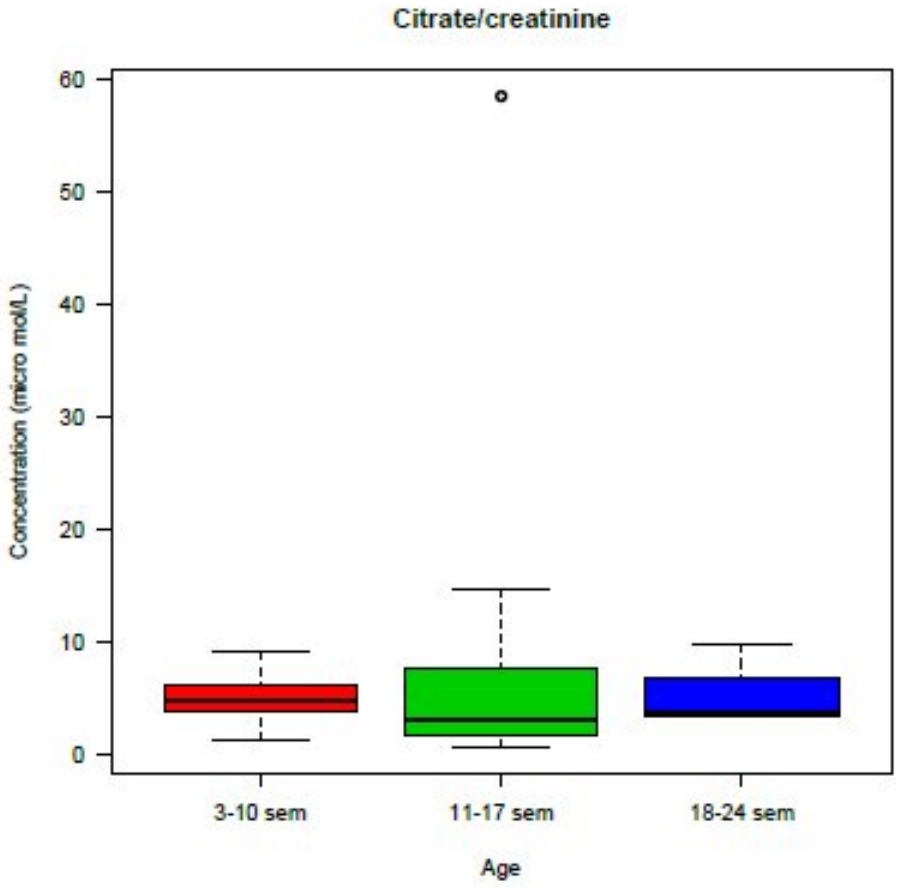
Figure 5. Urinary citrate/lactate ratio and nutrition (breastfed vs. formula).



Adjusted for creatinine citrate excretion and age

Excreted citrate/creatinine was similar in all different age groups (figure 6), without statistically significant differences (Kruskal-Wallis analysis: χ^2=0,89; p=0,640).

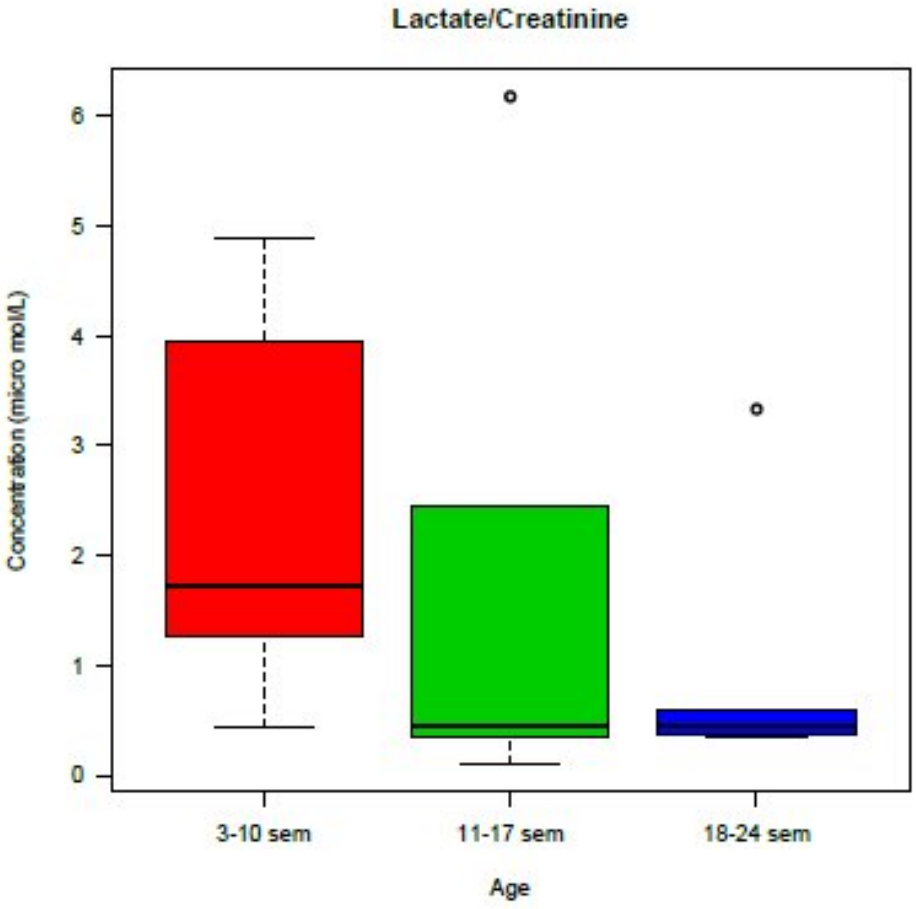
Figure 6. Citrate excretion (µmol/l) per creatinine excretion (µmol/l) and age (weeks)



Adjusted for creatinine lactate excretion and age

Excreted lactate/creatinine was reduced with age (figure 7), but statistically significant differences were not found (Kruskal-Wallis analysis: χ^2=4,04; p=0,133).

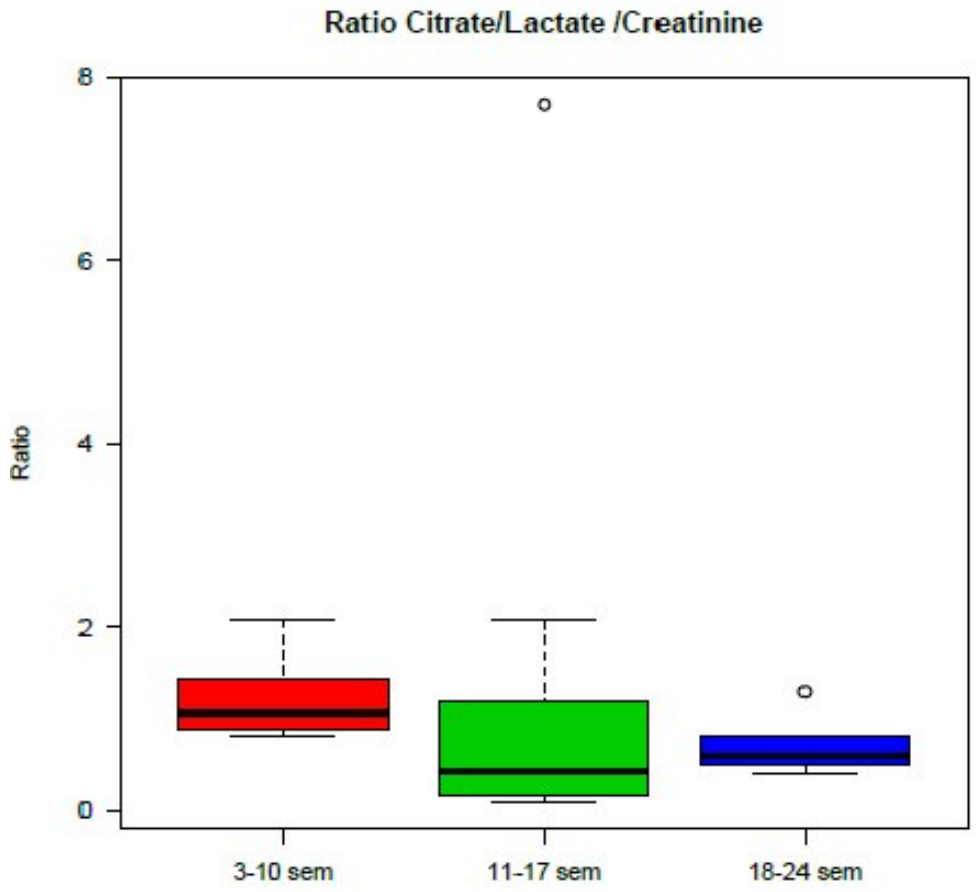
Figure 7. Lactate excretion (µmol/l) per creatinine excretion (µmol/l) and age (weeks)



Adjusted for creatinine citrate/lactate and age

When adjusted for creatinine excretion, citrate/lactate ratio was increased with age (figure 8). This association was close to be statistically significant (Kruskal-Wallis analysis: χ^2=5,21; p=0,074).

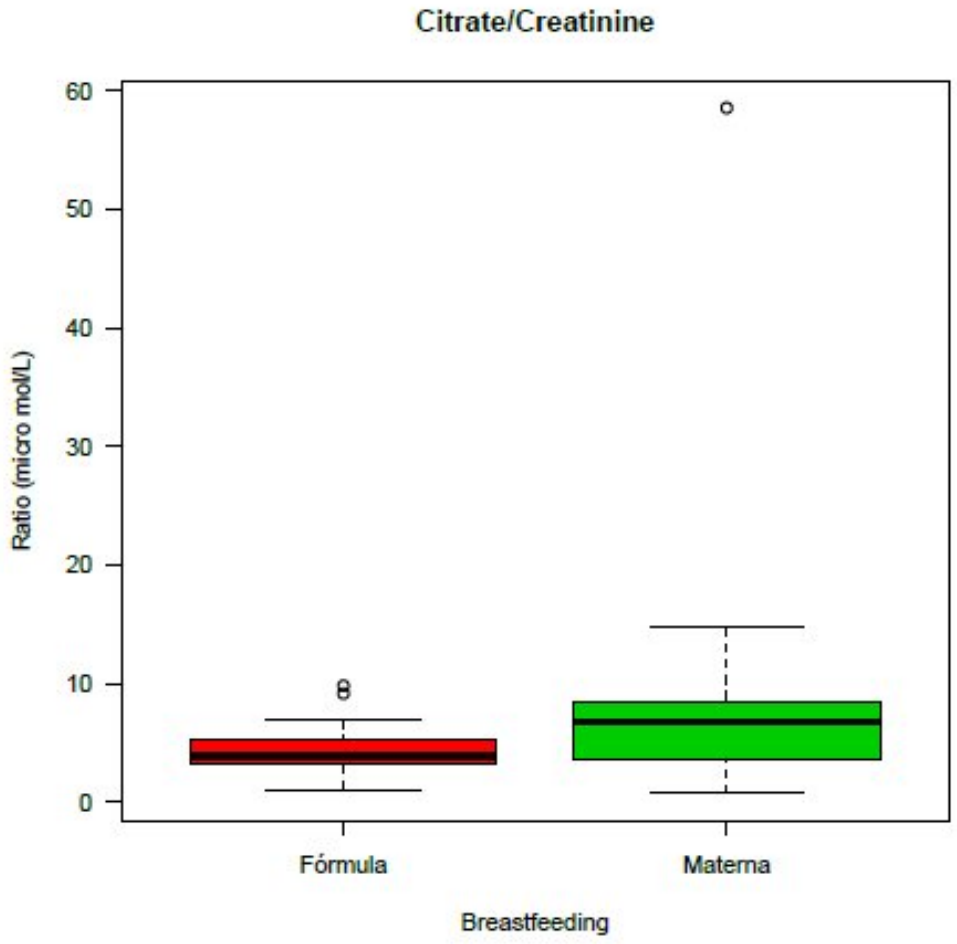
Figure 8. Citrate/lactate excretion (µmol/l) per creatinine excretion (µmol/l) and age (weeks)



Adjusted for creatinine citrate excretion and nutrition

Excreted citrate/creatinine was higher in the breastfed infants groups (figure 9). Differences were close to be statistically significant (Mann-Whitney analysis: W=110; p=0,055).

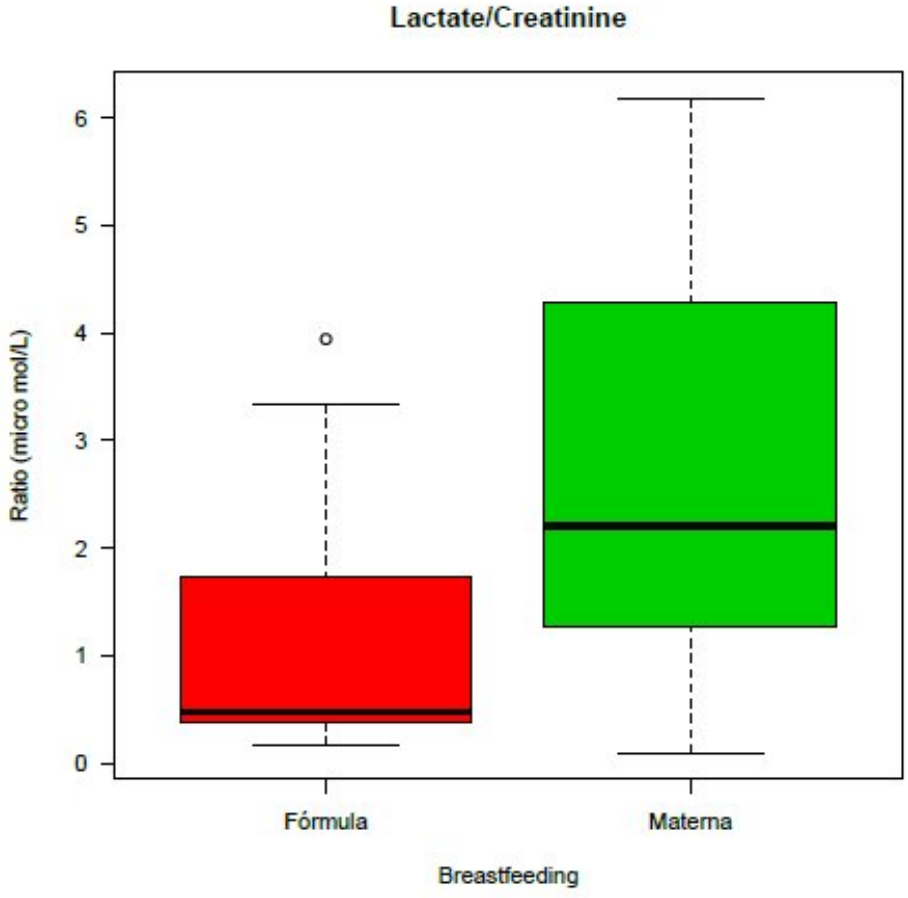
Figure 9. Citrate excretion (µmol/l) per creatinine excretion (µmol/l) and nutrition (breastfed vs. formula fed)



Adjusted for creatinine lactate excretion and nutrition

Excreted lactate/creatinine was higher in the breastfed infants groups (figure 10), but differences were not statistically significant (Mann-Whitney analysis: W=42; p=0,071).

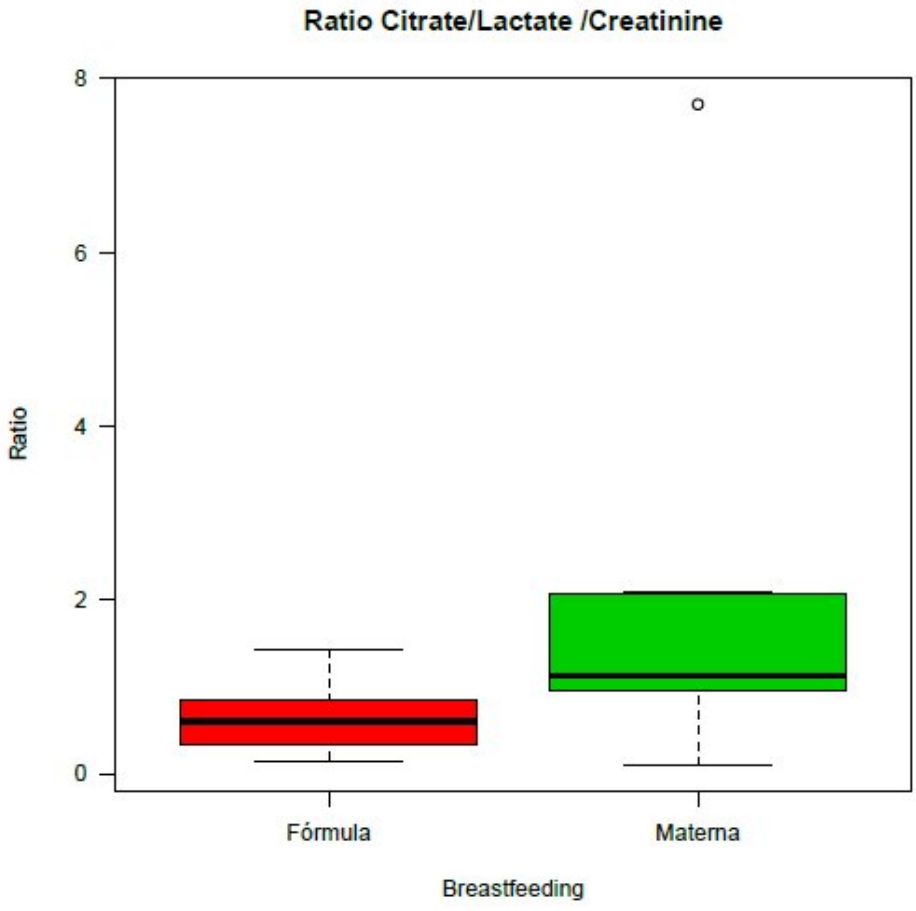
Figure 10. Lactate excretion (µmol/l) per creatinine excretion (µmol/l) and nutrition (breastfed vs. formula fed)



Adjusted for creatinine citrate/lactate ratio and nutrition

When adjusted for creatinine excretion, citrate/lactate ratio was increased in the breastfed infants (figure 11). This association was statistically significant (Mann-Whitney analysis: W=33; p=0,019).

Figure 11. Adjusted for creatinine (µmol/l) citrate/lactate excretion (µmol/l) ratio and nutrition (breastfed vs. formula fed)



Discussion

The urinary citrate/lactate quotient is supposed to behave as a potential biomarker of mitochondrial tubular function. As in previous studies, our results reveal changes in the excretion pattern of lactate and citrate during infant's maturation[3,5,11]. To date, no one had related both metabolites in a ratio, expressing a potential relationship of mitochondrial activity.

There is wide evidence in infants tubular functionality expressed in renal handling of sodium, pH and osmolarity. This improvement in the management of ions, acid-base balance and urine concentration is related to nutritional and maturational events[8,10]. These changes are associated with an enhanced mitochondrial function, demonstrated by TCA cycle intermediaries variations[6,11]. Our results suggest that urinary citrate/lactate ratio might be a useful biomarker of tubular maturation in the <6 months infants. This ratio has a dramatically increase form in the first four months (less than 17 weeks), being less evident from 4 to 6 months of life. This ratio is definitely conditioned by the kind of nutrition the baby receives. When adjusted for creatinine, the ratio is higher in breastfed infants, whereas without any adjustment is higher in bottle-fed babies.

Urinary lactate behavior

Let´s briefly revise lactate clearance and excretion; Lactate is avidly cleared from plasma to a non-urine compartment, or more likely metabolized. It has been demonstrated that intravenous lactate infusions elicited only a small increase in the plasma lactate concentration and no significant change in the urinary lactate excretion[11]. Filtered lactate is extensively reabsorbed in the proximal tubule, handled similarly to other small organic anions (Na^+/H^+ and Cl^-/organic anion exchange[11]. In the medulla, lactate metabolism is different, as higher concentrations are found. The medulla is relatively hypoxic and energy metabolism relies on glycolysis, with the consequent production of lactate[11]. This fact is clearly shown up in certain mitochondrial cytopathies, in which ATP generation is impaired and lactate tends to accumulate. Therefore, assuming that tubular maturation optimizes mitochondrial function and anions exchange, it results consistent to find a lesser amount of lactate in the older infant compared to a younger one. Both, enhanced reabsorption and decreased renal production might occur simultaneously. To sum up, better anion transporters (ions co-transport, monocarboxylate transporters, etc.) and/or improved cellular respiration in tubular cells may reflect renal maturation through a reduced excretion of lactate[11].

Urinary citrate behavior

Previous studies have demonstrated, in rats and humans, that infants have a significantly higher urinary citrate/creatinine ratio, as well as urinary citrate concentration, compared with adults[4]. The rate of intracellular metabolism of citrate

plays a major role in determining the amount of citrate excreted in the urine[5,9]. For a better understanding of citrate urinary excretion a brief explanation is required; Plasma levels of citrate are low and poorly influenced by dietary intake[3]. Changes in urinary citrate are predominantly conditioned by the acid-base status, and relatively little by plasma levels, being largely tubular reabsorption the main factor in citrate excretion[3,6,9]. Higher urine pH tends to be associated with higher urine citrate. Both intracellular and systemic acidosis may inhibit renal citrate output if the citrate reabsorption and metabolism in the proximal tubule becomes upregulated in order to counteract those disorders[3,5]. Systemic acid-base changes cause drastic changes in citrate clearance and metabolism by alteration in the pH gradient across the inner mitochondrial membrane and changes in the tricarboxylate carrier[4,6].

Citrate is freely filtered at the glomerulus and, up to 90%, reabsorbed by proximal tubule. In normal conditions there is no citrate secretion[3,5]. In the proximal tubule, citrate is reabsorbed by a membrane Na^+/dicarboxylate cotransporter. The citrate utilized by kidneys is supplied predominantly by reabsorbed citrate, and it is mostly metabolized to CO_2. Thus, citrate utilization represents an essential component of tubular oxidative metabolism[4,5].

Once it has been reabsorbed, citrate is metabolized by two potential pathways: cytoplasmic metabolism via citrate lyase or mitochondrial metabolism via TCA cycle. Both pathways generate oxaloacetate[3]. Fractional excretion of citrate can be increased either by increasing intracellular citrate synthesis from precursors or by inhibiting mitochondrial citrate metabolism[3,5]. Studies conducted in rats showed that the developing infant higher urinary citrate excretion is not due to a difference in renal proximal tubular Na^+/citrate cotransporter activity, nor renal cortical citrate synthase or ATP citrate lyase activities[5,6]. What is more, infant rat kidneys had significantly lower mitochondrial aconitase activity (responsible for metabolism to isocitrate) but not renal citrate concentration compared to adults[5]. This findings suggest that this higher urinary citrate is likely due to maturational differences in the proximal tubule expressing an enhanced oxidative metabolism and energy demand.

Urinary citrate/lactate ratio and tubular maturation

Our results point the same direction. Enhanced oxidative metabolism should be reflected both, in lower lactate or higher citrate, expressed clearly in the citrate/lactate ratio. Previous works show that total urinary citrate is increased with age, remaining plasmatic citrate stable[4]. In the other hand, urinary citrate adjusted for plasma creatinine does not change significantly with age[2]. These findings suggest a balanced maturation between glomerular and tubular function. As for uric acid, urinary citrate behavior is hard to interpret[8]. On one hand, an improvement in tubular function should imply an increase in proximal reabsorption, with a reduction in urinary citrate. But, on the other hand, urinary citrate raises, presumably meaning an increase in tubular cells mitochondrial metabolism. This fact is consistent with the urinary lactate reduction, suggesting a smaller reliance on anaerobic glycolysis, and thus, an energy metabolism optimization.

Citrate-lactate excretion and nutrition

Urinary citrate, lactate and citrate/lactate ratio are decreased in the breastfed infant when compared to the formula fed ones. This observation suggests a delayed maturation of mitochondrial pattern in the breastfed when compared with the bottle-fed. A more immature tubular pattern should be expected in the breastfed, which could be related to a fed pattern of free demand compared with the bottle fed with a more regular/structured pattern of intakes.

On the other hand, these parameters result increased in the breastfed (vs. the formula fed) when adjusted for creatinine excretion. As in newborns and younger infants creatinine is not only filtered but also excreted in proximal tubules, urinary creatinine is extremely variable during the first months of life. Creatinine is a marker of glomerular function but it is very influenced by muscular mass and activity.

Moreover, renal medullar maturation strictly depends on diet, with a faster maturation in higher protein intakes. All this functional changes are related to urinary acidification and concentration capacities. The impact of diet in body composition and renal maturation should be extremely apparent in renal excretion of substances as creatinine.

Thus, although we observe a direct influence of diet in citrate/lactate/creatinine parameters, it is not possible to conclude how diet influences the excretion of these metabolic products in order to establish a predictable pattern.

Conclusions

Our results demonstrate an increase in the citrate/lactate excretion ratio related to age. This ratio could reflect mitochondrial enhancement of renal tubules, and thus, behave as a tubular maturation biomarker. We also found a marked influence of the kind of feeding on this ratio, conditioned by creatinine levels. Breastfed infants showed a reduced pattern of excretion compared to the ones fed with formula, but when adjusted for creatinine this association appeared to be inverted. These findings confirm that renal development relies on dietary factors, supporting the idea of a maturational acceleration induced by formula feeding. In order to clarify how the urinary citrate/lactate ratio might work as a biomarker further investigation is needed.

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