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Updating on the lysinoalanine content of commercial infant formulae and beicost products

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Abstract

Since the safety issue of lysinoalanine (LAL) still remains unresolved, its concentration in infant formulae should be reduced to a minimum. Data collected in the 1980s indicated that LAL is formed in higher amounts in liquid than in powdered formulae. Recently the market of liquid infant formulae is increasing rapidly and there are no new data, so 23 commercial powdered or liquid samples were investigated. In powdered samples, LAL was below the detection limit, whereas liquid adapted formulae contained up to 86 μg/g protein, liquid follow-on formulae up to 390 μg/g protein, and liquid growing milks up to 514 μg/g protein. The concentration of LAL in liquid formulae is considerably lower than in the past; however, the level in a few products remains rather high, especially compared with normal UHT-treated milk. Great differences were observed among products of different companies, which suggests that labelling with the thermal treatment applied would be very advisable. The investigation of some beicost products indicates that LAL is present only in products certainly containing milk proteins. Considering the rather low levels in comparison with liquid infant formulae, the contribution of beicost products to the total LAL daily intake does not seem to be particularly relevant.

Keywords: Lysinoalanine; Infant formulae; Baby foods; Beicost; Thermal damage; Cross-linked amino acids

1. Introduction

Heat processing is essential for preserving infant formulae and other baby foods. Heating, however, induces a number of chemical changes that must be carefully considered, because infant formulae, in particular adapted ones, are the sole food given to new-born babies and should fulfil all their nutritional requirements.

The content of available lysine has been used as a measure of the nutritional quality after thermal treatments, either in cow's milk (Arteaga, Vàzquez-Arteaga, & Nakai, 1994; Hewedy, Kiesner, Meissner, Hartkopf, & Erbersdobler, 1994; Morales, Romero, & Jimènez-Pèrez, 1995) or in model systems (Baiser & Labuza, 1992; Morales, Romero, & Jimènez-Pèrez, 1995). More recently (Ferrer, Alegrìa, Farrè, Abellàn, & Romero, 2000), the effects of thermal processing and storage on infant formulae have been studied under conditions (ratios between temperature and time) that fit with those really applied in the preparation of infant formulae: the level of available lysine in infant formulae is about 20% lower than in raw milk.

The depletion of lysine depends mostly on two independent processes: (1) the reaction with reducing sugars, which produces Maillard reaction products (MRPs), and (2) the degradation of cystine, *O*-phosphorylserine or *O*-glycosylserine, which produces dehydroalanine residues that react with the ε amino group of lysine, giving lysinoalanine [LAL, N^{ε}-(*R,S*-2-amino-2-carboxyethyl)-*S*-lysine], which is considered a useful molecular marker of the thermal damage to food and milk in particular (Faist, Drusch, Kiesner, Elmadfa, & Erbersdobler, 2000). The major milk proteins are susceptible to the formation of LAL, caseins owing to the high concentration of *O*-phosphorylserine, α-lactalbumin and β-lactoglobulin, owing to the high level of sulfur amino acids (Friedman, 1999; Maga, 1984). A recent review (Friedman, 1999) lists the processes and foods which have been studied for the formation of LAL, with detailed references to baby foods.

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The reduced digestibility of proteins treated by heating and/or with alkali (Anantharaman & Finot, 1994; Berger, & Possompes 1987; Savoie, Parent, & Galibois, 1991; Sarwar, Peace, & Botting, 1989) depends on the fact that LAL impairs the action of digestive enzymes because it represents a cross-linker of the protein chains (Pellegrino, van Boekel, Gruppen, & Resmini, 1998). Its adverse effects on growth, protein digestibility, protein quality, and mineral bioavailability and utilisation have recently been reviewed (Sarwar, L'Abbe, Trick, Bottig, & Ma, 1999).

Some studies have also indicated some toxicological consequences, since LAL was shown to provoke lesions in rat kidney cells causing nephrocitomegaly (Friedman, Gumbmann, & Masters, 1984; Gould & MacGregor, 1977; Karayiannis, MacGregor, & Bjeldanes, 1980); however, at least some effects seem to be reversible, because they disappear when feeding with LAL-rich proteins is ceased (Struthers, Beilmaier, Raymond, Dahlgren, & Hopkins, 1980; Struthers, Dahlgren, & Hopkins, 1977). These observations have promoted investigations on humans, in particular on pre-term infants (Langhendries et al., 1992), which have shown more limited damage than in animals. Nevertheless, the safety issue of LAL is still considered unresolved (Friedman, 1999).

As the antinutritional effects of LAL are certainly more pronounced in sole-source foods such as infant formulae, many groups have quantified this heat-induced compound in these products in the 1980s. Levels of LAL in the range of 150–2100 μg/g protein have been reported (Bellomonte, Boniglia, Carratù, & Filasi, 1987; de Koning & van Rooijen, 1982; Friedman, 1999; Fritsch & Klostermeyer, 1981 Pompei, Rossi, & Marè, 1987), and it has been demonstrated that liquid formulae are much more susceptible to LAL formation than powdered ones.

In Italy, until some years ago, mothers used to buy only powdered infant formulae; however, in the last few years liquid formulae have become more and more popular, although they are more expensive, because they do not require any preparation except mild warming. Taking into account this change in the market and the complete absence of up-to-date data, we decided to determine the presence of LAL in current liquid and powdered commercial samples.

Excluding products for children with specific pathologies, roughly three kinds of products are now available on the market: adapted ones that are used for feeding healthy new-born babies in the first 6 months, follow-on formulae which are used afterwards and constitute the main liquid food during weaning, and growing-milk to be used by the 1-year old child. We collected samples of each class together with some beicost products used during weaning: the samples were produced by different companies, as it has been observed that the quality of commercial milk-based infant formulae varies considerably (Pompei et al., 1987).

2. Materials and methods

2.1. Samples

Samples were labelled with acronyms, without showing commercial names. Powdered formulae were nine: one adapted and eight follow-on formulae (three based on soybean proteins and two lactose free). The liquid samples were 14: six adapted, six follow-on and two growing milks. LAF1, LAF3, and LAF5 were adapted for pre-term infants, LAF2 was treated with poly-unsaturated fatty acids, LAF4 was treated with adapted proteins, LAF6 was enriched with Fe, LAF5 was UHT-sterilized. LFF1, LFF3, and LFF6 were defined as "easily digestible" and were enriched with Fe; LFF4 and LFF5 were UHT-sterilized and LFF5 contained soybean proteins instead of milk proteins. LGM1 and LGM2 did not present any particular indications on their label.

WF1 and WF2 were wet baby foods containing cheese, WF3 was a wet baby food based on rabbit meat; WF4 was a wet baby food based on chicken meat.

BC1 was a baby cheese. BB1 was a granulated biscuit and BB2 was a biscuit; both were enriched with milk proteins. MC1 and MC2 were milk cereals containing dried fruits and vitamins.

2.2. Materials

All chemicals for derivatisation, solid phase extraction and HPLC analyses were highly pure. 9-Fluorenylmethylchloroformate (FMOC-Cl) was purchased from Fluka. The SPE amino cartridges (Bakerbond, 500 mg/3 ml) were purchased from Baker, The Netherlands. The chromatographic column (Amino-Quant, 200 × 2.1 mm i.d.) and the guard column cartridge (ODS-Hypersil C18, 5 μm, 20 × 2.1 mm i.d.) were purchased from Hewlett Packard, Germany.

2.3. LAL determination

LAL was determined by a procedure, including acid hydrolysis of the sample with HCl 6 N, FMOC derivatisation of the amino groups, selective solid phase extraction (SPE) to purify of LAL derivatives, and subsequent HPLC analysis with fluorescence detection, which permits quantitation LAL at very low levels in cheese (Pellegrino, Resmini, de Noni, & Masotti, 1996). The HPLC elution solvents were prepared from stock solution I (0.5% tetrahydrofuran and 0.1% ethyl acetate in 30 mM potassium acetate; v/v/v) and stock solution II (80% acetonitrile in 100 mM sodium acetate;

v/v). The stock solutions were mixed (v/v) for preparing elution solvent A (I:II = 70:30) and elution solvent B (I:II = 25:75). Elution solvent C was 100% of stock solution II. An aliquot of 20 μl of the sample purified by SPE, was injected into the HPLC column protected by a guard column cartridge; the separation was carried out by a linear elution gradient. Fluorescence detection was used with excitation at 266 nm and emission at 310 nm. Under the conditions described, the two isomers *S,R*- and *S,S*-LAL were eluted as separated peaks with t_R 25 and 26 min, respectively. The standard deviation of the method was 3–4%, samples of different lots may have about 9–10% differences.

3. Results

In recent years, reversed phase HPLC has become the major method for determining LAL in foods and, in particular, Pellegrino et al. (1996) have proposed a method based on derivatisation with FMOC-Cl, solid phase extraction, reverse-phase chromatography and fluorescence detection, which allows quantification of LAL at very low levels in cheese. This method was selected for our study, because it is very sensitive, the threshold limit being around 0.5 μg/g protein, and accurate, because it avoids interferences. As indicated in the original paper, LAL appears in the chromatograms as a double peak, due to the presence of the *S,S*- and *S,R*-diastereoisomers.

In total, nine powdered and 14 liquid formulae were analysed (at least two samples of each product from different lots). Among the powdered formulae, one was adapted and eight were follow-on; in all them the LAL content was below the detection limit (data not shown).

The results for the liquid formulae are shown in Table 1. The samples labelled as LAF were liquid adapted formulae, some (LAF1, LAF2, and LAF3) contained 23–86 μg/g of LAL, whereas others (LAF4, LAF5, LAF6) contained only traces that could not be quantified. In general, higher levels were detected in liquid follow-on formulae (labelled as LFF): LFF1 and LFF3 contained 390 and 346 μg/g protein, respectively; others (LFF2, LFF4, and LFF5) were in the range 83–143 μg/g protein, whereas sample LFF6 contained only traces. Sample LFF5, the only sample based on soybean proteins, had a LAL content in line with milk based products. A great variability was observed in liquid growing milks (LGM1 and LGM2), which contained 514 and 98 μg/g protein, respectively. The enrichment with Fe did not seem to have any effect on LAL levels.

Only five samples reported indication of the thermal treatment applied for their microbiological stabilisation: LAF5, LFF4, LFF5 were labelled as UHT-sterilised and had a relatively low content of LAL, whereas LFF1 and LFF3, labelled as "sterilised" (probably meaning in-bottle sterilised) contained more than 300 μg/g protein. Unfortunately, LGM1, the third sample containing a very high level of LAL, did not report the thermal process applied.

Some beicost products were also analysed (Table 2): four wet products containing milk proteins or not, a baby cheese, two baby biscuits and two baby milk cereals. LAL was detected only in products containing milk proteins but, with the exception of WF1, the levels were relatively low in comparison to liquid infant formulae. Therefore the contribution of beicost products to the total LAL daily intake does not seem to be particularly relevant.

Table 1
LAL content of infant formulae (LAF = liquid adapted formulae, LFF = liquid follow-on formulae, LGM = liquid growing milk)

Entry	LAL content (μg/g protein)	Protein content (%) ($N \times 6.38$)	Description reported on the label
LAF1	86±5	2.4	For pre-term or low weight infants; containing 15.4% homogenised milk
LAF2	45±2	2.0	With polynsaturated fatty acids (fish fats); whey proteins/caseins ratio 51/49
LAF3	23±2	2.3	For pre-term or low weight infants; with polynsaturated fatty acids and hydrolysed proteins; whey proteins/caseins ratio 78/22
LAF4	Traces	1.5	With adapted proteins
LAF5	Traces	2.0	UHT-sterilised; for pre-term or low weight infants; with fish fats, ultrafiltered proteins,
LAF6	Traces	1.7	Added with Fe, with ultrafiltered proteins
LFF1	390±25	1.8	Sterilised; without sucrose; added with Fe; whey proteins/caseins ratio 50/50, "easy digestible"
LFF2	143±10	2.6	With rice starch
LFF3	346±32	2.1	Sterilised; without sucrose; added with Fe; "easy digestible"
LFF4	105±7	1.7	UHT-sterilised; whey proteins/caseins ratio 50/50
LFF5	83±5	1.8[a]	UHT-sterilised; soybean proteins based
LFF6	Traces	2.1[a]	Added with Fe
LGM1	514±35	2.2	Without any particular indication
LGM2	98±5	2.6	Added with Fe

[a] $N \times 6.25$.

Table 2
LAL content of beicost products (WF = wet baby foods, BC = baby cheeses, BB = baby biscuits, MC = milk cereals)

Entry	LAL content (μg/g protein)	Protein content (%) ($N \times 6.38$)	Description reported on label
WF1	201 ± 14	13	Homogenised baby cheese, based on milk proteins with low lipid content, sterilised and vacuum-packed.
WF2	68 ± 6	7.8	Homogenised cheese for babies; based on milk proteins.
WF3	n.d.	7.8[a]	Based on rabbit meal, sterilised and vacuum packed
WF4	n.d.	9[a]	Based on chicken meat, sterilised and vacuum-packed
BC1	Traces	13	Baby cheese, without any particular indication
BB1	80 ± 7	11[a]	Granulated biscuit with milk proteins
BB2	34 ± 3	12.5[a]	Biscuit with milk proteins
MC1	n.d.	15.5[a]	Milk cereals with dried fruit and vitamins
MC2	n.d.	13.4[a]	Milk cereals with dried fruit and vitamins

n.d. not detected.
[a] $N \times 6.25$.

4. Discussion

It is generally recognised that heat and/or alkaline treatments cause significant reduction in the digestibility of milk and soybean proteins (Anantharaman, & Finot, 1993; Berger & Possompes 1987; Savoie et al., 1991) and a drastic negative effect on protein quality due to the formation of cross-linked amino acids. Possible causes of protein damage include the destruction of essential amino acids, such as lysine and threonine, or semi-essential amino acids, such as cysteine, a reduced digestibility due to the formation of cross-links and D-amino acids, and loss of phosphorus from phosphoproteins (Friedman, 1999; Sarwar, L'Abbe, Trick, Bottig, & Ma, 1999). Animal studies have shown that there are adverse effects on mineral nutrition due to chelation of trace elements, such as copper, zinc and iron (Friedman, Gumbmann & Masters, 1984; Friedman & Pearce, 1989; Rehner & Walter, 1991). The mineral status of rats is compromised, and the kidney iron content of rats fed with heat-treated proteins is lower than that of rats fed with unheated proteins, whereas liver copper levels are up to three-fold higher (Sarwar, L'Abbe, Trick, Bottig, & Ma, 1999). In addition, LAL was shown to provoke lesions in rat kidney cells, causing nephrocitomegaly (Friedman, Gumbmann & Masters, 1984; Gould & MacGregor, 1977; Karayiannis, MacGregor, & Bjeldanes, 1980; Leegwater, 1978).

Human studies, however, have shown that higher levels of LAL in infant formulae compared to breast milk had no influence on creatinine clearance or electrolyte excretion and that there is no evidence of tubular damage, as determined by the urinary excretion of four kidney-derived enzymes, whereas a general increase in urinary microprotein levels is observed (Langhendries et al., 1992). Although it is reassuring that the effects on kidneys observed in rodents were not apparent in primates, so that the Food Protein Council decided not to fix the maximum acceptable level in foods (Codex Alimentarius, 1982), special attention should be paid to the preparation of foods for particular classes of consumers, such as infants, because the safety of LAL and related compounds is still considered unresolved (Friedman, 1999).

The main objective of our work was to collect up-to-date data on the LAL content of infant formulae and to compare them with those of products, which were on the market in the 1980s.

Starting with powdered formulas, that contain very low levels of LAL, it is immediately clear that a lot of work has been done by manufacturers to improve the situation in respect to the past, when the values were in the range 150–920 μg/g protein (Bellomonte et al., 1987; Friedman, 1999; Fritsch & Klostermeyer, 1981; de Koning & van Rooijen, 1982; Pompei et al., 1987). This is particularly relevant because the preparation of milk powder requires many steps involving heating (Westergaard, 1983): a preheat treatment of milk (typically 90–95 °C/30–120 s) to ensure product safety, an evaporation step which concentrates the solids from 13 to 46–48%, and the spray drying treatment at high temperature (typically 200–220 °C), that reduces the moisture content from about 54–6%.

In liquid formulae, values were in the range from traces to 514 μg/g protein, which demonstrates that the quality of current commercial products is much better than in the 1980s, when values were in the range 160–2120 μg/g protein (Bellomonte et al., 1987; Friedman, 1999; Fritsch & Klostermeyer, 1981; de Koning, & van Rooijen, 1982; Pompei et al., 1987). Considering the three categories of infant formulae, the average level is 26 μg/g protein in adapted formulae (range: traces–85 μg/g protein), 178 μg/g protein in follow-on formulae (range: traces–390 μg/g protein), and 306 μg/g protein in growing milk (range: 98–514 μg/g protein), demonstrating very clearly that, today, manufacturers are particularly attentive to the quality of products for new born babies (a comparison with the past is not possible because old separated data are not available). However, it may be observed that only three products out of 14

have levels higher than 150 μg/g protein (two follow-on formulas, LFF1 and LFF3, and a growing milk, LGM1). Excluding these samples, the average value of follow-on formulae was 110 μg/g protein (maximum value 143 μg/g protein), in very good agreement with the average content of LAL in UHT-treated milk (maximum value 186 μg/g protein; Faist et al., 2000). LFF1, LFF3 and LGM1 are in agreement with the values of in-bottle sterilised milk (average 428 μg/g protein, range 224–653 μg/g protein; Faist et al., 2000). Certainly, as in drinkable milk (Faist et al., 2000), also in these products a careful choice of the manufacturing procedures should allow considerable reduction of the formation of LAL.

Unfortunately, the indication of the thermal treatment applied to these kinds of products is optional and only a few products investigated in this work report this information. For example, of the three products, in this work, containing high levels of LAL, LFF1 and LFF3 were "sterilised", possibly meaning in-bottle sterilised, whereas LGM1 had no specific indication. We suggest that it is very advisable to make compulsory the labelling of infant formulae as is compulsory for drinkable milk.

In addition, mothers, often too busy or anxious, should be better informed about the differences between powdered and liquid products.

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