Does hydrolyzed formula reduce the risk of developing type 1 diabetes mellitus in infants at risk compared to non-hydrolyzed formula? – A systematic review and meta-analysis.

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Abstract

Objective: Evaluate T1DM development risk in genetically susceptible infants exposed to hydrolyzed versus conventional formula during early life.

Methods: A systematic review of randomized clinical trials (up to August 2021) included infants at high T1DM risk.

Primary outcome: T1DM development by WHO criteria. Secondary outcome: T1DM-related autoantibodies. Data were analyzed to determine the Risk Ratio (RR), 95% Confidence Interval (CI), and the test for an overall effect. The heterogeneity of the studies was tested using I^2.

Results: Three studies addressed T1DM risk in genetically susceptible infants exposed to hydrolyzed versus conventional formula during their early life. The metanalysis of studies (3297 infants: 1462 hydrolyzed, 1835 non-hydrolyzed) showed no significant difference in T1DM risk (RR: 1.12; 95% CI 0.86-1.47).

Conclusion: Hydrolyzed formula didn't reduce T1DM risk compared to non-hydrolyzed formula in genetically susceptible infants.

Keywords: Beta-cell autoimmunity, Dietary manipulation, Infants, Type 1 diabetes, Randomized controlled trials.

Abbreviations: T1DM: Type 1 Diabetes Mellitus; HLA: Human Leukocyte Antigen; WHO: World Health Organization; ICA: Islet Cell Antibodies; IAA: Insulin Autoantibodies, GAD: Glutamic Acid Decarboxylase; ZnT8: Zinc Transporter 8

Introduction

Type 1 Diabetes Mellitus (T1DM), one of childhood's most common chronic diseases, is defined by the autoimmune destruction of insulin-producing beta-cells in the pancreatic islets in genetically susceptible people. The incidence of T1DM is increasing more than it had previously among children, particularly in those younger than 5 years of age. The prevalence of T1DM in children in Canada is currently 1 in 300. Overt diabetes is preceded by an asymptomatic period of highly variable duration during which diabetes-associated autoantibodies appear in the peripheral circulation as markers of emerging beta-cell autoimmunity. Accumulating evidence suggests that autoimmune destruction of beta-cells may be induced during infancy. Type of feeds introduced in early life may modify the risk of T1DM later in life. A shorter duration of breastfeeding and early exposure to complex dietary proteins have been implicated as risk factors for T1DM. Early exposure to complex dietary proteins such as those found in regular cow's milk formula may increase the risk of beta-cell autoimmunity in children at genetic risk for T1DM. Hydrolyzed formula has less complex dietary protein compared with regular formula. Extensively hydrolyzed formulas do not contain intact proteins. Early nutritional intervention may help to prevent T1DM and has been reported to be successful in experimental models of autoimmune diabetes, although data are not consistent. A meta-analysis in older age groups also showed that comparing partially or extensive hydrolysed formula has no benefit to reduce the risk of allergic or autoimmune disease. Although the effect of early nutrition on the risk of developing T1DM and autoantibodies related to diabetes has been reported in numerous Randomized Control Trials (RCTs), there are currently no systematic reviews or updated meta-analyses that compare the use of hydrolyzed formula with non-hydrolyzed formula on the risk of developing

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T1DM. Therefore, this is the first study within the literature reporting on this topic [1].

Materials and Methods

A systematic review and meta-analysis were conducted as per the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. RCTs in which infants, up to two years of age, who are at increased risk of developing T1DM either by having a first-degree relative with T1DM or defined Human Leukocyte Antigen (HLA) genotype were exposed to either hydrolyzed formula or non-hydrolyzed formula. The primary outcome measure was a diagnosis of T1DM as defined by the World Health Organization (WHO) criteria up to 16 years of age. Secondary outcomes included-development of autoantibodies, including Islet Cell Antibodies (ICA), Insulin Autoantibodies (IAA), antibodies to Glutamic Acid Decarboxylase (GAD), antibodies to tyrosine phosphatase-like proteins such as insulinoma-associated protein (IA-2, ICA512), and antibodies to the zinc transporter 8 (ZnT8) [2].

Literature search

A systematic literature search strategy was developed by HLR together with subject experts. The search was conducted on August 31, 2021. Databases included were OVID MEDLINE, OVID EMBASE, OVID Cochrane Central Register of Controlled Trials, OVID Cochrane Database of Systematic Reviews, CINAHL, Scopus, and Web of Science. Randomized controlled trials published in English were included. No publication date limits were applied. The MEDLINE search used controlled vocabulary and keywords and subsequent searches were based on it [3].

Data extraction and management

Two reviewers (AA and PM) independently screened all search results for relevancy using keywords, titles, and abstracts. Data extraction was done by the two reviewers separately using standardized data abstraction forms. Both reviewers (AA and PM) independently examined all results, scientific contents, and conclusions. The information retrieved was compared, and any differences were resolved by consensus. When consensus could not be reached, a third reviewer (AL) cross-checked the data and resolved the disagreement. All articles and data abstraction forms were checked and reviewed by a third reviewer (AL). The choice between qualitative and quantitative analysis (meta-analysis) was based on the available data and heterogeneity found [4].

Methodological quality

Two reviewers (AA and PM) independently evaluated the methodological quality as per the Revised Cochrane Risk of Bias tool. These instruments assess clinical trial quality and risk of bias and include 10 and 7 items, respectively (Tables 1 and 2). The Newcastle-Ottawa quality assessment scale for RCTs was also used to assess the quality of the studies (Table 3). The reviewers were not masked to the authors, institutions, or journals. When further clarification was needed to conduct analysis, study authors were contacted by email or phone for additional information not available in the publications [5,6].

Table 1. *Risk of bias based on Cochrane tool.*

Bias	**Knip et al.**	**Vaarala et al.**	**Knip et al.**
Random sequence generation (selection bias)	+	+	+
Allocation concealment (selection bias)	+	+	+
Selective reporting (reporting bias)	+	+	+
Blinding of participants and personnel (performance bias)	+	+	+
Blinding of outcome assessment (detection bias)	+	+	+
Incomplete outcome data (attrition bias)	+	?	+
Other bias	+	+	+

Table 2. *Quality assessment of randomized controlled trials included in systematic review.*

	Knip et al.	**Vaarala et al.**	**Knip et al.**
Background and objectives			
Clearly articulated research hypothesis	Y	Y	Y
Case selection			

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Defined inclusion/exclusion criteria	Y	Y	Y
Described sampling time frame	Y	Y	Y
Data abstraction			
Blinded data collection procedure	Y	Y	Y
Standardized data abstraction protocol	Y	Y	Y
Reliability check for abstracted data	Y	Y	Y
Data management			
Established rules for missing data	Y	Y	Y
Defined outcome measures	Y	Y	Y
Described data analysis	Y	Y	Y
Confidentiality			
Local ethic board approval	Y	Y	Y
Final Score	10	10	10

Table 3. *Newcastle-Ottawa Scale (NOS) to assess the quality of randomized control trials.*

Study names	Selection	Comparability	Exposure
Knip et al.	****	*	**
Vaarala et al.	****	*	***
Knip et al.	****	*	***

Statistical analysis

The data was analyzed and combined using Review Manager Software (RevMan). Heterogeneity was tested using I^2. P value<0.05 was interpreted as statistically significant. Relative Risk (RR), Odds Ratio (OR), risk difference, and Number Needed to Treat (NNT) were calculated for dichotomous outcomes. All estimates of intervention effects were reported with 95% Confidence Intervals (CI). A forest plot was used for reported outcomes. A funnel plot looked for publication bias (Figure 1). Reporting of the results based on the PRISMA guidelines [7].

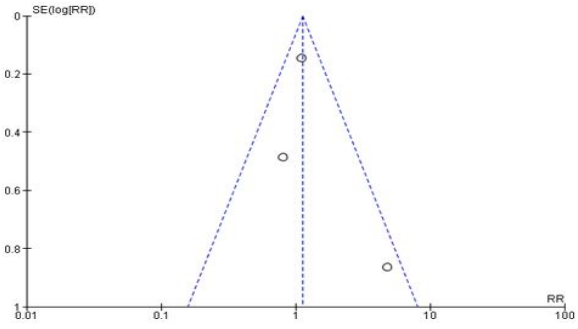

Figure 1. *Funnel plot of the studies.* ***Note:*** *Funnel plot to assess for publication bias. Each circle represents one study. Publication bias was not detected.*

Results

Study selection

The PRISMA flow diagram of study selection is shown in Figure 2. We identified 489 studies by literature search. After removing duplicates, 230 studies were analyzed, of which six studies were found to be eligible. The respective authors of these studies were contacted and confirmed either potential overlap, or lack of follow-up data in these studies, either due to being a feasibility trial, or due to lack of funding for follow-up. Because of either overlap with other studies or lack of follow-up data, three studies were excluded [8].

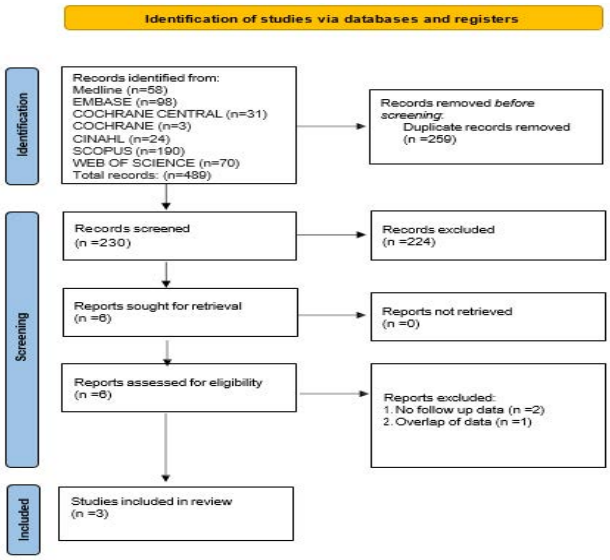

Figure 2. *PRISMA flow diagram.* ***Note:*** *It is depicting the selection process of studies included in this systematic review.*

Characteristics of the included studies

Table 4 summarizes the study characteristics. In this meta-analysis, 3297 infants were included, of which 1462 received hydrolyzed and 1835 received non-hydrolyzed formula [9].

Table 4. *Characteristics of included studies.*

Study ID	Methods	Participants	Interventions	Outcomes	Allocation concealment
Knip et al.	Prospective, multisite double-blind, randomized trial	Total 470 infants were eligible in the study	Intervention: An extensively hydrolyzed casein-based formula (Nutramigen, Mead Johnson Nutrition)	Primary outcome: Positivity for two or more diabetes-associated autoantibodies	Adequate
	Masking of allocation: Yes	A total of 230 infants were enrolled in the study	Control formula: composed of 80% intact milk protein (Enfamil) and 20% hydrolyzed milk protein	Secondary outcome: Clinical type 1 diabetes mellitus	
	Masking of intervention: Yes	Total 113 in the intervention group and 117 in the control group	Breastfeeding was encouraged, and the formula was given only when breast milk was not available		
	Masking of outcome assessment: Yes				
	Completeness of follow-up: Yes				
Vaarala et al.	Prospective, multisite double-blind pilot randomized control trial	Total 1113 infants eligible. Total 1104 infants were enrolled in the study. Total 908 infants were eligible in the study. Infants were allocated to Conventional Formula (CMF) (n=389) Whey-based Hydrolyzed Formula (WHF) (n=350), or whey-based FINDIA formula essentially free of bovine insulin (n=365)	Intervention 1: FINDIA formula	Primary outcome: beta-cell autoimmunity monitored at ages 3, 6, and 12 months and then annually until age three years	Adequate
	Masking of allocation: Yes		Intervention 2: Whey based hydrolyzed formula (Peptidi-Tutteli; Valio Ltd.) Control: Cow's milk formula (Tutteli; Valio Ltd.)	Secondary outcome: Type 1 diabetes mellitus	

	Masking of intervention: Yes		Breastfeeding was encouraged, and the formula was given only when breast milk was not available		
	Masking of outcome assessment: Yes				
	Completeness of follow-up: Yes				
Knip et al.	International prospective, multisite double-blind, randomized control trial	Total 5606 eligible infants. Total 5156 enrolled in study. Total 2159 completed study. Total 1081 in the intervention group and 1078 in the control group	Intervention: An extensively hydrolyzed casein-based formula (Nutramigen, Mead Johnson Nutrition) Control formula: composed of 80% intact milk protein (Enfamil) and 20% hydrolyzed milk protein	Primary outcome type 1 diabetes diagnosed according to World Health Organization criteria	Adequate
	Masking of allocation: Yes		Breastfeeding was encouraged, and intervention formula was given only when breast milk was not available	Secondary outcomes: Age at diabetes diagnosis and safety (adverse events)	
	Masking of outcome assessment: Yes				
	Completeness of follow-up: Yes				

Dietary intervention in infancy and later signs of beta cell autoimmunity: Knip et al. conducted a multi-center prospective RCT that included 230 infants with HLA-conferred susceptibility to T1DM and at least one family member with T1DM. This study was based at 15 hospitals in Finland, and recruitment was from February 1995 until November 1997. Infants were randomized to receive either extensively hydrolyzed casein-based formula (intervention), or conventional, cow's milk-based formula (control), whenever breast milk was unavailable during the first 6-8 months of life.

Removal of bovine insulin from cow's milk formula and early initiation of beta-cell autoimmunity in the FINDIA pilot study: Vaarala et al. performed a multi-center prospective RCT that included 1113 patients with HLA-conferred susceptibility to T1DM. These were randomly assigned to receive study infant formula, including a cow's milk formula, whey-based hydrolyzed formula, or whey-based FINDIA formula. This study was based at three pediatric hospitals in Finland from May 2002 until November 2005.

Effect of hydrolyzed infant formula vs. conventional formula on the risk of T1DM: Knip et al. conducted an international multi-center prospective RCT that included 2159 infants with HLA-conferred disease susceptibility and a first-degree relative with T1DM. This study involved 78 centers in 15 countries, with recruitment from May 2002 until January 2007. The participants received either a casein hydrolysate or a conventional adapted cow's milk formula composed of 80% intact milk protein (Enfamil) and 20% hydrolyzed milk protein.

Quality assessment of included studies

The Newcastle-Ottawa scale, which offers a star system for analysis, was used to assess the quality of the three RCTs (Table 3). All 3 studies included showed high quality for selection, exposure and comparability in their respective study groups. No significant heterogeneity was noted using I^2 statistics. On the revised Cochrane risk-of-bias tool for RCTs, all three studies included had a lower risk of bias and were rated as high quality (Table 1).

Development of T1DM

We assessed the development of T1DM in terms of exposure to either hydrolyzed and non-hydrolyzed formulas. Development of T1DM was reported in three studies which enrolled 3297 patients in total (Figure 3). No statistically significant difference was seen in the RR analysis showing no difference in outcome between intervention and control (RR: 1.12; 95% CI 0.86-201 202 1.47, p=0.39). A low level of heterogeneity was found among the studies (I^2=39%, p=0.19).

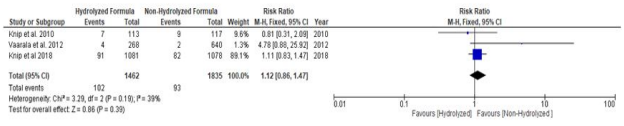

Figure 3. *Forest plot showing progression to T1DM as outcome.* **Note:** *Forest plot of (hydrolyzed group) vs. (non-hydrolyzed group) – (development of T1DM). Quantitative analysis showing the risk ratio in T1DM.*

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Development of autoantibodies related to T1DM

Secondary outcomes are reported in Table 5. According to Knip et al., there was a lower risk of seroconversion to positivity for islet cell antibodies, IA-2 autoantibodies, and at least one autoantibody (one or more of ICA, IAA, GAD, IA-2 and ZnT8 autoantibodies.) for those in the hydrolyzed formula exposure group. In their study, at least one autoantibody was developed in 17% and 30% of infants in the hydrolyzate and control groups, respectively. In the casein hydrolyzate group, 8/99 (8.1%), compared to 17/109 (15.6%) in the control group, tested positive for ≥ 2 antibodies. According to Vaarala et al., there was no apparent reduction in beta-cell autoimmunity among infants who received whey-based hydrolyzed formula. Beta cell autoimmunity was reduced in the FINDIA formula compared to other interventions. According to Knip et al., the autoantibody status based on the antibody type of both the casein hydrolyzate and the control formula was reported. Positivity for ≥ 1 autoantibody was reported in a previous study. This study shows that there is no significant difference between the study groups in the appearance of islet cell antibodies compared to the previous studies.

Table 5. *Secondary outcomes.*

Outcomes	Study	Hydrolyzed formula	Cow's milk formula	FINDIA formula	Hazard ratio of hydrolyzed formula (95% CI)
Development of ≥ 1 autoantibody	Knip et al.	17/99 (17%)	33/109 (30%)	N/A	0.54 (0.29-0.95)
	Vaarala et al.	11/187 (5.8%)	17/236 (7.2%)	4/229 (1%)	0.85 (0.41-1.76)
Development of ≥ 2 autoantibody	Knip et al.	8/99 (8%)	17/109 (15.5%)	N/A	0.52 (0.21-1.17)
Development of ICA	Knip et al.	394/1081 (36.5%)	373/1078 (34.8%)	N/A	Not reported
Development of IAA	Knip et al.	183/1081 (17%)	162/1078 (15.1%)	N/A	Not reported
Development of GADA+	Knip et al.	207/1081 (19.2%)	186/1078 (17.3%)	N/A	Not reported
Development of IA-2A	Knip et al.	115/1081 (10.7%)	102/1078 (9.5%)	N/A	Not reported

Discussion

Different authors studied cow's milk as a potential trigger for T1DM, with inconsistent results regarding the risk of developing T1DM and beta cell autoantibody formation. In this systematic review and meta-analysis, we found that hydrolyzed formula was not associated with a reduction in the incidence of T1DM, compared to a non-hydrolyzed formula in infants at risk of developing T1DM. This was also evident in the most recent and largest RCT-the TRIGR trial. There was no evidence of publication bias in our meta-analysis, and heterogeneity was low. In addition to the above outcomes, this study also attempted to look at exposure to different formulas and subsequent development of autoantibodies related to T1DM. Knip et al. demonstrated a reduction in beta-cell autoimmunity by the age of 10 years in their pilot study. In contrast, Vaarala et al. did not demonstrate this in their study. However, the hydrolyzed formula used in their study was whey-based, compared to the other 2 studies included in this review, which were casein-based. This may have some implications in the role of type of protein exposure and development of T1DM. Based on these results, there is no need to adjust dietary recommendations in infants at risk for T1DM.

Strength and limitations

This is the first systematic review and meta-analysis aiming to determine if hydrolyzed formula reduces the risk of developing T1DM in susceptible individuals. The strength of our systematic review is the inclusion of all recent trials. In addition, we used several methods to reduce bias (comprehensive literature search, duplicate data abstraction, specific search inclusion criteria, and specific criteria for analysis). Regarding enteral feeding, breast-feeding was encouraged in all 3 included studies, and the formula was given only when breast milk was unavailable.

Duration of breastfeeding was monitored, as well as exposure to formula between the groups. The hydrolyzed formula used in Vaarala et al. was notably whey-based, compared to Knip et al. and Knip et al. which were both casein-based. This review included term infants with risk factors for developing T1DM; hence the results may not apply to other populations, such as those without any risk factors for T1DM, or preterm infants.

Conclusion

Although the evidence is limited with three studies comparing hydrolyzed formula with non-hydrolyzed formula, the results of this meta-analysis suggest that changing to a hydrolyzed formula did not reduce the risk of developing T1DM compared to non-hydrolyzed formula among infants with genetic susceptibility to T1DM.

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Availability of Data and Materials

The authors declare that all data supporting the findings of this study are avalible from the corresponding author on reasonable request.

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Competing Interests

The authors declared that they have no competing interests.

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Helen Lee Robertson (HLR) contributed to the search strategy development and conducted parts of the literature search. Ms. Robertson received no financial compensation for this support. We declare no conflict of interest. Abdulaziz Abul (AA) performed the search strategies and quality assessments/data extraction and wrote the final paper. Abhay Lodha (AL) developed the protocol and contributed to writing the final paper. Amelie Stritzke (AL) contributed by writing and editing the final paper. Prashanth Murthy (PM) contributed by performing quality assessments/data extraction and contributed to writing the final paper. All authors approved the final version of the manuscript and submission.

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