

# Vitamin A Concentration in Umbilical Cord Blood of Infants from Three Separate Regions of the Province of Québec (Canada)

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## ABSTRACT

**Background:** Inuit women from Northern Québec have been shown to consume inadequate quantities of vitamin A. This study was conducted to evaluate the prevalence of blood vitamin A deficiency in newborns from 3 distinct populations of the province of Québec.

**Methods:** 594 newborns were included in this study (375 Inuit newborns from northern Québec (Nunavik), 107 Caucasian and Native newborns from the Lower Northern Shore of the Saint-Lawrence River (LNS) and 112 newborns from Southern Québec where clinical vitamin A deficiency is uncommon). Mothers were recruited at delivery and vitamin A (retinol) was analyzed from umbilical cord blood samples by reversed-phase high-pressure liquid chromatography.

**Results:** Nunavik and LNS newborns had significantly lower mean vitamin A concentrations in cord blood compared to Southern Québec participants (15.7 µg/dL, 16.8 µg/dL and 20.4 µg/dL respectively). The differences observed were similar when adjusted for sex and birthweight. Results also showed that 8.5% of Nunavik newborns and 12.2% of LNS newborns were below 10.0 µg/dL, a level thought to be indicative of blood vitamin A deficiency in neonates.

**Conclusion:** These data suggest that a carefully planned vitamin A supplementation program during pregnancy in Nunavik and LNS might be indicated to promote healthy infant development.

*La traduction du résumé se trouve à la fin de l'article.*

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Vitamin A influences the expression of many genes and is essential for normal growth. Its involvement in infant development, vision and cell differentiation is well known. Adequate maternal stores of vitamin A are essential during pregnancy to meet the needs of the fetus. Vitamin A deficiency during development and early childhood delays growth, impairs vision and increases the severity of infections, ultimately leading to many otherwise preventable deaths.<sup>1-4</sup>

Vitamin A deficiency among children is an important public health problem primarily in developing countries.<sup>4,5</sup> In Canada, Inuit infants are also at risk of vitamin A deficiency because Inuit women of childbearing age have been shown to consume inadequate quantities of vitamin A.<sup>6-8</sup> Canadian Inuit preschool children have been shown to have a high incidence of infections.<sup>9,10</sup> It is therefore important to determine if vitamin A deficiency, an important risk factor for infectious diseases, is prevalent in this population. This study attempts to document the prevalence of blood vitamin A deficiency in Inuit neonates. Comparison of vitamin A concentrations between studies has proven difficult because of variations in analytical methods. In this study, we compare the vitamin A concentrations in umbilical cord serum samples of Inuit neonates from the northern part of the province of Québec (Nunavik) with cord blood levels of two different populations from Québec, with all samples being analyzed in the same laboratory.

## METHODS

### Populations and recruitment

The participants in this study were recruited between 1993 and 1997 and came from three different populations in the province of Québec (Canada): 1) mothers and newborns from 14 small Inuit communities of Nunavik – a vast and remote area in northernmost Québec (Figure 1), 2) 15 villages in the Lower North Shore (LNS) region of the St-Lawrence River – remote and isolated from the rest of the province, and 3) women admitted for delivery in one of the 10 participating hospitals from 10 administrative regions of Québec, thought to be representative of the southern portion of the province where clinical vitamin A deficiency is uncommon.

The women were recruited originally for the evaluation of prenatal exposure to food-chain contaminants (heavy metals and organochlorines),<sup>11-13</sup> and a subset of each population were randomly selected and agreed to participate in the present study (Population 1: 419/491 (85.3%), Population 2: 108/467 (23.1%), Population 3: 112/1109 (10.1%)).

### Data collection and sample analysis

After signing the appropriate consent forms, all mothers answered a short questionnaire relating to their sociodemographic characteristics. Data on the newborns (weight, gestational age, APGAR, etc.) were gathered from the medical files. After delivery, 15 to 20 ml of cord blood were sampled by venipuncture, centrifuged and then frozen at  $-80^{\circ}\text{C}$ . The samples were then sent to the Institut National de Santé Publique du Québec (Québec City, Canada) for vitamin A (retinol) analysis. They were stored 1 to 3 months before being processed. Ethanol was added to the samples to denature proteins, and retinol was extracted from the solution with hexane. After concentration, retinol was redissolved in ethanol. Retinol concentration was then determined by reversed-phase high-pressure liquid chromatography using Waters pumps (model 600), a programmable diode array detector (model 996) and Waters uBondapak C18 columns with retinal acetate as the internal standard.<sup>14-16</sup> The laboratory technicians were "blinded" to the region of origin of the samples and the samples were processed in a random order. This project was approved by the ethics committee of the Laval University medical research centre.

### Statistical analysis

Arithmetic means  $\pm$  standard deviations are shown. We used Scheffé's test for multiple comparisons to compare unadjusted means among the 3 populations. Proportions were compared using the  $\chi^2$  statistic. We also performed multivariate regression to control for birthweight and sex; we controlled for birthweight rather than gestational age since birthweight was more closely associated with vitamin A concentrations in cord blood. The t-test was used in multivariate linear regression to compare adjusted means (with Scheffé's

**TABLE I**

### Characteristics of Participating Mothers and Their Infants According to Region of Residence

Characteristics	Nunavik (n = 375)	Lower North Shore (n = 107)	Southern Québec (n = 112)
Mothers			
Age (years)*	23.7 $\pm$ 5.3 <sup>a</sup>	24.4 $\pm$ 5.3 <sup>a</sup>	28.6 $\pm$ 4.5 <sup>b</sup>
Number of previous pregnancies*	3.7 $\pm$ 2.2 <sup>a</sup>	1.5 $\pm$ 1.6 <sup>b</sup>	1.2 $\pm$ 1.1 <sup>b</sup>
Ethnic origin <sup>f</sup>			
Caucasian (%)	0%	51.4%	95.5%
Inuit (%)	100%	0%	0%
Aboriginal other than Inuit (%)	0%	48.6%	0.9%
Other (%)	0%	0%	3.6%
Smoking during pregnancy (%) <sup>†</sup>	85.9% <sup>c</sup>	50.0% <sup>d</sup>	28.6% <sup>e</sup>
Infants			
Sex (% of males)	46.9% <sup>c</sup>	48.6% <sup>c</sup>	59.8% <sup>d</sup>
Birthweight (g)*	3491.3 $\pm$ 453.4	3537.6 $\pm$ 535.2	3439.9 $\pm$ 466.1
Gestational age (y)*	39.0 $\pm$ 1.4	39.5 $\pm$ 1.3	39.6 $\pm$ 1.3

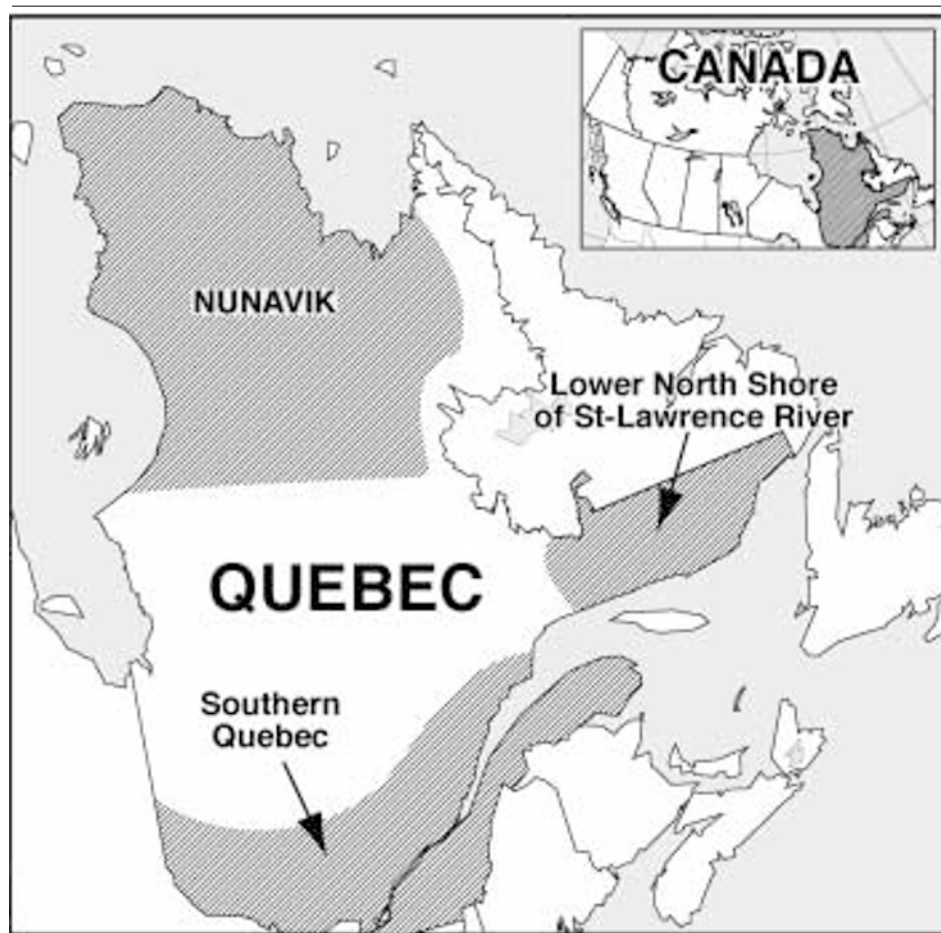
\* Means  $\pm$  standard deviations.

<sup>†</sup> Information on 59 subjects was missing for smoking during pregnancy.

<sup>a, b</sup> Means with different letters are significantly different (Scheffé's test for multiple comparisons).  $\alpha=0.05$

<sup>c, d, e</sup> Proportions with different letters are significantly different ( $\chi^2$ ).  $p<0.05$

<sup>f</sup> The ethnic distribution is significantly different in every region.  $p<0.05$



**Figure 1.** Location of Nunavik, Lower North Shore of the Saint-Lawrence River and Southern Québec.

adjustment for multiple comparisons when necessary). All statistical analyses were performed with the SAS software (SAS Institute, Cary, NC, USA). By convention,  $p<0.05$  was considered significant.

### RESULTS

A total of 639 women/infant pairs were recruited for the present study. Due to missing values (insufficient blood sample,

TABLE II

## Vitamin A Concentrations in Cord Blood According to Region of Residence

Region	Mean µg/dL	CI 95% µg/dL	5th percentile µg/dL	25th percentile µg/dL	Median µg/dL	75th percentile µg/dL	95th percentile µg/dL
Nunavik	15.7 ± 4.9 <sup>a</sup>	15.2 – 16.2	8.8	12.2	15.2	18.2	24.3
Lower North Shore	16.8 ± 5.6 <sup>a</sup>	15.8 – 17.9	7.7	12.9	17.2	20.6	25.1
Southern Québec	20.4 ± 5.8 <sup>b</sup>	19.3 – 21.5	11.4	16.2	19.5	24.4	29.8

CI 95% = 95% confidence interval of the mean.

<sup>a, b</sup> Means with different letters are significantly different (Scheffe's test from multiple comparisons).  $\alpha=0.05$ 

TABLE III

## Proportions of Infants in the Different Cord Serum Vitamin A Categories According to Region of Residence

Region	Proportion of the Population in Each Vitamin A Category					global $\chi^2$ p-values
	Less than 10.0 µg/dL	10.0 - 14.9 µg/dL	15.0 - 19.9 µg/dL	20.0 - 24.9 µg/dL	25.0 µg/dL and more	
Nunavik	8.5%	38.3%	35.5%	13.7%	4.0%	Each region is significantly different than the two others (p<0.01)
Lower North Shore	12.2%	26.2%	29.9%	26.2%	5.6%	
Southern Québec	2.7%	11.6%	41.1%	21.4%	23.2%	

TABLE IV

## Adjusted Means\* and Confidence Intervals of Vitamin A in Cord Serum According to Sex and Birthweight for Each Region of Residence

Determinants	Nunavik (µg/dL)			Lower North Shore (µg/dL)			Southern Québec (µg/dL)	
All infants	15.6	[15.1 – 16.1] <sup>a</sup>		16.7	[15.7 – 17.6] <sup>a</sup>		20.7	[19.8 – 21.7] <sup>b</sup>
Sex								
Male	14.5	[13.8 – 15.2]	p<0.01	16.3	[14.8 – 17.8]	p=0.32	19.6	[18.2 - 20.9]
Female	16.8	[16.1 – 17.4]		17.4	[15.9 – 18.8]		21.6	[20.0 - 23.3]
Birthweight								
Vitamin A level Increase for each 100 g increase in birthweight	3.06	[2.01 – 4.11]	p<0.01	2.91	[0.95 – 4.87]	p<0.01	2.99	[0.71 – 5.26]

\* Means are adjusted for birthweight and sex using multivariate linear regression.

<sup>a, b</sup> Means with different letters are significantly different (t-test on adjusted means with Scheffe's adjustment from multiple comparisons).  $\alpha=0.05$ 

sociodemographic questionnaire not completed), we excluded 45 participants (6.9%) from the statistical analysis. Table I shows the characteristics of the 594 mother/infant pairs included in the analysis according to their region of residence. Mothers from Nunavik were younger than their counterparts from Southern Québec. They also had more previous pregnancies than the mothers from LNS and Southern Québec. As expected, the ethnic origins were considerably different according to the region of residence. The Nunavik participants were exclusively Inuit while 51% of the mothers from LNS were Caucasians and 49% were Aboriginal. The Southern Québec region was comprised of mostly Caucasians with a few mothers with other ethnic origins. Women from LNS gave birth to slightly heavier babies but this difference was not significant. Maternal smoking during pregnancy was much more prevalent in Nunavik (85.9%) than in the LNS (50.0%) and in Southern Québec (28.6%). Maternal smoking, maternal age and the number of previous pregnancies did not significantly affect the

vitamin A levels in either region (data not shown) and were thus excluded from the multivariate analyses.

The concentrations of vitamin A in cord serum were normally distributed in each region with a slightly lower standard deviation in Nunavik. Table II presents the mean vitamin A concentrations and the quartiles boundaries for each region. The mean concentration of vitamin A in Nunavik was 15.7 ± 4.9 µg/dL compared to 16.8 ± 5.6 µg/dL in LNS and 20.4 ± 5.8 µg/dL in Southern Québec. The differences were significant between Nunavik and Southern Québec and between LNS and Southern Québec. In LNS, the newborns with Aboriginal origins had a slightly higher mean vitamin A concentration than the Caucasians newborns (17.5 µg/dL vs. 16.2 µg/dL), but the difference was not significant (p=0.21). Data from newborns of both ethnic origins in LNS were therefore merged in the remaining analyses.

In Nunavik, 47.5% of the samples were below 15.0 µg/dL (Table III). This proportion was higher than that observed in LNS (38.3%, p=0.06) and in Southern

Québec (14.3%, p<0.0001). As much as 8.5% of the samples in Nunavik fell below 10.0 µg/dL (Table III). This proportion was slightly lower as compared to that of LNS (12.2%, p=0.46) but was significantly higher than that in Southern Québec (2.7%, p=0.02).

Table IV shows the mean concentrations of vitamin A among the regions adjusted for sex and birthweight using multivariate regression. The mean vitamin A concentration in Nunavik adjusted for sex and birthweight was significantly lower than the mean concentration in Southern Québec (15.6 µg/dL vs. 20.7 µg/dL, p<0.0001). The adjusted difference was higher than the unadjusted difference (5.1 µg/dL vs. 4.7 µg/dL) principally because of the confounding effect of sex. The adjusted mean concentration in Nunavik was slightly lower than in LNS (15.6 µg/dL vs. 16.7 µg/dL) but the difference was not significant (p=0.15). Table IV also shows the independent effects of sex and birthweight on vitamin A in cord serum. Females had higher concentrations of vitamin A than males, especially in Nunavik. Birthweight was also

positively associated with vitamin A in all three groups.

## DISCUSSION

In this study, we found that newborns from Nunavik and the Lower North Shore of the St-Lawrence River had lower neonatal concentrations of vitamin A than their counterparts from regions of Southern Québec. Mean concentrations of vitamin A in cord blood vary widely among studies and populations. Other studies have reported similar concentrations,<sup>17-19</sup> lower concentrations<sup>20</sup> and higher concentrations<sup>21-24</sup> in cord blood of healthy term newborns as compared to those presented here. Many factors can affect the comparability among studies. In this study, to increase the comparability among the different regions, the same laboratory processed all the samples and used the same analytical method. We also controlled for sex and birthweight in the statistical analysis since both of these factors were associated with vitamin A concentration at birth.

Lifestyle and nutrition are likely to be responsible for the lower vitamin A concentrations seen in Nunavik. Blanchet et al. investigated the diet of Inuit women from Nunavik and found that their daily intake of vitamin A fell below the recommended intake level.<sup>7</sup> Vitamin-A rich traditional food items such as ringed seal liver were consumed infrequently and contributed little to the vitamin A intake over the entire year.<sup>7,8,25</sup> Fruits and vegetables are less available and often more expensive in Nunavik communities as compared to Southern Québec and are therefore consumed less frequently in northern regions. Inuit women from other regions of northern Canada have also been shown to have a vitamin A-deficient diet.<sup>6,8</sup> Newborns from LNS had similar cord blood vitamin A concentrations to the Nunavik newborns. As in Nunavik, inhabitants from LNS are isolated, live in harsh climatic conditions and reside in small coastal communities. Because of this, as well as other cultural and traditional reasons, their main source of food comes from the sea. Fruits, vegetables and fortified milk contribute less to their daily diet than these country food items.

The region of Nunavik is inhabited almost exclusively by Inuit while the

Southern Québec participants were mainly of Caucasian descent. One could speculate that race and genetic factors could be responsible for the differences observed here. In 1996, Godel et al. observed lower concentrations of vitamin A in cord plasma among Inuit newborns as compared to Caucasian newborns in a northern Canadian population.<sup>21</sup> The concentrations observed were higher than the ones in this study but the difference between Inuits and Caucasians was similar. Based on a food frequency questionnaire, the authors concluded that the difference was most likely due to environmental and nutritional factors rather than ethnic origin itself. Looker et al. studied the differences in vitamin A status between Mexican-Americans, non-Hispanic blacks and whites in the U.S.<sup>26</sup> They too concluded that nutrition and lifestyle factors explained most of the differences they observed. We believe that in the present study, the differences in vitamin A concentrations observed among the regions are due mostly to nutritional characteristics rather than race.

Data on the supplementation of vitamin A during pregnancy in these three populations are scarce. Multivitamin tablets containing vitamin A are often recommended during pregnancy but the extent of the implementation of this measure among these populations is not known. Knowing, however, that compliance is often problematic in Nunavik, it is likely that part of the difference in the vitamin A levels observed in this study was due to some variation in multivitamin supplement intake among the participating populations.

There is no clear consensus on the "cut-off concentration" for vitamin A deficiency in cord blood. Godel et al. suggested that the cutoff for vitamin A deficiency should be the same for both adults and newborns.<sup>21</sup> Our results from Southern Québec, where malnutrition and vitamin A clinical deficiency are rare, suggest that vitamin A concentrations among healthy babies can be well below the normal adult standard of 20 µg/dL. Several studies have shown that cord plasma vitamin A concentrations in non-deficient populations averaged about 50% of values in maternal plasma.<sup>18,19,27-29</sup> Linblad et al. also observed a clear relationship between age

and vitamin A concentration in the first two years of life and suggested a cut-off concentration for deficiency of 10 µg/dL for one-month-old infants.<sup>24</sup> These studies and our results suggest that a cut-off value of 10 µg/dL in cord blood is appropriate for the detection of vitamin A deficiency at birth. Therefore, according to this value, as many as 8.5% of the Nunavik infants and 12.2% of the LNS newborns included in our study were born with deficient concentrations of circulating vitamin A.

This study shows that the prevalence of blood vitamin A deficiency is high in Nunavik and LNS and could affect as many as one in ten newborns. Since vitamin A deficiency is associated with important health problems, studies addressing clinical deficiency should be initiated to determine whether a supplementation program during pregnancy should be considered by public health officials.

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## RÉSUMÉ

**Contexte :** Il a été démontré que les femmes inuites du Nord du Québec consomment des quantités insuffisantes de vitamine A. Notre étude a été entreprise pour évaluer la prévalence de la carence en vitamine A chez les nouveau-nés de trois populations distinctes de la province de Québec.

**Méthodes :** L'étude a porté sur 594 nouveau-nés (375 nouveau-nés inuits du Nord du Québec [Nunavik], 107 nouveau-nés caucasiens et autochtones de la Basse-Côte-Nord du Saint-Laurent [BCN] et 112 nouveau-nés du Sud du Québec où la carence en vitamine A est peu fréquente). Les mères ont été recrutées lors de l'accouchement, et la vitamine A (rétinol) a été dosée dans le sang du cordon ombilical par chromatographie liquide à haute pression en phase inversée.

**Résultats :** Les nouveau-nés du Nunavik et de la BCN avaient une concentration moyenne en vitamine A significativement plus basse que ceux du Sud du Québec (15,7 µg/dL, 16,8 µg/dL et 20,4 µg/dL respectivement). Les différences observées étaient semblables après ajustement selon le sexe de l'enfant et le poids à la naissance. Les résultats ont aussi montré que 8,5 % de nouveau-nés du Nunavik et 12,2 % des nouveau-nés de la BCN avaient une concentration inférieure à 10,0 µg/dL, un niveau que l'on croit être indicatif d'une carence néonatale en vitamine A.

**Conclusion :** Ces données suggèrent qu'un programme de supplémentation bien planifié en vitamine A visant les femmes enceintes du Nunavik et de la BCN pourrait être nécessaire.