



Number of colony-forming unit-Hill colonies among children and teenagers with obesity, dyslipidemia and breastfeeding history

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Abstract *Background and aims:* The number of colony-forming unit (CFU)-Hill colonies has been proposed as a biomarker of vascular function and cardiovascular risk in adults but information about its role in children is scarce. This study evaluates the associations between obesity, cardiovascular risk factors and breastfeeding history with the numbers of CFU-Hill colonies in a sample of young people.

Methods and results: We selected 49 children and teenagers between ages 10 and 17 (65.3% boys) from Mexican Health Care system. Physical activity and Anthropometric measures data were registered. CFU-Hill colonies were cultured from mononuclear cells obtained from venous blood. We detected inverse associations between the formation of CFU-Hill colonies and body mass index (BMI; $\beta = -1.53$; 95% confidence interval [CI], $-1.92, -1.13$), triglycerides ($\beta = -0.26$; 95% CI = $-0.34, -0.18$), total cholesterol ($\beta = -0.13$; 95%CI = $-0.17, -0.08$), Low Density Lipoprotein (LDL) ($\beta = -0.20$; 95%CI = $-0.31, -0.09$) and glucose ($\beta = -0.37$; 95%CI = $-0.55, -0.18$) using multivariate models. Breastfeeding duration showed a 1.46-colony increase for each month of breastfeeding (95%CI = $0.73, 2.18$).

Conclusions: CFU-Hill colony-forming capacity in children and teenagers was inversely associated with obesity, dyslipidemia and high blood levels of glucose. In contrast a longer breastfeeding duration was directly associated with an increased number of CFU-Hill colonies. However these results must be confirmed with further studies. Our findings support the importance of promoting breastfeeding and monitoring nutritional and metabolic status at an early age to prevent chronic disease development.

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Introduction

Overweight and obesity during childhood have been associated with cardiovascular (CV) diseases development

at a young age. It has been suggested that childhood obesity is associated with early endothelial and vascular dysfunction, which can be considered as an initial state of atherosclerosis development [1,2]. Additionally associations between breastfeeding and adiposity childhood and CV function have been reported [3,4].

Evidence indicates that vascular integrity is partially preserved by circulating endothelial progenitor cells (EPCs) which promote angiogenesis and vasculogenesis that repair endothelial damage and form new vascular tissue

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[5]. Several assays to assess the capacity of circulating putative EPCs to proliferate and form colonies *in vitro* in adult subjects have reported that the number of colony-forming units (CFUs) of these cells is associated with CV risk factors [6,7]. Particularly it has been proposed that the number of CFUs formed *in vitro* by the Hill assay (CFU-Hill) can be used as a biomarker of vascular function and CV risk in adults. This is supported by the fact that there is a strong correlation between the number of CFU-Hill colonies and Framingham risk score and vascular function in healthy adults [6].

The number of CFUs is reduced in adults with overweight, obesity and hyperleptinemia [8,9]. In addition data support a decrease CFUs in obese adults that is correlated to elevated levels of triglycerides and low levels of high-density lipoprotein (HDL) [10].

Recently CFU number was reported to be lower in children with familial hypercholesterolemia compared to healthy children. An association between the number of CFUs and endothelial function in healthy subjects (including children) was also shown in this study [11]. However the relationship between CFU-Hill number as an indicator of vascular function and other CV risk factors at an early age has not yet been determined.

The aim of this study was to evaluate the association between CV risk factors and factors related to breastfeeding history with the number of CFU-Hill colonies formed *in vitro*.

Methods

Study population

We conducted a cross-sectional study in users of an outpatient clinic from Mexican Social Security Institute (IMSS) between May 2012 and July 2013. We selected 49 children and teenagers of both sexes aged between 10 and 17 years old by simple random sampling. All participants' parents signed a written consent form and all subjects were provided with an assent form. Children and teenagers suffering acute illness during the last 4 weeks and those with chronic diseases were excluded from the study. Obese children under medical supervision were also excluded. The protocol was approved by The Ethics and Research Committee from IMSS and The National Institute of Public Health as well.

Co-variables

Anthropometric data were registered by measuring height and weight by standard procedures [12] and body mass index (BMI) was calculated according to tables from the Center for Disease Control (CDC) adjusted to age and sex [13]. Previously standardized questionnaires for children in Mexican population were used to assess physical activity [14]. Metabolic equivalents (METs) were used to calculate energy expenditure. As cutoff points a value of 3–5 METs/hour/day was considered moderate physical activity and a value of more than 5 METs/hour/day was

considered vigorous activity. Gynecological and obstetric histories of the participant's mothers including pregnancies number, delivery type, birth weight, feeding type and breastfeeding duration were registered.

A venous blood sample was obtained after a 12-h fasting to measure glucose and lipids (total cholesterol, triglycerides, HDL-cholesterol and low-density lipoprotein [LDL]-cholesterol) using commercial kits and for performing the CFU-Hill assay described in [Appendix A](#).

Determining the number of CFU-Hill colonies

An EndoCult Liquid Medium Kit (StemCell Technologies, Vancouver, British Columbia, Canada) was used for cultivation and quantification of CFU-Hill colonies following the manufacturer's instructions ([Appendix A](#)).

Statistical analysis

We used a nonparametric Kruskal–Wallis ranked analysis of variance (ANOVA) with multiple comparisons and the results are shown as median values and 25th and 75th percentiles. We used Pearson correlation coefficients to assess the correlation between continuous variables. To explore the relationship between the number of CFU-Hill colonies and risk factors we used multiple linear regression models adjusted for potential confounders such as age, sex, height and physical activity. Confidence intervals at 95% were used for statistical significance conclusion. All information was analyzed in Stata 13.1.¹

Results

We studied 49 patients aged 10–17 years, with a median age of 13 years, weight of 61.4 kg and height of 154 cm. The biochemical measurements of the participants showed abnormal values in lipid profiles which suggest the presence of atherogenic risk in this sample ([Table 1](#)). Lower medians of CFU-Hill colony numbers were found in children with overweight and obesity than in normal BMI children (6 vs. 16; $p < 0.01$ for boys; 3 vs. 18.5; $p = 0.01$ for girls) ([Table 2](#)). Physical activity showed no difference between intensity categories ($p = 0.36$). Participants with a history of positive breastfeeding showed a higher number of CFU-Hill colonies than those who were not breastfed (11 vs. 2; $p = 0.02$). Participants with high cholesterol and triglycerides showed lower numbers of CFU-Hill colonies compared to those with normal values (5 vs. 17; $p < 0.01$ in both cases) ([Fig. 1](#)). The number of CFU-Hill colonies cultured *in vitro* was negatively correlated with current weight, glucose, triglycerides, total cholesterol and LDL. Birth weight, breastfeeding duration and HDL were positively correlated ([Table 3](#)).

¹ StataCorp. 4905 Lakeway Drive College Station, Texas 77845 USA 800-STATA-PC. <http://www.stata.com>.

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