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Prenatal Maternal Physical Activity and Stem Cells in Umbilical Cord Blood

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Abstract

Purpose—Early life processes, through influence on fetal stem cells, affect postnatal and adult health outcomes. This study examines effects of physical activity before and during pregnancy on stem cell counts in umbilical cord blood.

Methods—We isolated mononuclear cells from umbilical cord blood samples from 373 singleton full-term pregnancies and quantified hematopoietic (CD34⁺, CD34⁺CD38⁺, CD34⁺c-kit⁺), endothelial (CD34⁺CD133⁺, CD34⁺CD133⁺VEGFR2⁺, CD34⁺VEGFR2⁺, and CD133⁺VEGFR2⁺), and putative breast (EpCAM⁺, EpCAM⁺CD49f⁺, EpCAM⁺CD49f⁺CD117⁺, CD49f⁺CD24⁺, CD24⁺CD29⁺, and CD24⁺CD29⁺CD49f⁺) stem/progenitor cell subpopulations by flow cytometry. Information on physical activities before and during pregnancy was obtained from questionnaire. Weekly energy expenditure was estimated based on the metabolic equivalent task (MET) values.

Results—Pre-pregnancy vigorous exercise was associated positively with levels of the endothelial CD34⁺CD133⁺, CD34⁺CD133⁺VEGFR2⁺, CD34⁺VEGFR2⁺, and CD133⁺VEGFR2⁺ progenitor cell populations (p=0.02, 0.01, 0.001, and 0.003, respectively); the positive associations were observed in samples from the first births and those from the second or later births. Pre-pregnancy moderate and light exercise and light exercise during the first trimester were not

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significantly associated with any stem/progenitor cell population. Light exercise during the second trimester was positively associated with CD34⁺VEGFR2⁺ endothelial progenitor cells ($p=0.03$). In addition, levels of the EpCAM⁺CD49f⁺ and CD49f⁺CD24⁺ breast stem cells were significantly lower among pregnant women who engaged in vigorous or moderate exercise during pregnancy ($p=0.05$ and 0.02 , respectively).

Conclusion—Vigorous exercise before pregnancy increases endothelial progenitor cell numbers in umbilical cord blood and thus could potentially enhance the endothelial function and improve cardiovascular fitness in the offspring. Findings of a lower level of putative breast stem cell sub-populations could have implication on exercise and breast cancer prevention. Prenatal effects of exercise on fetal stem cells warrant further studies.

Keywords

breast stem cells; cardiovascular fitness; early-life determinants; endothelial function; pregnancy; prenatal exposure

Introduction

Provided that pregnancy is normal and healthy, the current American College of Obstetrics and Gynecology (ACOG) guidelines promote continuation of pre-pregnancy exercise activities and recommend that sedentary women start exercising during pregnancy if they are free of medical complications (2). In guidelines on physical activity issued in 2008 by the U.S. Department of Health and Human Services, a reference was also made that healthy pregnant women should begin or continue at least 150 minutes of moderate-intensity physical activity a week (33).

These guidelines are supported by substantial evidence showing that physical activities during pregnancy are safe and beneficial to both the mother and the child (25). Being physically active before becoming pregnant and continuing to exercise during pregnancy may help minimize the risk of developing gestational diabetes mellitus, hypertensive disorders including preeclampsia, and abnormal fetal growth (25). Other benefits include a shorter labor and decreased incidence of cesarean section. Maternal physical activity may help limit gestational weight gain to the recommended targets and reduce the child's risk of excessive and restricted growth, an effect that may confer adaptive benefits for the child later in life (11).

It is known that exercise can cause several hematologic changes, including levels of stem cell populations. Endothelial progenitor cells (EPC) increase in healthy young men cycling four hours continuously (23), in patients with peripheral arterial disease under supervised exercise training (32), in sixth-grade students receiving daily school exercise lesson (35), as well as in mice on running wheel (21). Exercise also affects the level of hematopoietic progenitor cells in amateur runners training for an average of 93.8 km per week (6) and neural stem cell populations in mice exercised voluntarily with 21 days of running (5). However, potential effect of prenatal maternal exercise on levels of fetal stem cells has not been reported. The aim of this study is to assess whether exercise before and during pregnancy are associated with stem cell concentrations in umbilical cord blood samples.

Methods

Study subjects

The study protocol was approved by the Institutional Review Boards of the University of Massachusetts Medical School, Worcester, MA and Tufts Medical Center, Boston MA. Study subjects were recruited from October 2004 to April 2012 among pregnant woman who delivered at the Tufts Medical Center. Subjects were recruited in two cycles. Study cycle 1 included those recruited from October 2004 to April 2006, and study cycle 2 from November 2006 to April 2012. Written informed consent was obtained from all subjects. Subjects were >18 years old with a singleton, full term pregnancy, negative for human immunodeficiency, hepatitis B and C viruses, with a fetus free of structural anomalies by ultrasound examination and no complication of preeclampsia. Infants were delivered according to standard obstetric practices. After birth, labor and delivery data were abstracted from medical records.

Umbilical cord blood samples

Umbilical cord blood was collected at delivery from the umbilical vein into a sterile bag containing 35 ml of citrate phosphate dextrose anticoagulant (Fenwal, Lake Zurich, IL). Samples were processed for mononuclear cells (MNC) using a Ficoll-Paque (STEMCELL Technologies, Vancouver, Canada) density gradient within 24 hour of birth as described previously (28).

Physical activities

Women consenting to participate in the study were asked during their stay in the labor and delivery wards to provide information on their age, height, pre-pregnancy weight, maximum weight gain after pregnancy, race, education, number of prior pregnancies and live births, and physical activities. Information on physical activities was sought in a semi-quantitative questionnaire, modeled after the International Physical Activity Questionnaire (8) for adaptation to pregnant women, seeking exercise information separately for the 12 months before pregnancy, during first trimester, and during second trimester. For pre-pregnancy period, the respondents reported on the time spent participating in five groups of physical activities: vigorous ("strenuous") exercises (e.g., swimming laps, aerobics, calisthenics, running, jogging, basketball, cycling on hills), moderate exercises (e.g., brisk walking, golf, volley ball, cycling on level streets, recreational tennis), casual walking, housework, and standing or walking at work. Casual walking, doing housework, and standing or walking at work, were classified as light exercise. Time spent on sitting and sleeping was also recorded. For vigorous and moderate exercise during pregnancy, covering both the first and second trimester, only a binary response (yes or no) was sought from the participants, without specifying the time spent on the activities. For light exercise during pregnancy, the time spent on activities (casual walking, doing housework, and standing or walking at work) was recorded separately for the first and the second trimester. To calculate weekly energy expenditure from the physical activities, the amount of time spent in each of these five groups of activities was multiplied by its corresponding metabolic equivalent task (MET) values (which reflects the intensity levels of that group of activities), and the resulting score expressed as MET-hours per week was summed over the five groups. Each group of the

activities was assigned a MET value based on the widely used compendium of physical activities (1): vigorous exercise (7 METs), moderate exercise (4 METs), casual walk (3.5 METs), housework (3 METs) and standing or walking at work (3 METs).

Flow cytometry

Flow cytometric analyses were carried out as described previously (28). Briefly, 1×10^6 umbilical cord blood-derived MNC were incubated for 30 min on ice in the dark with the following fluorochrome-conjugated antibodies: anti-CD34-fluorescein isothiocyanate (FITC) (Clone 581; BD BioSciences Pharmingen, San Diego, CA), anti-CD38 phycoerythrin (PE) (Clone HIT2; BD BioSciences Pharmingen), anti-CD133 allophycocyanin (APC) (Clone AC133; Miltenyi Biotechnology, Auburn, CA), anti-VEGFR-2 PE (Clone 89106; Research & Diagnostics systems, Inc, Minneapolis, MN), anti-EpCAM FITC (Clone VU-1D9; STEMCELL Technologies, Vancouver, Canada), anti-CD49f PE (Clone GoH3; BD BioSciences Pharmingen), anti-CD117 APC (Clone YB5.B8; BD BioSciences Pharmingen), anti-CD24 FITC (Clone SN3; Antibodies-online, Aachen, Germany), anti-CD29 APC (Clone MAR4; BD BioSciences Pharmingen), or the combination of these antibodies. Samples with vehicle in place of primary antibody served as negative controls. Cells were washed, fixed with 4% paraformaldehyde, and analyzed using a FACSCalibur flow cytometer (BD Biosciences Immunocytometry Systems, San Jose, CA). Hematopoietic ($CD34^+$, $CD34^+CD38^-$, and $CD34^+c-kit^+$) (31), endothelial ($CD34^+CD133^+$, $CD34^+CD133^+VEGFR2^+$, $CD34^+VEGFR2^+$, and $CD133^+VEGFR2^+$) (3, 13, 22), and putative breast ($EpCAM^+$, $EpCAM^+CD49f^+$, $EpCAM^+CD49f^+CD117^+$, $CD49f^+CD24^+$, $CD24^+CD29^+$, and $CD24^+CD29^+CD49f^+$) (28) stem/progenitor cell subpopulations were quantified from the gated MNC populations using the FlowJo software program (Tree Star, Ashland, OR). The number of cells was normalized to 10^3 MNC.

Statistics

Statistical analysis was conducted using STATA (version 12.0, STATA Corporation, College Station, TX). We applied multiple regression analysis to examine the association between natural log-transformed concentration of each stem cell measurement (dependent variable) and energy expended on different intensities of physical activity (independent continuous variable, referred as MET-hours/week). We categorized pre-pregnancy exercises into two groups depending on the intensity: vigorous and non-vigorous exercise (as the sum of moderate and light exercise). For the period of pregnancy, we analyzed the effect of vigorous/moderate exercise (yes/no) during pregnancy and light exercise (MET-hour/week) in the first and second trimester. We adjusted for potential confounding factors from maternal and neonatal variables, including maternal age, maternal pre-pregnancy weight and height, parents' ethnicity, number of previous live births, gestational age, time of delivery (day or night), newborn birth weight, gender, and study cycle (1 or 2). Maternal age, maternal pre-pregnancy weight and height, number of previous live births, and birth weight were treated as continuous variables. The fitted coefficients from the regression analysis were exponentiated to obtain the estimated proportional change in outcome associated with each independent variable. Additional analyses were conducted separately for samples from participants delivering the first birth and those delivering the second or later birth to

examine potential effect modification by the life-style and physiological differences between nulliparous and parous women.

Results

A total of 373 pregnant women, including 199 from study cycle 1 and 174 from study cycle 2, were recruited during the study periods. Table 1 describes the maternal and newborn characteristics of the study samples.

Total weekly energy expended on vigorous, moderate, and light exercise before pregnancy, and light exercise during the first and second trimester were calculated separately. For vigorous and moderate exercise during pregnancy, only a binary response is available (yes or no). Information on different measures of physical activities is summarized in Table 1. We also quantified the percentages of the different stem cell populations in umbilical cord blood-derived MNC based on specific stem and progenitor cell markers; the summary statistics (n, mean, standard deviation, and range) of each stem cell population assayed are presented in Table 1.

To investigate whether mother's exercise before pregnancy affects the number of different stem cells in umbilical cord blood, we conducted multiple linear regression analysis taking into account potential confounding factors related to maternal and neonatal characteristics, such as maternal age, maternal height, maternal pre-pregnant weight, parental race, parity, gestation duration, newborn's gender, newborn's birth weight, time of delivery time, and study cycle. The amount of pre-pregnancy vigorous exercise was found to have positive and significant relations with levels of CD34⁺CD133⁺, CD34⁺CD133⁺VEGFR2⁺, CD34⁺VEGFR2⁺, and CD133⁺VEGFR2⁺ EPC ($p=0.02$, 0.01 , 0.001 , and 0.003 , respectively; Table 2). No significant associations were observed between the amount of pre-pregnancy vigorous exercise and levels of hematopoietic stem cell populations. The positive associations between pre-pregnancy vigorous exercise and EPC were observed in samples from the first births and those from the second or later births (Table 2). We found no significant associations between MET from pre-pregnancy non-vigorous (moderate and light) exercise and umbilical cord blood stem/progenitor cells among all samples (Table 3).

Associations between exercise during pregnancy and stem cell populations were similarly examined (Tables 4-6). Light exercise during the first trimester was not significantly associated with any stem/progenitor cell population. Endothelial CD34⁺VEGFR2⁺ progenitor cells had a positive relation with the amount of light exercise during the second trimester ($p=0.03$; Table 5). In addition, levels of EpCAM⁺CD49f⁺ and CD49f⁺CD24⁺ breast stem cell subpopulations were significantly lower among pregnant women who engaged in vigorous or moderate exercise during pregnancy in comparison with those who did not ($p=0.05$ and 0.02 , respectively; Table 6).

Discussion

To our knowledge, this is the first report that assessed the effect of exercise before and during pregnancy on stem cell counts in the umbilical cord blood samples. Our results showed that the amount of vigorous exercise before pregnancy has a strong positive

association with levels EPC and vigorous/moderate exercise during pregnancy had a negative association with levels of putative breast stem cells.

Circulating EPC have been used as biological markers for vascular function and risk of cardiovascular disease (17). We, therefore, measured four previously reported populations of EPC (CD34⁺CD133⁺, CD34⁺CD133⁺VEGFR2⁺, CD34⁺VEGFR2⁺, and CD133⁺VEGFR2⁺) (3, 13, 22), and pre-pregnancy vigorous exercise increased the levels of all four populations of EPC in the umbilical cord blood samples. Consistent with our results, it has been reported that regular physical exercise increases the number of EPC in the peripheral blood of both healthy adults (23, 34) and children (35). While the mechanism for this change is not clear, it has been shown that the increased nitric oxide (NO) production and the release of vasoactive substances prostacyclin (PGI₂) and endothelin-1 (ET-1) and cytokine vascular endothelial growth factor (VEGF) contribute to this effect (9).

It is of interest to note that vigorous, but not moderate or light (non-vigorous), exercise before pregnancy was found to have a strong positive relationship with the number of EPC in the umbilical cord blood. In after-exercise comparison between those with and without hypoxic training, only the hypoxic regimen increased the number of CD34⁺KDR/VEGFR2⁺CD117⁺ EPC (36). Similarly, the number of circulating EPC has been reported to increase after intensive or 30 min of moderate exercise, but not after 10 min of moderate exercise (20). A hypoxic situation caused by high-intensity exercise, therefore, plays an important role in inducing the proliferation of EPC, possibly via the up-regulation of the angiogenic factors VEGF, stromal cell-derived factor-1 (SDF-1), and matrix metalloproteinases (MMPs) (18, 30, 38). Alternatively, a questionnaire study tends to measure vigorous activity better than moderate or light activity, and the differences in measurement error associated with different exercise intensities could be the reason for the different findings.

The number of circulating EPC is influenced by several clinical conditions. In adult peripheral blood, they are increased with significant coronary artery disease, type-2 diabetes mellitus, and bacterial pneumonia (10, 16, 37). In umbilical cord blood, levels of EPC are decreased in pregnancies complicated with preeclampsia (24, 29) and in premature newborns with bronchopulmonary dysplasia (BPD) (4). Interestingly, exercise during pregnancy has been reported to prevent most of the pathological features of preeclampsia, likely from the effect of reduced levels of circulating soluble fms-like tyrosine kinase 1 (sFlt1), as observed in a hypertension mouse model (14), and offspring of exercise-trained swine were found to have a higher endothelial function than that of sedentary swine (26). Based on our results that maternal physical activities before pregnancy increased the number of EPC in umbilical cord blood, it is thus possible that vigorous exercise before pregnancy, by inducing the proliferation of EPC and enhancing endothelial function, will not only prevent pregnancy-related complications like preeclampsia in the mothers but also prematurity-related diseases (e.g., BPD) in the newborns by improving their cardiovascular fitness.

It is not clear why vigorous exercise before pregnancy, but not during pregnancy, had strong effects on the levels of EPC. One of the study limitations is that our questionnaire had asked

for fewer details on exercises during pregnancy. Anticipating that a pregnant woman would be less likely to continue vigorous exercise during pregnancy, our questionnaire asked only whether a woman had participated in vigorous or moderate exercise during pregnancy, without separating the two. The effect estimate observed for the vigorous/moderate exercise during pregnancy could thus be estimating largely the effect associated with moderate exercise, with a smaller effect on the stem cell quantities. Since about half (46.4%) of those who engaged in pre-pregnancy vigorous exercise also participated in moderate or vigorous exercise during pregnancy among our participants, future studies on cord blood stem cells should seek more detailed information on physical activities during pregnancy.

Light exercise during the second trimester also induced an increase of all four EPC sub-populations and significantly so for CD34⁺VEGFR2⁺. This effect was not observed for the light exercise during the first trimester. Leisure time physical activity, similar to the pattern for vigorous activity, tends to decrease in intensity and duration from the beginning toward the end of pregnancy (27). Whether light exercise in later (versus early) stages of gestation might be more effective in increasing cord blood levels of EPC requires further studies.

Our data showed that vigorous/moderate exercise during pregnancy were associated with lower levels of putative EpCAM⁺CD49f⁺ and CD24⁺CD29⁺breast stem cells in umbilical cord blood. This finding is of interest in relation to the hypothesis that susceptibility to breast cancer can be influenced prenatally and that stem cells are potential targets of this influence (15, 28, 29). Higher levels of physical activity during the adolescent period or adulthood have been linked to lower risk of breast cancer (12, 19). While there is a lack of evidence relating maternal exercise to breast cancer risk in the offspring, experimental studies have shown that maternal exercise during pregnancy reduces tumorigenesis in rat offspring (7). Our present findings on breast stem cells are thus intriguing and further investigations on prenatal effects of exercise on fetal stem cells are warranted.

Stratifying the analysis by the first or later births, the associations between exercise variables and EPC remained largely consistent with that from all samples together. For hematopoietic and breast stem cells, exercise variables had varied, mostly non-significant effect estimates in the stratified analysis. While difference in life-style and physiology between nulliparous and parous women could contribute to these variations, with the small number of samples in the current analysis, findings from stratified analysis remain to be confirmed.

Conclusions

Vigorous exercise before pregnancy increases endothelial progenitor cell numbers in the cord blood. Pre-pregnancy physical activity thus could potentially enhance the endothelial function and improve cardiovascular fitness in the offspring. Findings of a lower level of putative breast stem cell sub-populations could have implication on exercise and breast cancer prevention. Prenatal effects of exercise on fetal stem cells warrant further studies.

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Table 1
Summary of the maternal and newborn characteristics, exercise variables, and stem cell measurements

	N	Mean $\pm$ SD or %	Range
Maternal and newborn characteristics			
Maternal age (years)	371	30.3 $\pm$ 5.9	18 - 44
Mother's height (cm)	367	164.0 $\pm$ 6.8	144.5 - 188.0
Mother's pre-pregnancy weight (kg)	363	63.7 $\pm$ 13.7	40.8 - 120.2
Parity			
First	165	46.1	
Second	107	29.9	
Third	54	15.1	
Fourth and over	32	8.9	
Unknown	15		
Gestational age (weeks)	330	39.6 $\pm$ 1.1	37 - 41.7
Newborn gender			
Male	177	50.1	
Female	176	49.9	
Race of parents			
Both Caucasian	209	57.1	
Both African-American	43	11.8	
Both Asian	52	14.2	
Both Hispanic	30	8.2	
Other	32	8.7	
Unknown	7		
Newborn birth weight (g)	370	3,393.0 $\pm$ 442.8	1973 - 4701
Umbilical cord blood volume (ml) ^a	372	95.7 $\pm$ 26.2	44.5 - 216
Exercise measures			
Pre-pregnancy vigorous exercise (MET-hr/week)	364	11.5 $\pm$ 19.2	0 - 84
Pre-pregnancy moderate exercise (MET-hr/week)	363	12.4 $\pm$ 14.4	0 - 48
Daily activity ^c before pregnancy (MET-hr/week)	371	105.6 $\pm$ 79.2	3.3 - 494
Moderate exercise + Daily activity ^c before pregnancy (MET-hr/week)	360	118.2 $\pm$ 83.8	3.8 - 514
Vigorous or moderate exercise during pregnancy			
Yes	120	32.2	
No	248	66.5	
Daily activity ^c during 1 st trimester (MET-hr/week)	367	101.1 $\pm$ 78.1	1.5 - 529.8
Daily activity ^c during 2 nd trimester (MET-hr/week)	363	92.6 $\pm$ 76.6	1.5 - 529.8
Umbilical cord blood cell populations			
Initial total nucleated cells (TNC) ^a	371	15.74 $\pm$ 4.86	6 - 34.32
Initial total mononuclear cells (MNC) ^a	371	7.35 $\pm$ 2.65	2.06 - 20.88
Hematopoietic stem cells			

	N	Mean $\pm$ SD or %	Range
CD34 ⁺ ^b	372	8.29 $\pm$ 6.25	0.38 - 56.7
CD34 ⁺ CD38 ⁻ ^b	373	3.29 $\pm$ 2.65	0.17 - 23.75
CD34 ⁺ c-kit ⁺ ^b	199	7.42 $\pm$ 4.66	0.21-29.17
Endothelial progenitor cells			
CD34 ⁺ CD133 ⁺ ^b	81	3.99 $\pm$ 3.82	0.22 - 15.9
CD34 ⁺ CD133 ⁺ VEGFR2 ⁺ ^b	81	0.17 $\pm$ 0.42	0 -2.44
CD133 ⁺ VEGFR2 ⁺ ^b	81	0.19 $\pm$ 0.46	0 - 2.57
CD34 ⁺ VEGFR2 ⁺ ^b	81	0.43 $\pm$ 0.77	0.008 - 4.09
Breast stem cells			
EpCAM ⁺ ^b	128	3.23 $\pm$ 3.82	0.18 - 19.8
EpCAM ⁺ CD49f ⁺ ^b	125	1.66 $\pm$ 1.81	0.049 - 9.7
EpCAM ⁺ CD49f ⁺ CD117 ⁺ ^b	53	0.52 $\pm$ 1.13	0 - 7.35
CD49f ⁺ CD24 ⁺ ^b	70	13.87 $\pm$ 11.79	0.17 - 48.1
CD24 ⁺ CD29 ⁺ ^b	73	8.30 $\pm$ 8.63	0.097 - 46.2
CD49f ⁺ CD24 ⁺ CD29 ⁺ ^b	70	6.97 $\pm$ 7.80	0.011 - 44.4

^a $\times 10^6$ per ml

^b per 1000 MNC

^c Daily activity consisted of causal walking, doing housework, standing or walking at work; these activities were classified as light exercise. Sitting and sleeping were not included in the calculation of MET-hr/week.

MET: metabolic equivalent task

Table 2

Association between energy expended in pre-pregnancy vigorous exercise (in MET-hr/week) and umbilical cord blood stem cell.

Umbilical cord blood cell population	All samples			First-birth samples			Second or later-birth samples		
	N	% difference (95% CI)	p	N	% difference (95% CI)	p	N	% difference (95% CI)	p
TNC	276	1.6 (-2.6, 6.0)	0.47	125	1.4 (-4.0, 7.1)	0.62	151	1.8 (-5.0, 9.1)	0.61
MNC	276	1.7 (-2.9, 6.6)	0.47	125	0.7 (-5.4, 7.2)	0.83	151	2.7 (-4.7, 10.7)	0.48
Hematopoietic stem cells									
CD34 ⁺	277	5.5 (-4.9, 16.9)	0.31	125	12.7 (-3.6, 31.8)	0.13	152	-5.9 (-18.0, 8.0)	0.38
CD34 ⁺ CD38 ⁻	278	3.8 (-6.4, 15.1)	0.48	126	7.6 (-7.1, 24.7)	0.33	152	-4.8 (-17.9, 10.4)	0.51
CD34 ⁺ c-kit ⁺	129	3.9 (-10.9, 21.3)	0.62	59	7.5 (-17.7, 40.3)	0.59	70	1.7 (-18.0, 26.2)	0.87
Endothelial progenitor cells									
CD34 ⁺ CD133 ⁺	64	43.7 (6.0, 94.9)	0.02	34	115.6 (40.5, 230.9)	0.001	30	41.7 (-16.9, 141.7)	0.19
CD34 ⁺ CD133 ⁺ VEGFR2 ⁺	64	102.1 (16.1, 251.7)	0.01	34	90.8 (-42.7, 535.6)	0.28	30	317.7 (82.8, 854.4)	0.002
CD34 ⁺ VEGFR2 ⁺	64	98.0 (33.2, 194.4)	0.001	34	60.2 (-18.0, 213.0)	0.16	30	163.8 (24.0, 461.2)	0.02
CD133 ⁺ VEGFR2 ⁺	64	104.0 (29.6, 221.1)	0.003	34	109.5 (-11.7, 396.9)	0.09	30	197.5 (30.5, 578.2)	0.01
Breast stem cells									
EpCAM ⁺	107	-2.8 (-20.0, 18.2)	0.78	52	-5.5 (-29.7, 27.0)		55	5.1 (-26.2, 49.5)	0.78
EpCAM ⁺ CD49f ⁺	104	-1.0 (-18.8, 20.6)	0.92	52	-11.0 (-32.1, 16.6)	0.39	52	21.6 (-15.4, 75.0)	0.28
EpCAM ⁺ CD49f ⁺ CD117 ⁺	44	61.1 (-12.9, 197.8)	0.12	27	-20.9 (-72.0, 123.5)	0.63	17	122.1 (-86.6, 3594.0)	0.43
CD49f ⁺ CD24 ⁺	55	11.9 (-30.0, 78.9)	0.63	31	-32.4 (-60.0, 14.4)	0.13	24	629.6 (47.9, 3499.5)	0.02
CD24 ⁺ CD29 ⁺	58	-33.3 (-59.4, 9.7)	0.11	33	-55.7 (-76.0, -18.3)	0.01	25	39.2 (-71.3, 576.6)	0.65
CD24 ⁺ CD29 ⁺ CD49f ⁺	55	-45.4 (-70.9, 2.4)	0.06	31	-67.3 (-83.5, -35.0)	0.003	24	-29.3 (-96.7, 1413.8)	0.81

Results from a multiple regression analysis, adjusting for MET-hr/week from pre-pregnancy moderate and light exercise, MET-hr/week from light exercise during the first trimester, MET-hr/week from light exercise during the second trimester, vigorous/moderate exercise (yes/no) during pregnancy, mother's age, parents' ethnicity (both Caucasian versus other), number of previous live births, gestation duration, gender of baby, time of delivery (day or night), birth weight, and study cycle. The analyses included subjects with no missing information on the variables in the regression models.

% difference: estimated proportional change in stem cell levels associated with each SD increase in MET-hr/week; TNC: total nucleated cells; MNC: mononuclear cells; MET: metabolic equivalent task

Table 3

Association between energy expended in pre-pregnancy moderate and light exercise (in MET-hr/week) and umbilical cord blood stem cell.

Umbilical cord blood cell population	All samples			First-birth samples			Second or later-birth samples		
	N	% difference (95% CI)	p	N	% difference (95% CI)	p	N	% difference (95% CI)	p
TNC	276	2.1 (-4.2, 8.8)	0.52	125	4.6 (-6.4, 17.0)	0.42	151	-1.0 (-9.0, 7.6)	0.81
MNC	276	1.7 (-5.2, 9.2)	0.63	125	5.7 (-7.0, 20.2)	0.39	151	-1.2 (-9.8, 8.2)	0.79
Hematopoietic stem cells									
CD34 ⁺	277	-9.2 (-22.1, 5.9)	0.22	125	-4.9 (-30.8, 30.8)	0.76	152	-12.8 (-26.2, 3.0)	0.11
CD34 ⁺ CD38 ⁻	278	-4.3 (-17.9, 11.8)	0.59	126	-0.9 (-26.6, 33.8)	0.95	152	-7.1 (-22.4, 11.2)	0.42
CD34 ⁺ c-kit ⁺	129	3.4 (-19.2, 32.1)	0.79	59	6.7 (-34.8, 74.5)	0.79	70	-7.2 (-33.8, 29.9)	0.66
Endothelial progenitor cells									
CD34 ⁺ CD133 ⁺	64	-7.9 (-46.9, 59.8)	0.77	34	-13.0 (-54.6, 66.9)	0.66	30	-38.8 (-73.6, 41.8)	0.23
CD34 ⁺ CD133 ⁺ VEGFR2 ⁺	64	-47.6 (-80.8, 43.0)	0.20	34	-52.7 (-92.4, 194.4)	0.40	30	-79.1 (-94.3, -23.0)	0.02
CD34 ⁺ VEGFR2 ⁺	64	-39.7 (-70.6, 23.7)	0.16	34	-32.4 (-75.6, 87.1)	0.43	30	-68.7 (-90.5, 3.0)	0.06
CD133 ⁺ VEGFR2 ⁺	64	-16.0 (-63.1, 91.2)	0.67	34	-44.3 (-85.0, 107.1)	0.36	30	-48.7 (-86.0, 88.0)	0.29
Breast stem cells									
EpCAM ⁺	107	5.8 (-27.8, 55.1)	0.77	52	35.7 (-30.6, 165.2)	0.36	55	-11.1 (-48.0, 52.0)	0.66
EpCAM ⁺ CD49f ⁺	104	10.0 (-25.9, 63.2)	0.63	52	6.1 (-42.6, 96.1)	0.85	52	3.2 (-41.6, 82.6)	0.91
EpCAM ⁺ CD49f ⁺ CD117 ⁺	44	9.3 (-63.9, 231.1)	0.87	27	44.5 (-60.6, 430.5)	0.55	17	-87.3 (-100.0, 45085.9)	0.48
CD49f ⁺ CD24 ⁺	55	29.2 (-37.8, 168.3)	0.48	31	97.3 (-1.5, 294.9)	0.05	24	-69.0 (-92.4, 26.3)	0.09
CD24 ⁺ CD29 ⁺	58	21.7 (-44.0, 164.6)	0.61	33	84.9 (-24.0, 349.9)	0.16	25	-22.2 (-80.7, 213.4)	0.70
CD24 ⁺ CD29 ⁺ CD49f ⁺	55	59.1 (-40.2, 323.4)	0.34	31	78.2 (-27.9, 340.5)	0.20	24	-7.2 (-93.7, 1275.4)	0.95

Results from a multiple regression analysis, adjusting for MET-hr/week from pre-pregnancy vigorous exercise, MET-hr/week from light exercise during the first trimester, MET-hr/week from light exercise during the second trimester, vigorous/moderate exercise (yes/no) during pregnancy, mother's age, parents' ethnicity (both Caucasian versus other), number of previous live births, gestation duration, gender of baby, time of delivery (day or night), birth weight, and study cycle. The analyses included subjects with no missing information on the variables in the regression models.

% difference: estimated proportional change in stem cell levels associated with each SD increase in MET-hr/week; TNC: total nucleated cells; MNC: mononuclear cells; MET: metabolic equivalent task

Table 4

Association between energy expended in light exercises (in MET-hr/week) during the first trimester and umbilical cord blood stem cells.

Umbilical cord blood cell population	All samples			First-birth samples			Second or later-birth samples		
	N	% difference (95% CI)	p	N	% difference (95% CI)	p	N	% difference (95% CI)	p
TNC	276	-1.7 (-8.5, 5.6)	0.64	125	-2.2 (-13.9, 11.0)	0.73	151	-1.1 (-10.1, 8.7)	0.81
MNC	276	0.26 (-7.4, 8.6)	0.95	125	4.3 (-9.8, 20.7)	0.56	151	-1.7 (-11.4, 9.0)	0.74
Hematopoietic stem cells									
CD34 ⁺	277	8.2 (-9.0, 28.6)	0.37	125	17.6 (-17.2, 67.0)	0.36	152	4.8 (-13.4, 26.8)	0.63
CD34 ⁺ CD38 ⁻	278	4.4 (-12.3, 24.2)	0.63	126	14.4 (-17.9, 59.3)	0.42	152	1.3 (-17.5, 24.4)	0.90
CD34 ⁺ c-kit ⁺	129	4.9 (-23.8, 44.2)	0.77	59	-1.0 (-42.1, 69.2)	0.97	70	14.2 (-28.7, 82.8)	0.57
Endothelial progenitor cells									
CD34 ⁺ CD133 ⁺	64	-20.8 (-66.4, 86.8)	0.59	34	-45.8 (-83.7, 80.4)	0.30	30	-55.0 (-89.6, 94.4)	0.26
CD34 ⁺ CD133 ⁺ VEGFR2 ⁺	64	-25.6 (-84.4, 255.3)	0.71	34	6.5 (-96.4, 3020.7)	0.97	30	-40.9 (-93.9, 470.1)	0.63
CD34 ⁺ VEGFR2 ⁺	64	-54.9 (-85.2, 38.1)	0.16	34	-42.0 (-91.1, 280.5)	0.55	30	-77.0 (-97.1, 82.1)	0.15
CD133 ⁺ VEGFR2 ⁺	64	-68.5 (-91.2, 13.3)	0.08	34	-41.9 (-94.9, 556.1)	0.64	30	-68.1 (-96.7, 205.8)	0.30
Breast stem cells									
EpCAM ⁺	107	28.3 (-18.2, 101.3)	0.28	52	18.9 (-45.1, 157.5)	0.65	55	37.8 (-26.9, 159.7)	0.31
EpCAM ⁺ CD49f ⁺	104	24.2 (-21.8, 97.3)	0.36	52	13.6 (-44.0, 130.6)	0.72	52	25.6 (-35.8, 145.6)	0.50
EpCAM ⁺ CD49f ⁺ CD117 ⁺	44	-61.4 (-95.7, 249.4)	0.38	27	22.4 (-91.2, 1602.6)	0.87	17	-100.0 (-100.0, ∞)	0.37
CD49f ⁺ CD24 ⁺	55	-0.2 (-73.1, 270.4)	1.00	31	105.1 (-58.8, 922.6)	0.36	24	23.7 (-88.5, 1235.8)	0.85
CD24 ⁺ CD29 ⁺	58	20.2 (-64.3, 303.0)	0.76	33	145.2 (-50.4, 1112.3)	0.25	25	4.3 (-89.7, 956.0)	0.97
CD24 ⁺ CD29 ⁺ CD49f ⁺	55	71.4 (-70.4, 892.7)	0.54	31	665.1 (-5.8, 6114.2)	0.06	24	-79.2 (-99.8, 1903.7)	0.46

Results from a multiple regression analysis, adjusting for MET-hr/week from pre-pregnancy vigorous exercise, MET-hr/week from pre-pregnancy moderate and light exercise, MET-hr/week from light exercise during the second trimester, vigorous/moderate exercise (yes/no) during pregnancy, mother's age, parents' ethnicity (both Caucasian versus other), number of previous live births, gestation duration, gender of baby, time of delivery (day or night), birth weight, and study cycle. The analyses included subjects with no missing information on the variables in the regression models.

% difference: estimated proportional change in stem cell levels associated with each SD increase in MET-hr/week; TNC: total nucleated cells; MNC: mononuclear cells; MET: metabolic equivalent task

Table 5

Association between energy expended in light exercises (in MET-hr/week) during the second trimester and umbilical cord blood stem cells.

Umbilical cord blood cell population	All samples			First-birth samples			Second or later-birth samples		
	N	% difference (95% CI)	p	N	% difference (95% CI)	p	N	% difference (95% CI)	p
TNC	276	-1.7 (-7.2, 4.1)	0.56	125	-2.9 (-11.3, 6.4)	0.53	151	-1.4 (-9.1, 7.0)	0.74
MNC	276	-3.6 (-9.6, 2.7)	0.25	125	-9.0 (-18.1, 1.0)	0.08	151	-1.8 (-10.1, 7.3)	0.69
Hematopoietic stem cells									
CD34 ⁺	277	-3.0 (-15.6, 11.5)	0.67	125	-8.9 (-29.5, 17.6)	0.47	152	0.5 (-14.5, 18.2)	0.95
CD34 ⁺ CD38 ⁻	278	-2.1 (-14.9, 12.6)	0.76	126	-8.7 (-28.3, 16.2)	0.46	152	0.8 (-15.3, 20.1)	0.93
CD34 ⁺ c-kit ⁺	129	-6.9 (-25.3, 15.8)	0.52	59	-0.6 (-36.4, 55.5)	0.98	70	-15.5 (-38.7, 16.4)	0.30
Endothelial progenitor cells									
CD34 ⁺ CD133 ⁺	64	30.8 (-45.9, 215.9)	0.54	34	123.8 (-36.6, 689.2)	0.20	30	204.5 (-20.4, 1064.9)	0.10
CD34 ⁺ CD133 ⁺ VEGFR2 ⁺	64	94.9 (-60.9, 870.4)	0.41	34	40.2 (-95.9, 4742.2)	0.84	30	650.0 (-6.1, 5890.8)	0.06
CD34 ⁺ VEGFR2 ⁺	64	248.3 (10.4, 998.4)	0.03	34	217.8 (-55.7, 2181.7)	0.24	30	807.7 (36.0, 5956.9)	0.03
CD133 ⁺ VEGFR2 ⁺	64	266.5 (-1.5, 1264.2)	0.05	34	196.8 (-76.6, 3670.3)	0.38	30	480.3 (-26.9, 4507.4)	0.09
Breast stem cells									
EpCAM ⁺	107	-18.3 (-42.5, 16.1)	0.26	52	-17.1 (-55.1, 53.2)	0.54	55	-10.7 (-46.0, 47.6)	0.65
EpCAM ⁺ CD49f ⁺	104	-24.1 (-46.9, 8.5)	0.13	52	-1.8 (-44.0, 72.3)	0.95	52	-22.5 (-53.9, 30.4)	0.33
EpCAM ⁺ CD49f ⁺ CD117 ⁺	44	157.6 (-76.8, 2763.5)	0.43	27	-23.8 (-94.8, 1026.3)	0.83	17	∞(-100.0, ∞)	0.29
CD49f ⁺ CD24 ⁺	55	-1.5 (-70.5, 228.7)	0.98	31	-64.5 (-91.9, 55.4)	0.16	24	412.0 (-37.9, 4120.2)	0.12
CD24 ⁺ CD29 ⁺	58	-15.9 (-74.7, 180.0)	0.77	33	-70.1 (-94.1, 51.4)	0.14	25	130.1 (-71.5, 1758.5)	0.40
CD24 ⁺ CD29 ⁺ CD49f ⁺	55	-36.0 (-87.2, 221.5)	0.58	31	-87.4 (-98.2, -13.7)	0.04	24	699.3 (-86.1, 45779.7)	0.28

Results from a multiple regression analysis, adjusting for MET-hr/week from pre-pregnancy vigorous exercise, MET-hr/week from pre-pregnancy moderate and light exercise, MET-hr/week from light exercise during the first trimester, vigorous/moderate exercise (yes/no) during pregnancy, mother's age, parents' ethnicity (both Caucasian versus other), number of previous live births, gestation duration, gender of baby, time of delivery (day or night), birth weight, and study cycle. The analyses included subjects with no missing information on the variables in the regression models.

% difference: estimated proportional change in stem cell levels associated with each SD increase in MET-hr/week; TNC: total nucleated cells; MNC: mononuclear cells; MET: metabolic equivalent task

Table 6

Association between vigorous/moderate exercise during pregnancy (yes versus no) and umbilical cord blood stem cells.

Umbilical cord blood cell population	All samples			First-birth samples			Second or later-birth samples		
	N	% difference (95% CI)	p	N	% difference (95% CI)	p	N	% difference (95% CI)	p
TNC	276	-3.6 (-11.2, 4.7)	0.39	125	-5.0 (-14.6, 5.8)	0.35	151	-4.4 (-16.4, 9.3)	0.51
MNC	276	-1.6 (-10.2, 7.8)	0.73	125	-3.0 (-14.3, 9.7)	0.62	151	-1.2 (-14.7, 14.3)	0.87
Hematopoietic stem cells									
CD34 ⁺	277	-1.6 (-19.4, 20.2)	0.88	125	-12.0 (-35.3, 19.6)	0.41	152	16.9 (-10.6, 52.9)	0.25
CD34 ⁺ CD38 ⁻	278	-3.2 (-20.8, 18.4)	0.75	126	-17.2 (-38.0, 10.7)	0.20	152	17.7 (-11.9, 57.1)	0.27
CD34 ⁺ c-kit ⁺	129	-23.5 (-42.4, 1.8)	0.07	59	-18.7 (-49.5, 30.7)	0.38	70	-25.8 (-51.8, 14.3)	0.17
Endothelial progenitor cells									
CD34 ⁺ CD133 ⁺	64	7.9 (-35.8, 81.2)	0.77	34	-12.0 (-53.6, 66.7)	0.68	30	9.3 (-53.5, 157.0)	0.83
CD34 ⁺ CD133 ⁺ VEGFR2 ⁺	64	13.9 (-55.7, 192.8)	0.78	34	-24.4 (-87.4, 355.5)	0.75	30	-45.2 (-85.4, 106.3)	0.35
CD34 ⁺ VEGFR2 ⁺	64	-29.4 (-64.1, 38.8)	0.31	34	-45.0 (-79.8, 49.4)	0.23	30	-37.1 (-81.2, 111.1)	0.43
CD133 ⁺ VEGFR2 ⁺	64	28.5 (-40.7, 178.5)	0.52	34	-29.6 (-80.6, 155.5)	0.58	30	-12.7 (-76.7, 227.0)	0.83
Breast stem cells									
EpCAM ⁺	107	-21.1 (-48.0, 19.9)	0.26	52	-7.8 (-50.0, 69.9)	0.79	55	-26.4 (-65.0, 55.0)	0.41
EpCAM ⁺ CD49f ⁺	104	-35.1 (-57.5, -0.9)	0.05	52	-18.9 (-53.7, 42.0)	0.45	52	-56.8 (-79.9, -7.5)	0.03
EpCAM ⁺ CD49f ⁺ CD117 ⁺	44	-19.6 (-73.2, 141.5)	0.69	27	-21.2-79.8, 206.9)	0.71	17	154.6 (-98.4, 41013.7)	0.60
CD49f ⁺ CD24 ⁺	55	-56.8 (-78.5, -13.3)	0.02	31	-32.7 (-65.4, 30.8)	0.22	24	-76.3 (-94.2, -3.0)	0.05
CD24 ⁺ CD29 ⁺	58	-17.0 (-60.2, 73.2)	0.61	33	59.7 (-29.9, 263.9)	0.25	25	-34.7 (-83.0, 150.3)	0.50
CD24 ⁺ CD29 ⁺ CD49f ⁺	55	-26.9 (-71.2, 85.9)	0.50	31	13.8 (-52.2, 170.5)	0.76	24	-34.6 (-95.6, 880.1)	0.73

Results from a multiple regression analysis, adjusting for MET-hr/week from pre-pregnancy vigorous exercise, MET-hr/week from pre-pregnancy moderate and light exercise, MET-hr/week from light exercise during the first trimester, MET-hr/week from light exercise during the second trimester, mother's age, parents' ethnicity (both Caucasian versus other), number of previous live births, gestation duration, gender of baby, time of delivery (day or night), birth weight, and study cycle. The analyses included subjects with no missing information on the variables in the regression models.

% difference: estimated proportional change in stem cell levels contrasting those who engaged in vigorous or moderate exercise during pregnancy with those did not; TNC: total nucleated cells; MNC: mononuclear cells; MET: metabolic equivalent task