Fetal Nutrition and Muscle Oxygen Supply in Childhood

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Fetal undernutrition may increase the risk of insulin resistance syndrome in adult life and low birth weight carries with it the risk of endothelial dysfunction. We studied 59 prepubertal and adolescent children whose birth weight exceeded 2.5 kg. We related their birth weight and ponderal index (PI) (weight/length^3) at birth to muscle oxygen supply (assessed by near infrared spectroscopy [NIRS]) and to variables associated with increased risk of developing the insulin resistance syndrome (ie, abdominal subcutaneous and visceral fat volumes, percentage body fat, fasting leptin, insulin, and glucose levels). Birth weight was not associated with muscle oxygen supply or any other independent variable measured. In the prepubertal group, there was a significant inverse relation between PI and muscle reoxygenation half-time in both the male ($R = .58$, $P = .03$) and female ($R = .72$, $P = .009$) groups. This relationship was not present in either the male ($R = .03$, $P = .90$) or female ($R = .09$, $P = .75$) adolescent groups. In a population in which low birth weight subjects were excluded, low PI at birth is associated with poor muscle oxygen supply in early childhood, but this relationship is not present in adolescence.

There is an association between fetal undernutrition and the prevalence in adult life of the insulin resistance syndrome (hypertension, type 2 diabetes, obesity and hyperlipidemia).[1,2] Other studies have shown that fetal undernutrition, as assessed by birth weight, relates to the degree of hypertension[3,4] and insulin resistance[5] from as young as 4 years of age. Endothelial dysfunction is present in 20-year-olds with a history of a low birth weight (<2.5 kg).[6] It is not known whether fetal nutrition remains a significant associate of vascular regulation if this low birth weight group is excluded from the analysis. Both birth weight and ponderal index (PI, weight/length^3) at birth are used as measures of fetal nutrition. A low PI implies the baby's growth has been disproportionate resulting in a thin neonate with relatively less muscle and adipose tissue. A study of adult females over the age of 50 years showed that fetal nutrition, assessed by PI at birth, was related to the regulation of muscle circulation, assessed by near infrared spectroscopy (NIRS).[7] This association could have persisted since its putative development *in utero* or it could have developed postnatally, possibly as a response to other metabolic changes within the muscle, such as altered activation of glycogenolysis during exercise.[8]

NIRS is a noninvasive technique that assesses the changes in oxygenated hemoglobin (Hb) and myoglobin (Mb) in muscle in vivo. NIRS can be used to determine relative muscle oxygen supply by measuring the rate of increase in levels of oxygenated Hb and Mb after ischemic exercise.[9,10] NIRS has detected abnormalities in oxygen supply in conditions such as peripheral vascular disease or heart failure where muscle perfusion is reduced.[11,12] NIRS data show a strong linear correlation with forearm deep vein oxygen saturation during exercise in humans, and the technique has been validated against venous occlusion plethysmography, including during intra-arterial perfusions of nitroprusside and noradrenaline.[13,14]

The aim of the current study was to examine the association between muscle microcirculatory regulation and 2 indices of fetal nutrition in subjects with a birth weight greater than 2.5 kg. We measured the rate of muscle reoxygenation after ischemic exercise, using NIRS, and related this to both PI and birth weight in prepubertal and adolescent groups of healthy males and females. We included other anthropometric and biochemical measures known to be risk factors for the development of insulin resistance in adult life to see whether these variables contribute to any association between size at birth and muscle reoxygenation rate.

SUBJECTS AND METHODS

All subjects and their parents gave written informed consent, and the study was approved by the ethics committees at The Children's Hospital at Westmead, Sydney and at the University of Sydney. There was neither personal history of diabetes nor parental history of type 1, type 2, or gestational diabetes in any subject group. Birth details were obtained from hospital-completed health records accompanying each subject. We only recruited subjects whose birth weight was greater than 2.5 kg and with gestation not less than 35 weeks.

Subject Recruitment

Twelve prepubertal females and 14 prepubertal males were recruited from local primary schools. Fifteen adolescent females and 18 adolescent males were recruited from local high schools. Initially, the principal of each school was contacted, then a letter sent to parents of appropriately aged children. Interested parents were asked to contact us whereupon, after obtaining informed consent, the following tests were performed at The Children's Hospital at Westmead, Sydney. All subjects were of European Caucasian origin.

Analysis

For ethical reasons related to the age of these subjects, hyperinsulinemic euglycemic clamps were not performed. Instead a fasting 5-mL blood sample was collected from the antecubital vein of each subject. Blood was not taken from 3 prepubertal males and 2 adolescent females due to technical difficulties or subject refusal. Plasma glucose concentrations were measured by the glucose oxidase method,[15] and serum insulin and leptin levels were quantified using specific double antibody radioimmunoassays (Linco Research, St Charles, MO). Homeostasis model assessment (HOMA) of insulin sensitivity was calculated using

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Table 1. Subject Characteristics

	Prepubertal		Adolescent	
	Male	Female	Male	Female
No.	14	12	18	15
Age (yr)	8.3 ± 0.8	7.9 ± 0.8	17.0 ± 1.1	16.5 ± 0.5
Birth weight (kg)	3.7 ± 0.7	3.5 ± 0.4	3.6 ± 0.6	3.4 ± 0.5
PI (kg/m^3)	26.7 ± 3.0	26.3 ± 1.8	25.3 ± 3.2	26.2 ± 3.5
BMI (kg/m^2)	18.3 ± 3.7	16.6 ± 2.2	23.7 ± 4.6	21.8 ± 2.9
BMI SD score	0.5 ± 1.3	0.0 ± 0.9	0.4 ± 1.1	0.0 ± 0.7
Muscle reoxygenation halftime (s)	11.8 ± 3.9	11.0 ± 4.1	13.0 ± 8.4	16.4 ± 6.7
Abdominal subcutaneous fat volume (cm^3)	73.6 ± 64.7	67.5 ± 40.2	147.6 ± 156.1	142.4 ± 72.2
Visceral fat volume (cm^3)	11.0 ± 5.9	10.0 ± 7.9	23.1 ± 21.4	14.4 ± 7.1
% body fat	19.2 ± 5.8	19.9 ± 4.5	14.2 ± 4.6	23.2 ± 6.4*
Fasting insulin (pmol/L)	76.8 ± 18.6†	75.6 ± 30.0	102.0 ± 64.2	103.8 ± 36.0‡
Fasting glucose (mmol/L)	5.7 ± 0.4†	5.3 ± 0.3	4.6 ± 0.8	4.6 ± 0.7‡
HOMA	3.2 ± 0.8†	3.0 ± 1.3	3.8 ± 3.0	3.7 ± 1.7‡
Fasting leptin (ng/mL)	8.3 ± 5.6†	6.2 ± 3.7	6.7 ± 9.1	17.7 ± 12.0‡*

NOTE. Data are represented as means ± SD.

*$P < .05$ compared to relevant equivalently aged male group (Student's *t* test).

†n = 11.

‡n = 13.

the formula (fasting glucose [mmol/L] × fasting insulin [μU/mL]/22.5).[16,17] This method is commonly used in clinical and population-based studies as a relative index of insulin resistance.[15]

Anthropometry

All anthropometric measurements were performed in duplicate by one investigator using standardized techniques.[18] Body mass index (BMI) was calculated as weight/height^2 (kg/m^2). To compensate for age-related changes in BMI, all measurements were expressed as standard deviation (SD) scores from a reference population.[19] Skinfold thickness was measured (± 0.1 mm) on the right side of the body at 4 sites (triceps, biceps, subscapular, and supraspinale) using a Harpenden caliper (British Indicators, St Albans, Hertsfordshire, UK). Percent body fat (%BF) was calculated from skinfold thicknesses.[20]

Magnetic Resonance Imaging

Cross-sectional magnetic resonance images of the abdomen at the fourth lumbar (L4) region were generated on a Philips 1.5 Tesla magnetic resonance scanner (Best, The Netherlands). Using a software program (ANALYZE, Mayo Clinic, Rochester, MN), subcutaneous and visceral regions of a single slice (1.0 cm thick) at the level of mid-L4 were distinguished and labeled, and the volumes (cross-sectional area × 1 cm) of the labeled lipid compartments were determined.

Near Infrared Spectroscopy

NIRS was performed using a commercial apparatus (RunMan; NIM, Philadelphia, PA) to determine the half time of Hb and Mb reoxygenation after ischemic exercise. A light source was placed over the forearm muscle, flexor digitorum superficialis, of the dominant arm and reflected light was measured at 760 nm and 850 nm. Changes in tissue saturation at these wavelengths are attributed to changes in the saturation of both Hb and Mb and NIRS receives signals mostly from hemoglobin in the arterioles, capillaries, and postcapillary venules.[14] Each subject performed exercise by squeezing a ball at a rate of 30 times/minute for 60 seconds. A cuff was inflated around the upper arm to 50 mm Hg above systolic blood pressure as exercise continued for a further 15 to 20 seconds until maximal desaturation of Hb and Mb was achieved. The cuff was rapidly released 5 seconds after cessation of exercise. Half-time of Hb and Mb reoxygenation, a measure of oxygen supply to the tissue, was determined using the point of cuff deflation as the start of metabolic recovery. The coefficient of variation of this technique in our hands is 10.8% (J Ward, unpublished data), which compares well with literature values of 16.4%.[13] No subject's supine systolic blood pressure was above 110 mm Hg.

Statistics

Statistical analyses were performed using SPSS/PC version 8.0 (SPSS, Chicago, IL). Data were expressed as mean (± SD) and checked for normality. An unpaired Student *t* test was used to test for differences between male and female groups. Pearson correlation coefficients (*R*) were calculated to determine significant relations. A *P* value $< .05$ was defined as a significant correlation within a group or difference between groups. In view of the significant intervariable correlations that exist within this study, stepwise multiple linear regression analysis was applied to the relationships between muscle reoxygenation rate and ponderal index, HOMA, leptin, percent body fat, abdominal visceral, and subcutaneous fat volumes. This allowed definition of those variables that, when combined, have significant predictive associations with muscle reoxygenation half-time.

RESULTS

Birth details, current anthropometry, and age, as well as details of muscle perfusion, muscle lipid levels, abdominal subcutaneous and visceral fat volumes, insulin sensitivity indices, and plasma leptin are presented in Table 1. As expected, there were significant differences in percent body fat ($P = .0001$) and leptin levels ($P = .005$) between the adolescent male and female groups, but otherwise no significant differences were seen between the equivalently-aged male and female groups in any of these variables.

A significant inverse correlation was present between PI and muscle reoxygenation half-time within the male prepubertal group and within the female prepubertal group (Fig 1). In other

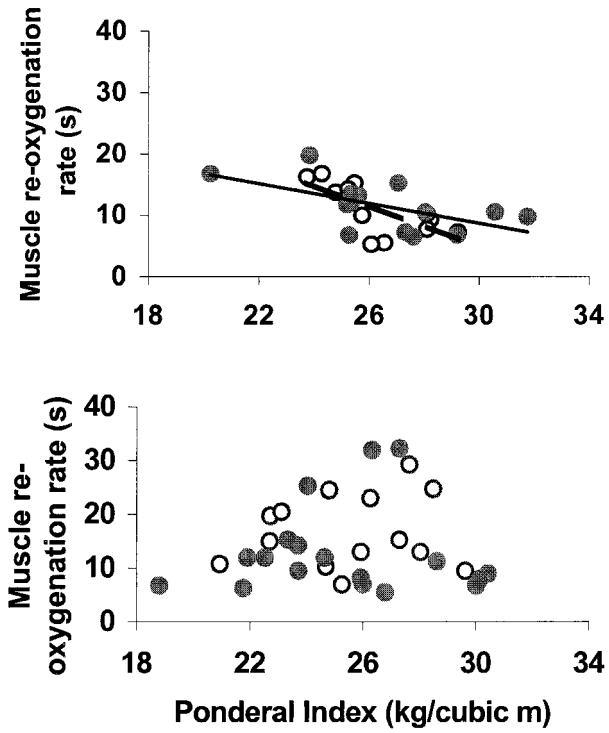

Fig 1. Muscle reoxygenation rate as a function of PI at birth in (A) 26 prepubertal subjects (●, solid line, males, $R = -0.58$, $P = .03$; ○, dotted line, females $R = -0.72$, P = .009) and in (B) 33 adolescent subjects (●, males, $R = 0.03$, $P = .90$; females, $R = 0.09$, $P = .75$).

words, the lower the PI, the longer the muscle reoxygenation rate.

This relationship remained even if these males and females were analyzed as 1 group ($R = -.60$, $P = .001$). None of these relationships was improved upon stepwise multiple regression analysis during addition of other variables known to relate to insulin sensitivity or to the risk of developing diseases associated with the insulin resistance syndrome (ie, HOMA, leptin, % body fat, abdominal visceral, and subcutaneous fat volumes).

In the adolescent subjects, there was no correlation between PI and muscle reoxygenation rate within either male or female groups (Fig 1) nor in the group as a whole, nor did a significant correlation appear when multiple regression analysis was performed and PI was combined with the other anthropometric and biochemical variables.

Birth weight did not relate to muscle reoxygenation rate in either the prepubertal or adolescent groups. There were no significant associations between PI and HOMA, plasma leptin level, % body fat, abdominal visceral or subcutaneous fat volumes in any group. This suggests that muscle reoxygenation is not merely a surrogate for regional adiposity or insulin sensitivity.

DISCUSSION

We have demonstrated a relationship between muscle oxygen supply and PI in prepubertal subjects. The slower muscle reoxygenation rates were seen in those children with a low PI at birth. This supports the reported association between fetal malnutrition, as assessed by birthweight, and large vessel endothelial dysfunction, as assessed by flow-related brachial artery dilation in 9- to 11-year-olds.[21] Unlike that study, we did not recruit any subjects with a birth weight less than 2.5 kg because endothelial dysfunction is known to be present in these subjects.[6] Even so, those subjects with a birth weight over 2.5 kg, but still characterized by relative fetal undernutrition (as shown by a lower PI), have slower muscle reoxygenation times.

In exercising muscle, there is a close correlation between venous oxygen saturation and differential absorption of light in the near infrared range.[14] The exact metabolic phenomenon monitored by NIRS after ischemic exercise is uncertain, but it is influenced by many interacting variables. Slow muscle reoxygenation is seen in peripheral vascular disease,[12] conditions associated with increased sympathetic nervous system vasoconstrictive activity (such as heart failure or those taking beta blockers)[11,22] and in those conditions associated with endothelial dysfunction (either defective nitric oxide release or action).[23,24] The inverse relationship reported in the present study implies that a decrease in the supply of oxygen to the muscle tissues may be a direct effect of relative fetal undernutrition (eg, endothelial dysfunction) or altered muscle growth, structure, or fiber type differentiation perinatally. Alternatively, reduced muscle perfusion may represent an adaptive response to other, as yet undiscovered, metabolic sequelae of relative fetal undernutrition. Pancreatic and cerebral vascularization are reduced in an animal model of fetal undernutrition, although capillary density of muscle was not assessed.[25] There has only been 1 study in humans of the relationship of muscle vascularity to PI, and no association was seen.[7] An important, although difficult, study would be to relate the PI at birth to prepubertal muscle capillary density, derived from analysis of a muscle biopsy.

PI is a measure of the relative size or shape of the neonate and, if low, is thought to reflect those infants who became undernourished in mid to late gestation, but maintained linear growth at the expense of other tissues such as skeletal muscle. Birth weight may be a less sensitive index of fetal nutrition[26] and, indeed, reduced birth weight might indicate prematurity or sustained fetal undernutrition. If a population is studied in which there is a wide range of birth weights, including those with a birth weight less than 2.5 kg, fetal undernutrition and large vessel endothelial dysfunction are related prepubertally and in 19- to 20-year-olds.[21,27] There was no association of birth weight with muscle reoxygenation rate in either the prepubertal or adolescent subjects in the current study. Our population did not include any subjects with a birth weight less than 2.5 kg suggesting that the absence of associations may be due to our recruitment criterion limiting the birth weight range.

The association of PI and muscle reoxygenation rate is absent in adolescent males and females and, indeed, becomes a positive association in older adults.[7] Our findings are different from those of Leeson et al[21,27] reported above in which endothelial dysfunction apparently persists into at least young adulthood. We cannot readily explain this difference in findings. Apart from the use of different measures of fetal nutrition (PI at birth and birthweight), the difference between our findings and those of others[21,27] may arise from the differing circulatory

beds examined, the variation in techniques used to examine vasoregulation in each study, or the exclusion of a low birth weight cohort, with known endothelial dysfunction, from our study. If present at all in adolescence, the relationship between muscle reoxygenation and PI at birth is less strong than in young childhood. Other unknown or unmeasured metabolic influences upon vascular regulation that are activated at puberty could obscure this relationship in this age group.

The comparatively reduced muscle oxygen supply arising from relative fetal undernutrition in normal birth weight subjects is not sustained throughout life. The positive association between muscle oxygen supply and PI seen in adults in their sixth decade is not present in either age group assessed in the present study. Presumably the facilitation of muscle oxygen supply to those with a history of relatively reduced fetal nutrition is an adaptive response to delayed activation of glycogenolysis[8] or other metabolic dysfunction in mature subjects at risk of developing the insulin resistance syndrome. If endothelial dysfunction is still present in these older adults, other vascular regulatory mechanisms (eg, sympathetic tone) may be involved in producing the observed relative increase in muscle oxygen supply.

In conclusion, poor muscle oxygen supply in early childhood is associated with low PI at birth. While endothelial dysfunction is a recognized complication of low birth weight, it may also be present in subjects of a normal birth weight, but whose intrauterine environment has resulted in a low PI at birth.

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REFERENCES

1. Barker DJP, Osmond C: Infant mortality, childhood nutrition and ischaemic heart disease in England and Wales. Lancet 1:1077-1081, 1986

2. Hales CN, Barker DJP: Type 2 (non-insulin-dependent) diabetes mellitus: The thrifty phenotype hypothesis. Diabetologia 35:595-601, 1992

3. Taylor SJ, Whincup PH, Cook DG, et al: Size at birth and blood pressure: Cross sectional study in 8-11 year old children. BMJ 314: 475-480, 1997

4. Law CM, de Swiet M, Osmond C, et al: Initiation of hypertension in utero and its amplification throughout life. BMJ 306:24-27, 1993

5. Yajnik CS, Fall CHD, Vaidya U, et al: Fetal growth and glucose and insulin metabolism in four-year-old Indian children. Diabet Med 12:330-336, 1995

6. Goodfellow J, Bellamy MF, Gorman ST, et al: Endothelial function is impaired in fit young adults of low birth weight. Cardiovasc Res 40:600-606, 1998

7. Thompson CH, Sanderson AL, Sandeman D, et al: Fetal growth and insulin resistance in adult life: Role of skeletal muscle morphology. Clin Sci 92:291-296, 1997

8. Taylor DJ, Thompson CH, Kemp GJ, et al: A relationship between impaired fetal growth and reduced muscle glycolysis revealed by ^{31}P magnetic resonance spectroscopy. Diabetologia 38:1205-1212, 1995

9. Wilson JR, Mancini DM, McCully K, et al: Noninvasive detection of skeletal muscle under perfusion with near-infra-red spectroscopy in patients with heart failure. Circulation 80:1668-1674, 1989

10. Sahlin K: Non-invasive measurements of O_2 availability in human skeletal muscle with near infra-red spectroscopy. Int J Sports Med 13:S157-S160, 1992

11. Hanada A, Okita K, Yonezawa K, et al: Dissociation between muscle metabolism and oxygen kinetics during recovery from exercise in patients with chronic heart failure. Heart 83:161-166, 2000

12. Komiyama T, Shigematsu H, Yasuhara H, Muto T: Near-infrared spectroscopy grades the severity of intermittent claudication in diabetics more accurately than ankle pressure measurement. Br J Surg 87:459-466, 2000

13. Van Beekvelt M, Colier W, Wevers R, et al: Performance of near-infrared spectroscopy in measuring local O_2 consumption and blood flow in skeletal muscle. J Appl Physiol 90:511-519, 2001

14. Mancini DM, Bolinger L, Li H, et al: Validation of near-infrared spectroscopy in humans. J Appl Physiol 77:2740-2747, 1994

15. Kaplan LA, Pesce AJ: Clinical Chemistry: Theory, Analysis and Correlation. St Louis, MO, Mosby, 1984

16. Mathews DR, Hosker JP, Rudenske AS, et al: Homeostasis model assessment: Insulin resistance and beta cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 412-419, 1985

17. Bonora E, Targher G, Alberiche M, et al: Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: Studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diabetes Care 23:57-63, 2000

18. Norton K, Whittingham N, Carter L, et al: Measurement techniques in anthropometry, in Norton K, Olds T (eds): Anthropometrica. A Textbook of Body Measurements for Sports and Health Courses. Sydney, Australia, University of New South Wales, 1996, pp 25-75

19. World Health Organization: Reference data for weight and height of children, in Measuring Change in Nutritional Status. Geneva, World Health Organization, 1983, pp 61-101

20. Deurenberg P, Pieters JJ, Hautvast JG: The assessment of the body fat percentage by skinfold thickness measurements in childhood and young adolescents. Br J Nutr 63:293-303, 1990

21. Leeson CPM, Whincup PH, Cook DG, et al: Flow medicated dilation in 9- to 11-year old children: The influence of childhood and intrauterine factors. Circulation 96:2233-2238, 1997

22. Maekawa K, Kuboki T, Miyawaki T, et al: Effect of intravenous infusion of a beta-adrenergic blocking agent on the haemodynamic changes in human masseter muscle induced by cold-pressor stimulation. Arch Oral Biol 44:475-483, 1999

23. Sander M, Chavoshan B, Harris SA, et al: Functional muscle ischaemia in neuronal nitric oxide synthase-deficient skeletal muscle of children with Duchenne muscular dystrophy. Proc Natl Acad Sci USA 97:13818-13823, 2000

24. Arrowsmith FE, Ward J, Pronk K, et al: Predictors of muscle oxygen supply in adolescents. Diabetologia 44:A166, 2001 (suppl 1, abstr)

25. Bennis-Taleb N, Remacle C, Hoet JJ, et al: A low-protein isocaloric diet during gestation affects brain development and alters permanently cerebral cortex blood vessels in rat offspring. J Nutr 129:1613-1619, 1999

26. Hays D, Patterson RM: A comparison of fetal biometric ratios to neonatal morphometrics. J Ultrasound Med 6:71-73, 1987

27. Leeson CPM, Kattenhorn M, Morley R, et al: Impact of low birth weight and cardiovascular risk factors on endothelial function in early adult life. Circulation 103:1264-1268, 2001