Contribution of Various Dietary Constituents to the Acid Base Status: Interest of Animal Models of Latent Metabolic Acidosis

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Abstract: Animal models of metabolic acidosis are generally based on drastic treatments and are poorly suitable as models of latent metabolic acidosis (LMA). Specific rodent diets have been evaluated as LMA models, together with their responsiveness to dietary alkalinizing factors. Using this model, the possibility to obtain LMA states with moderate levels of protein intake was identified, pointing to the critical role of anions in the mineral moiety of the diet. The potential role of large intestine fermentations to acid-base control has also been examined with this model.

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INTRODUCTION

During the last years, attention has been increasingly paid to health consequences of latent metabolic acidosis, which are a consequence of westernized diet habits. The determinism of LMA is relatively complex, namely a desequilibrium between the availability of acid-yielding nutrients (essentially sulfur amino acids) and that of alkalinizing compounds (essentially potassium salts of organic anions present in plant foods). Additionnal factors may contribute to LMA emergence: worsening role of salt excess [1]), aged-related decline in the renal capacity to eliminate acidity [2]. Consequences of LMA are multiple: Ca mobilization from bones and subsequent hypercalciuria, activation of protein catabolism, reduction of the responsiveness to trophic hormones [3,4]. Most of the studies in this domain have been effected in humans, but animals models are still useful as regards, for example, multiple comparisons of diets or nutrients or studies requiring tissue samplings. On the other hand, investigators working on mineral or protein metabolism, or on long-term effects of trophic hormones, are seldom aware of the effects of diets on the acid-base status of experimental animals and the potential consequences on results.

ANIMAL MODEL OF LATENT METABOLIC ACIDOSIS

In contrast to humans westernized diets, resulting in a majority of the population with a permanent status of an at least modest H$^+$ retention [5], rodent diets are generally well-balanced and even overprotective against the risk of LMA since they rich in potassium (partly as citrate) as well as in calcium and magnesium and limited in sodium. Therefore, even if they are relatively rich in proteins (around 20%), chow diets and most of the semi-purified diets are poor models of modern human nutrition but are rather reminiscent of the features of hunter-gatherers food habits [6].

The consequences of this is illustrated by data obtained in mice adapted to complex diets, either a standard diet or a 'westernized' chow diet [7]. The standard diet provided a relatively high level of K together with a low Na level, whilst the westernized diet provided a higher level of Na, hence a drastic difference in the K/Na ratio (2.16 and 0.43 respectively). The overall composition of the diets resulted in a marked hypocitraturia (suggestive of LMA) in animals fed the 'westernized diet'. Various physiological parameters were also modified in mice adapted to the 'westernized diet', especially adiposity which was strongly raised in a fraction of these animals. Bone mineral content as well as mineral density were very significantly reduced in mice fed the 'westernized diet' but there was a trend, like in humans, to a better bone status in obese animals (Table **1**). This nutritional model is complex and physiological responses are certainly multifactorial, but it shows that standard diets, optimized from growth and reproduction, are poor models for contemporary human nutrition.

Until recently, experimental designs for studies of the consequences of metabolic acidosis in rodents have frequently been based on drastic treatments : chiefly NH$_4$Cl in drinking water and/or gavage, or other less usual treatments such as acetazolamide. These treatments generally lead to overt metabolic acidosis with marked alterations of acid-base parameters in blood (in contrast to LMA) and, in addition, NH$_4$Cl will interfere with nitrogen metabolism. These considerations prompted studies on the feasability of rat model of LMA by introducing dietary alterations liable to chronically disturb the acid-base balance, namely (i) changes in dietary protein level (hence sulfur AA provision) and/or sulfur amino acid supplementation, (ii) changes in the mineral moiety of the diet, especially the balance between inorganic and organic K salts [8,9] and (iii) strict limitation of alkalinizing anions (carbonate, citrate). In these models, high dietary protein levels (from 20 to 48%) were found to elicit a marked rise of urinary net acid excretion, together with a greater calciuria and a severe hypocitraturia. This model was also shown to be responsive to a supplementation of alkalin-

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Table 1. **Long-Term Effects of a 'Western' Diet on Body Fat, Bone and Urinary Parameters in a Mouse Model (OF1 Female), with a Distinction between Mice Developping or Not an Obese Phenotype**

	Standard Diet n = 25	Western Diet Non Obese n = 14	Western Diet Obese n = 23
Body weight (g)	35.6 ± 1.5 [a]	36.5 ± 1.7 [a]	59.4 ± 1.5 [b]
Body fat (% body weight)	12.8 ± 1.2 [a]	14.1 ± 1.6 [a]	32.6 ± 1.2 [b]
Whole body bone mineral content (mg)	725 ± 15 [b]	494 ± 20 [a]	551 ± 15 [ab]
Femur mineral density (mg/cm^2)	111 ± 2 [b]	83 ± 3 [a]	93 ± 2 [ab]
Vertebra mineral density (mg/cm^2)	81 ± 1 [b]	62 ± 2 [a]	72 ± 1 [ab]
Urine sodium excretion (μmoles/d*)	31 ± 5 [a]	111 ± 7 [b]	100 ± 5 [b]
Urine potassium excretion (μmoles/d*)	67 ± 5 [b]	47 ± 3 [a]	46 ± 3 [a]
Urine citrate excretion (μmoles/d*)	5.6 ± 0.3	≈ 0	≈ 0

*Values are means **±** SEM *
a, b, ab **Values not sharing the same superscript are significantly different (P < 0.05); superscript ^a for the lowest values and **^b for the highest.**
****Data are expressed per μmoles creatinine*
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ising K salts (malate or citrate) in the diet, which reduced Ca and Mg urine excretion and restored citraturia [9]. Such rodent models are quite useful for studies on the effects of alkalinizing compounds or complex foods on LMA [10] and its consequences, for example on bone status or on the risk of renal lithiasis.

ROLE OF PROTEINS

Sulfur amino acids catabolism is the major source of sulfate anions, which essentially circulate as Na salts in plasma but, following ultrafiltration and Na reabsorption by kidneys, sulfate anions have to be buffered in urine by endogenous cationic species (Ca, Mg and/or NH_4^+) or metabolically generated alcalinizing compounds ($KHCO_3$ from dietary organic K salts). In principle, excreted SO_4 anions are proportional to dietary protein level (other sulfur derivatives such as taurine represent a minor part of sulfur excretion, [11]), even if there are some differences in the sulfur AA content of the dietary proteins, animal protein being generally higher in sulfur AA.

Minimizing SO_4 production and excretion could be achieved through a reduction of protein intake, but with a risk to disturb growth or protein renewal, and bone/muscle protein matrix are altered by protein deficiency [12]. In fact, the dietary protein intake in western countries (around 1 g/d/kg bw) is higher than the maintenance requirements, and the question arises as to whether this level will frequently promote LMA or whether other diet constituents will modulate this effect. In fact, data in the literature have frequently ascribed a favourable effect of protein consumption on bone health [12,13] but this point has been qualified in other investigations, especially as regards the balance between plant and animal proteins [14-16]. In rats, it has been observed that LMA takes place in animals adapted to high (26%) but also in those adapted to a moderate (13%) casein diet, if the mineral moiety of the diet is poorly alkalinizing. On the other hand, the high protein diet acidifying effect was blunted if the diet also contained a sufficient amount of K citrate [9]. This indicates that intake of relatively high levels of protein would not lead to acid-base desequilibrium if accompanied by sufficient amounts of K organic salts, as observed with well-balanced omnivorous diets.

ROLE OF FIBERS AND FERMENTABLE ANIONS

At present, little is known about the actual consequences of large intestine fermentations on acid-base balance, except when excessively acidic fermentations leads to malabsorption of water and minerals. Yet, the bacterial breakdown of fibers and related compounds generates everyday some 5 to 15 g short chain fatty anions (SCFA) in humans, hence 3-10 fold more than polycarboxylate anions (malate, citrate) from fruits and vegetables. If SCFA anions were to be absorbed together with cations, they could exert a substantial alkalinizing effect. Nevertheless, this is probably limited by the fact that a large part of SCFA is absorbed as protonated form or as $SCFA^-$ anion exchanged with HCO_3^- [17]. In this view, it turned out in a rat model that fiber supplementation had little effect on acid-base balance, but elicited a marked improvement of Mg balance in synergy with K citrate (Mg could participate to alcalinization, together with K) [18]. It has also been observed that a K salt of galacturonate, an anion not absorbed in the small intestine which arises from pectinolysis, was practically as effective as K citrate as an alkalinizing agent whereas K tartrate (partly absorbed) was almost uneffective [19]. Since galacturonate is metabolized to SCFA in the large intestine, this indicates that SCFA may exert an alkanizing effect when absorbed together with K (and possibly other cations such as Mg or Na), even when SCFA absorption is delayed compared to K absorption. This observation suggests that some minor anions, if available as K salts, could participate in the alkalinizing fonctionnality of plant foods besides well-identified anions (K malate and K citrate).

CONCLUSION

Even if the consequences of LMA can be investigated in humans using moderately invasive approaches, animal models can also be useful for mechanistics investigations or to separately evaluate nutrients or foods, as illustrated above for the comparison beween various K organic anion salts. Awareness of the role of LMA as a factor of hypercatabolism during ageing is clearly important for human nutrition, but should also be taken into consideration for animal models of ageing since usual rodent diets are frequently overprotective against LMA, in contrast to 'western' human diets.

Table 2. Example of a Westernized Diet Derived from a AIN-93 Maintenance Rat Diet, through Alterations of the Protein Level, of the Na/K Supply and of the Anionic Profile (Omission of Citrate and CO_3 Anions) of the Diet

Composition (g/kg diet)	AIN-93M	Westernized	Westernized + K3 citrate
Casein[1]	140	200	200
Cornstarch[2]	463	440	435
Dyetrose	155	0	0
Sucrose	100	150	150
Cellulose	50	50	50
Soybean Oil[3]	40	100	100
Ca CO_3	12,5	0	0
$CaHPO_4$	0,0	21,6	21,6
KH_2PO_4	8,75	0	0
KCl	0	2,98	2,98
K_3 citrate(H_2O)[4]	0,98	0	4,98
NaCl	2,59	10,0	10,0
K_2SO_4[5]	1,63	0	0
MgO	0,84	0,84	0,84
Trace Elements Mix (AIN-93M type)	10	10	10
Vitamin Mix (AIN-93M type)	10	10	10
L-Cystine	1,8	2,5	2,5
Choline Bitartrate	2,5	2,5	2,5
Na	*1,01*	*3,93*	*3,93*
K	*3,12*	*1,56*	*3,12*
Mg	*0,50*	*0,50*	*0,50*
Ca	*5,00*	*5,00*	*5,00*
P (mineral mix)	*1,62*	*2,52*	*1,26*
P (casein)	*1,12*	*1,60*	*1,60*
P(total)	*2,74*	*4,12*	*2,86*
Ca/P	*1,82*	*1,21*	*1,75*
Na/K	*0,32*	*2,52*	*1,26*

[1]casein, 0.8% P.
[2]qs for 1000 g.
[3]numerous alternative in the westernized diet as to (i) fat origin, (ii) dietary level and (iii) possible addition of cholesterol.
[4]possibility to also use malate or a citrate/malate mix.
[5]should be replaced by an other K salt if urinary sulfate excretion is measured as a reflect of sulfur amino acid oxidation.

Various types of 'westernized' diets have been proposed, based on complex products or on purified ingrdients and an example, derived from a classical AIN-93 rat diet is proposed in Table **2**.

ABBREVIATIONS

LMA = Latent metabolic acidosis

Na = Sodium

K = Potassium

Mg = Magnesium

Ca = Calcium

AA = Aminoacid

SCFA = Short-chain fatty acids

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