



Original/*Pediatría*

# Introduction of pasteurized/raw cow's milk during the second semester of life as a risk factor of type 1 diabetes mellitus in school children and adolescents

Edna F. Villagrán-García<sup>1</sup>, Erika F. Hurtado-López<sup>1</sup>, Edgar M. Vásquez-Garibay<sup>2</sup>, Rogelio Troyo-Sanromán<sup>1</sup>, Liuba M. Aguirre-Salas<sup>3</sup>, Alfredo Larrosa-Haro<sup>1</sup> and Ruth V. León-Robles<sup>1</sup>

<sup>1</sup>Instituto de Nutrición Humana, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara (Jalisco). <sup>2</sup>Salvador Quevedo y Zubieta 750, SL. Guadalajara, Jalisco. <sup>3</sup>Hospital Civil de Guadalajara, Guadalajara, Jalisco (México).

## Abstract

**Objective:** to demonstrate that type 1 diabetes mellitus (T1DM) in school children and adolescents is associated with the early introduction of pasteurized/raw cow's milk in the second semester of life.

**Material and methods:** this non-probabilistic study included 150 subjects (75 patients and 75 controls), divided according to sex and age (range, 6 to 16 years). T1DM was considered to be a dependent variable, and pasteurized/raw cow's milk (P/RCM) was considered to be an independent variable in the study. The statistical analyses included chi-squared test, odds ratio and 95% confidence intervals.

**Results:** the subjects were 51% male, age  $11 \pm 3.2$  years, and 80% were breastfed, 18% were exclusively breastfed, and 13% received pasteurized/raw cow's milk. The children receiving P/RCM had a higher risk of T1DM [OR, 3.9 (1.2-12.8)]. The presence of T1DM was three times higher in those consuming P/RCM vs. those receiving follow-up formula [RM, 3.2 (1.03-10.07)].

**Conclusions:** introducing pasteurized/raw cow's milk in the second semester of life increased by four times the likelihood of developing T1DM in children and adolescents.

(Nutr Hosp. 2015;32:634-637)

DOI:10.3305/nh.2015.32.2.9247

Key words: *Pasteurized/raw cow milk. Type 1 diabetes mellitus.*

## INTRODUCCIÓN DE LECHE ENTERA PASTEURIZADA/NO PASTEURIZADA DE VACA EN EL SEGUNDO SEMESTRE DE LA VIDA Y RIESGO DE DIABETES MELLITUS TIPO 1 EN ESCOLARES Y ADOLESCENTES

## Resumen

**Objetivo:** demostrar que la diabetes mellitus tipo 1 (DMT1) en escolares y adolescentes se asocia a una temprana introducción de leche entera pasteurizada/no pasteurizada en el segundo semestre de vida.

**Material y métodos:** en este estudio no probabilístico de casos y controles se incluyeron 150 participantes (75 pacientes y 75 controles), divididos de acuerdo a la edad y el sexo de 6 a 16 años de edad. Se consideró DMT1 como una variable independiente. El análisis estadístico incluyó la prueba de Ji cuadrada y razón de momios con su intervalo de confianza del 95%

**Resultados:** los participantes fueron 51% varones, con edades de  $11 \pm 3.2$  años y el 80% alimentados al pecho materno, 18% en forma exclusiva, y el 13% recibieron leche entera pasteurizada/no pasteurizada. Los niños que recibieron leche entera pasteurizada/no pasteurizada tuvieron un riesgo mayor de DMT1 [OR, 3,9 (1,2-12,8)]. La presencia de DMT1 fue tres veces más elevada en quienes consumieron leche entera pasteurizada/no pasteurizada que en aquellos que recibieron fórmula de seguimiento [RM, 3,2 (1,03-10,07)].

**Conclusión:** la introducción de leche entera pasteurizada/no pasteurizada en el segundo semestre de vida incrementó cuatro veces la probabilidad de desarrollo de DMT1 en escolares y adolescentes.

(Nutr Hosp. 2015;32:634-637)

DOI:10.3305/nh.2015.32.2.9247

Palabras clave: *Leche entera cruda y pasteurizada. Diabetes mellitus tipo 1.*

**Correspondence:** Erika F. Hurtado López.  
Instituto de Nutrición Humana,  
Hospital Civil de Guadalajara Juan I. Menchaca,  
Salvador Quevedo y Zubieta 750, SL., CP. 44340,  
Guadalajara, Jalisco (México).  
E-mail: erika26hurtado@gmail.com

Recibido: 12-V-2015.  
Aceptado: 18-VI-2015.

## Introduction

Type 1 diabetes mellitus (T1DM) is one of the most common endocrine and metabolic diseases in children. It is associated with damage to the pancreas and decrease in insulin secretion by autoimmune mechanisms, particularly antibodies against the enzyme glutamic acid decarboxylase (GAD), insulin, and islet cells (ICA 512/IA-2)<sup>1-2</sup>. These antibodies may be induced by five cow's milk proteins: casein,  $\beta$ -lactoglobulin, lacto-albumin,  $\gamma$ -globulin and bovine serum albumin<sup>1,4</sup>. The presence of antibodies GAD and ICA512/IA-2 predicts the development of T1DM in a very high percentage (>60%) of children and adolescents in the following five to ten years<sup>3-5</sup>.

Newborns have an increased permeability of the intestinal mucosa, allowing the interaction of cow's milk proteins and peptides with the immune system, thereby increasing the risk of an autoimmune response<sup>1,6-7</sup>. The infant who is exclusively breastfed is not exposed to these proteins and the sensitization risk is minimized. Additionally, mother's milk IgA is a protective factor against foreign proteins<sup>8-9</sup>.

The low prevalence of breastfeeding during the first year of life has led to the early introduction of infant formula and, in low socioeconomic levels, pasteurized or raw whole cow's milk<sup>10,11</sup>. These feeding practices may expose the infant to the emergence of various immunologic diseases, including T1DM. Our aim was to evaluate the association of T1DM with early exposure to raw or pasteurized whole cow's milk in children from a low socioeconomic level in which this type of feeding practice is frequent.

## Patients and methods

This case control study included 75 outpatient children and adolescents six to 16 years of age with T1DM (cases) and 75 children from the same age groups without chronic diseases, attended by acute infections (controls); the control subjects were otherwise healthy. The exposure variable was the introduction of P/RCM in the first year of life. The study period was September 2012 to February 2013. Children whose birth weight was <2,500 g or >4,100 g were not included in the study.

The diagnosis of T1DM was established in children and adolescents with classical symptoms, e.g., fasting glucose >100 mg/dL, postprandial glucose >140 mg/dL or >200 mg/dL anytime<sup>12,13</sup>. The control group of subjects were recruited from the hospital inpatient or outpatient areas during the same period.

A trained interviewer applied an *ad hoc* questionnaire designed to record the dietary history in the first 12 months of life in a face-to-face interview with the parents or guardians. The health status of the control subjects was evaluated by consulting the clinical files, including the laboratory and radiographic data by the primary author.

The statistical analysis was performed using the SPSS Statistics v. 18. The data were analyzed using 2  $\times$  2 tables with  $\chi^2$  and odds ratios; the p value and 95% confidence interval results represented significance tests. The protocol was approved by Hospital Civil de Guadalajara's Bioethics and Research Committees of the Civil (#1222/22). A written informed consent was signed by the parents of both study groups.

## Results

Overall, 80% (n=123) of the subjects were breastfed in the first six months of life. Approximately 18% (n= 29) of the subjects were exclusively breastfed for at minimum four months. The mean age of exclusive breastfeeding in the subjects with T1DM was lower ( $2.4 \pm 2.2$  months) than in the control group ( $3.1 \pm 2.3$  months) with a non-significant trend (p=0.09). In six to twelve months, 32% (n=48) of children in both groups were breastfed; however, the comparisons of nursing time in both groups showed no difference.

Sixty-seven percent (n=99) of mothers used a substitute for human milk (infant formula in the majority of the cases) to feed infants in the first six months of life in both groups, with no difference. At this stage of life, unprocessed whole milk or pasteurized milk was not used. In the second half of life, 13% of the children studied were exposed to pasteurized/raw cow's milk before one year of age, and this percentage more than doubled when whole milk powder was included.

Table I shows the difference in association between the frequency of cases (DM-1) who were exposed to pasteurized/raw cow's milk vs. human milk in the second semester of life [OR, 3.9 (1.2-12.8), p = 0.01]. The same table shows the difference in the frequency of cases (DM-1) who were exposed to pasteurized/raw cow's milk vs. follow-up formula [OR, 3.2 (1.03-10.07), p = 0.04]. The difference in the frequency of cases (DM-1) among those who were exposed to pasteurized/raw cow milk vs. whole milk powder showed no significant association.

## Discussion

The epidemiological studies in the last 15 years concerning the early introduction of cow's milk proteins in children under one year of age stimulated a growing concern worldwide because such exposure may be a risk factor for later development of DM-1. The first evidence supporting this hypothesis emerged in the mid-1980s when Borch-Johnsen et al.<sup>14</sup> demonstrated an inverse association between the duration of breastfeeding and the incidence of T1DM in Norway and Sweden. Other studies extended this observation by reporting a positive correlation between the incidence of T1DM and the per capita consumption of cow's milk<sup>6,15-16</sup>.

**Table I**

Comparison of feeding methods during second semester of life in 75 children and adolescents with type I diabetes and 75 controls. The subgroups include human milk, infant formula, pasteurized/raw cow milk and powdered whole cow's milk. The results are presented as frequencies and percentages. Statistics included odds ratio and 95% confidence intervals

Type of milk	Type I Diabetes mellitus		Controls		OR	(CI 95%)
	n	(%)	n	(%)		
Human milk	19	(55.9)	25	(83.3)	<b>3.9</b>	<b>(1.2-12.8)</b>
Pasteurized/raw cow's milk	15	(44.1)	5	(16.7)		
Total	34		30			
Human milk	19	(41.3)	25	(46.3)	1.2	(0.5-2.9)
Follow-up infant formula	27	(58.7)	29	(53.7)		
Total	46		54			
Follow-up infant formula	27	(64.2)	29	(85.3)	<b>3.2</b>	<b>(1.03-10.07)</b>
Pasteurized/raw cow's milk	15	(35.8)	5	(14.7)		
Total	42		34			
Powdered whole cow's milk	12	(44.4)	12	(70.6)	3.0	(0.7-13.4)
Pasteurized/raw cow's milk	15	(55.6)	5	(29.4)		
Total	27		17			

Breastfeeding appears to protect newborns from infection by viruses, thus decreasing the risk of developing T1DM. Another protective effect of human milk is the rapid decrease in intestinal permeability; in contrast, early exposure to proteins in cow's milk increases the permeability of the intestinal mucosa favoring inflammation and results in the deregulation of the immune response to proteins in cow's milk<sup>10,17</sup>.

In Mexico, the practice of breastfeeding has declined significantly in recent decades; however, this study found that more than 80% of the children were breastfed in the first months of life, although not exclusively. In the present study, we observed how dramatically the practice of breastfeeding in the second semester of life decreased because only 30% of these children were breastfed. This observation implies a high risk to the infants because mothers often prefer to introduce pasteurized/raw cow's milk most likely because of the high cost of infant formula. Controversy exists concerning whether the early introduction of cow's milk protein is associated with the development of T1DM in later life and whether studies are based on an early introduction of cow's milk proteins in infant formulas<sup>18,19</sup>.

However, in this study, we found a risk factor for the introduction of pasteurized/raw cow's milk in the second semester of life, which occurred more frequently in schoolchildren with T1DM than in healthy children, and we found the risk of developing this disease increased by four-fold. Strikingly, we observed that introducing pasteurized/raw cow's milk in the second semester of life increases by three-fold

the risk of developing T1DM compared with the follow-up formula.

These findings demonstrated a clear association between the early introduction of pasteurized/raw cow's milk with T1DM in contrast to other studies whereby the introduction of proteins in cow's milk occurs with the use of modified infant formula<sup>19</sup>.

Little is known about the natural development of antibodies vs. oral antigens in early life. Previous studies have shown that the early introduction of cow's milk protein induces both humoral and cellular immune responses<sup>3,20,21</sup> although the mechanism of the action of nutritional factors that play a role in the development of autoimmunity against  $\beta$  cells in the pancreas is not known. Studies have shown that early exposure to cow's milk modifies the permeability of the intestinal mucosa. This phenomenon would cause a reduction in the integrity of the physical barrier, and the immune system would be exposed to more antigens and would be more reactive<sup>22</sup>. One of the theoretical mechanisms suggesting the combination of exposure of cow milk and T1DM might be an immunological cross-reactivity between cow's milk proteins and auto-cell antigens. In fact, homologous sequences were observed between cow's milk proteins and pancreatic islet auto antigens<sup>23</sup>.

Vaarala et al.<sup>3</sup> demonstrated that infants who were exposed early to cow's milk proteins have high titers of antibodies vs. bovine insulin, a normal component of cow's milk. In another study, Simpson et al. (2008) found higher levels of immunoglobulins, such as immunoglobulin A (IgA) and immunoglobulin (IgG),

against bovine lacto-globulin in children who were given cow's milk before 12 months of age and who later developed T1DM compared with those who were autoantibody negative and disease free at the time of follow-up<sup>24</sup>.

The present study demonstrated that the control group continued breastfeeding for a longer period compared to the children with T1DM. This finding suggested that prolonged breastfeeding would help prevent the generation of antibodies against bovine  $\beta$ -casein, as demonstrated in a previous study<sup>7</sup>.

Saukkonen et al.<sup>20</sup> showed that children with T1DM have higher concentrations of antibodies against the proteins of cow's milk anti-bovine serum albumin (BSA) and  $\beta$ -lactoglobulin compared to the matched controls. These high concentrations of antibodies would behave as risk markers for T1DM. The increase of the humoral immune response against the number of proteins in cow's milk (increasing concentrations of IgG anti- $\beta$ -lactoglobulin and IgA against the proteins from cow's milk) in the infant stage is associated with children progressing to T1DM.

The strength of the study is that the population was obtained from a site concentration for poor people in the state of Jalisco, which includes the metropolitan area of Guadalajara. The primary limitation would be related to the sample size that was insufficient to demonstrate other associations with a clear non-significant trend. Our findings suggested that children with type 1 diabetes mellitus may also share similar immunological alterations that occur in other parts of the world and that the introduction of unprocessed whole cow's milk and pasteurized milk during the second semester of life is likely to increase the risk of developing T1DM.

### Authors' contributions

Conceived and designed the experiments: EFVG, EFHL, EMVG. Performed the experiments: EFVG, LMAS, RVLRL, Analyzed data: EFHL, EMVG, RTS, ALH. Wrote the paper: EFHL, EMVG, ALH. All authors read and approved the final manuscript.

### Interest conflict

We declare that there are no conflicts of interest to disclose.

### References

1. Leung KC, Sauve RS. Whole cow's milk in infancy. *Paediatr Child Health* 2003; 8:419-21.
2. Wu YL, Ding YP, Gao J, Tanaka Y, Zhang W. Risk factors and primary prevention trials for type 1 diabetes. *Int J Biol Sci*. 2013; 9: 666-79.

3. Virtanen S, Knip M. Nutritional risk predictors of  $\beta$  cell autoimmunity and type 1 diabetes at a young age. *Am J Clin Nutr* 2003; 78:1053-67.
4. Vaarala O, Hyöty H, Akerblom HK. Environmental factors in the aetiology of childhood diabetes. *Diabetes Nutr Metab* 1999;12:75-85.
5. Schrezenmeir J, Jagla A. Milk and diabetes. *J Am Coll Nutr* 2000; 19:176-190.
6. Simpson M, Norris J. Mucosal immunity and type 1 diabetes: looking at the horizon beyond cow's milk. *Pediatr Diabetes* 2008; 9:431-3.
7. Hermann E, Wasmuth and Hubert Kolb. Cow's milk and immune-mediated diabetes. *Proceedings of the Nutrition Society* 2000; 59:573-579.
8. Luopajarvi K, Savilahti E, Virtanen SM, Ilonen J, Knip M, Akerblom HK, et al. Enhanced levels of cow's milk antibodies in infancy in children who develop type 1 diabetes later in childhood. *Pediatr Diabetes* 2008; 9:434-441.
9. Knip M, Virtanen SM, Akerblom HK. Infant feeding and the risk of type 1 diabetes. *Am J Clin Nutr* 2010; 91:1506S-1513S.
10. Skrodeniene E, Marciulionyte D, Padaiga Z, Jasinskiene E, Sadauskaite-Kuehne V, Ludvigsson J. Environmental risk factors in prediction of childhood prediabetes. *Medicina* 2008;44: 56-63.
11. McKinney PA, Parslow R, Gurney K, et al. Perinatal and neonatal determinants of childhood type 1 diabetes. *Diabetes Care* 1999; 22:928-932.
12. World Health Organization. IDF. Definition and diagnosis of Diabetes Mellitus and Intermediate hyperglycaemia. 2006. Available at: [http://whqlibdoc.who.int/publications/2006/9241594934\\_eng.pdf](http://whqlibdoc.who.int/publications/2006/9241594934_eng.pdf).
13. American Diabetes Association. Standards of Medical Care in Diabetes 2011. *Diabetes Care* 2011; 34. Obtenido el: 15 de diciembre de 2011. Available at: [http://care.diabetesjournals.org/content/34/Supplement\\_1/S11.full.pdf+html](http://care.diabetesjournals.org/content/34/Supplement_1/S11.full.pdf+html).
14. Borch-Johnsen K, Joner G, Mandrup-Poulsen T, Christy M, Zachau-Christiansen B, Kastrup K, Nerup J. Relation between breast-feeding and incidence rates of insulin-dependent diabetes mellitus. A hypothesis. *Lancet* 1984; 2:1083-6.
15. Dahl-Jorgensen K, Joner G, Hanssen KF. Relationship between cows' milk consumption and incidence of IDDM in childhood. *Diabetes Care* 1991; 14:1081-3.
16. Scott FW. Cow milk and insulin-dependent diabetes mellitus: is there a relationship? *Am J Clin Nutr* 1990; 51:489-91.
17. Wadsworth EJK, Shield JPH, Hunt LP, et al. A case-control study of environmental factors associated with diabetes in the under 5s. *Diabet Med* 1997; 14:390-396.
18. Tiittanen M, Paronen J, Savilahti E, Virtanen SM, Ilonen J, Knip M, Akerblom HK, Vaarala O; Finnish TRIGR Study Group. Dietary insulin as an immunogen and tolerogen. *Pediatr Allergy Immunol* 2006; 17:538-43.
19. Erkki Savilahti, Kristiina M. Saarinen. Early infant feeding and type 1 diabetes. *Eur J Nutr* 2009; 48: 243-249.
20. Saukkonen T, Virtanen SM, Karppinen M, Reijonen H, Ilonen J, Räsänen L, Akerblom HK, Savilahti E. Significance of cow's milk protein antibodies as risk factor for childhood IDDM: interactions with dietary cow's milk intake and HLA-DQB1 genotype. Childhood Diabetes in Finland Study Group. *Diabetologia* 1998; 41:72-8.
21. Pérez-Bravo F, Oyarzun A, Carrasco E, Albala C, Dorman JS, Santos JL. Duration of breast feeding and bovine serum albumin antibody levels in type 1 diabetes: a case-control study. *Pediatric Diabetes* 2003; 4:157-161.
22. Bosi E, Molteni L, Radaelli MG et al. Increased intestinal permeability precedes clinical onset of type 1 diabetes. *Diabetologia* 2006; 49: 2824-2827.
23. Pozzilli P. Beta-casein in cow's milk: a major antigenic determinant for type 1 diabetes? *J Endocrinol Invest* 1999; 22: 562-7.
24. Simpson M, Norris J. Mucosal immunity and type 1 diabetes: looking at the horizon beyond cow's milk *Pediatr Diabetes*. 2008; 9: 431-3.