

# Circulation

JOURNAL OF THE AMERICAN HEART ASSOCIATION



## **Impact of Low Birth Weight and Cardiovascular Risk Factors on Endothelial Function in Early Adult Life**

C. P. M. Leeson, M. Kattenhorn, R. Morley, A. Lucas and J. E. Deanfield

*Circulation* 2001;103:1264-1268

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 72514

Copyright © 2001 American Heart Association. All rights reserved. Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://circ.ahajournals.org/cgi/content/full/103/9/1264>

Subscriptions: Information about subscribing to *Circulation* is online at

<http://circ.ahajournals.org/subscriptions/>

Permissions: Permissions & Rights Desk, Lippincott Williams & Wilkins, a division of Wolters Kluwer Health, 351 West Camden Street, Baltimore, MD 21202-2436. Phone: 410-528-4050. Fax: 410-528-8550. E-mail:

[journalpermissions@lww.com](mailto:journalpermissions@lww.com)

Reprints: Information about reprints can be found online at

<http://www.lww.com/reprints>

# Impact of Low Birth Weight and Cardiovascular Risk Factors on Endothelial Function in Early Adult Life

C.P.M. Leeson, MB, PhD; M. Kattenhorn, BSc; R. Morley, MB;  
A. Lucas, MD, FRCP; J.E. Deanfield, FRCP

**Background**—Low birth weight is related to increased risk of coronary heart disease in adults and recently has been associated with vascular endothelial dysfunction in children. We investigated whether the relation between birth weight and endothelial function was still present in early adult life and whether there was an interaction with emerging risk factors.

**Methods and Results**—In 315 adults (165 women, 150 men, aged 20 to 28 years), high-resolution ultrasound was used to determine endothelium-dependent and -independent vascular responses of the brachial artery. Vascular measures were related to classic risk factors (smoking history, lipid profile, blood pressure, fasting insulin, exercise capacity, body mass index, and combined risk score) and birth weight. Low birth weight was associated with reduced flow-mediated dilation (coefficient=0.18 kg<sup>-1</sup>, 95% CI 0.004 to 0.35, *P*=0.04) but not with endothelium-independent dilation. The difference in flow-mediated dilation between the top and bottom fifths of birth weight was the same as between smokers and nonsmokers. Increasing levels of acquired risk factors overwhelmed the association, and there was a significant interaction of risk score with the birth weight–endothelial function relation (coefficient of interaction term [birth weight×risk score] = −0.12, 95% CI −0.22 to −0.03, *P*=0.01).

**Conclusions**—Low birth weight is associated with endothelial dysfunction in young adults. This is most marked in individuals with lower risk factor profiles and may be relevant to the pathogenesis of atherosclerosis in later life. (*Circulation*. 2001;103:1264-1268.)

**Key Words:** endothelium ■ cardiovascular diseases ■ epidemiology

Coronary heart disease remains the leading cause of mortality and morbidity in the world.<sup>1</sup> Landmark epidemiological studies have identified key factors in adult life that increase the risk of coronary events,<sup>2,3</sup> and yet, these still only account for a proportion of the disease burden.<sup>4</sup> Recently, evidence has accumulated that cardiovascular disease is also predicted by antenatal events.<sup>5–7</sup> Low birth weight has been related to mortality from coronary disease and to the development of hypertension and diabetes.<sup>8–10</sup> These associations may be due to an effect of prenatal growth directly on the pathogenesis of early atherosclerosis, by “programming” the development of risk factors,<sup>5</sup> or due to altered “stability” of established atherosclerotic lesions.

Vascular endothelial dysfunction is a key event early in atherosclerosis and is important in the development of all the cardiovascular diseases associated with early growth.<sup>11–13</sup> Recently, we<sup>14</sup> have shown there is a relation between birth weight and endothelium-dependent flow-mediated dilation (FMD) in children in the first decade of life before the acquisition of a significant risk factor burden. This supports

the concept that prenatal factors have a direct effect on the vascular wall that is relevant to atherogenesis from remarkably early in life.

The interaction, however, between the effect of prenatal influences and classic postnatal risk factors on vascular function remains unclear. With aging, prenatal influences could act to amplify the effects of classic risk factors, similar to the interaction between raised cholesterol level with smoking,<sup>15</sup> or be overwhelmed in their presence. We therefore investigated the relative impact of prenatal and classic influences on endothelium-dependent arterial relaxation in a large group of 20- to 28-year-olds for whom weight measurements at birth and during infancy were available from maternity and child health records.

Our findings demonstrate a continued association between birth weight and endothelial function into the third decade of life. This influence, however, is progressively reduced in the presence of an increased cardiovascular risk profile. This suggests that the impact of prenatal growth on vascular disease does not operate through modification of acquired

Received August 30, 2000; revision received November 10, 2000; accepted November 16, 2000.

From the Vascular Physiology Unit (C.P.M.L., M.K., J.E.D.), Great Ormond St Hospital for Children NHS Trust, London, UK; Medical Research Council Childhood Nutrition Research Centre (C.P.M.L., M.K., A.L.), Institute of Child Health, London, UK; and the Menzies Centre for Population Health Research (R.M.), Tasmania and Clinical Epidemiology and Biostatistics Unit, University of Melbourne Department of Paediatrics, Royal Children's Hospital, Melbourne, Australia.

Correspondence to Professor J.E. Deanfield, Vascular Physiology Unit, Great Ormond St Hospital, London WC1N 3JH, UK.

© 2001 American Heart Association, Inc.

*Circulation* is available at <http://www.circulationaha.org>

risk factors or by a protective effect in their presence. Low birth weight may adversely influence endothelial function and the pathogenesis of atherosclerosis in subjects with low risk profiles to a similar degree as the effect of more widely recognized acquired risk influences, such as smoking.

## Methods

### Study Design

We studied adults born in Mill Road Maternity Hospital, Cambridge, UK, between 1969 and 1975 for whom birth and early growth measures were available from medical records. Subjects were traced via their general practitioner or from the National Health Service Central Register and were invited to attend a clinic, where they had an evaluation of cardiovascular risk profile based on a blood sample, questionnaire, physical measures, and an assessment of their vascular function and exercise capacity.

To maximize inclusion rate, invitations included stamped addressed envelopes and flexible appointment times, and subjects who did not respond to the letter were followed up. Ethical approval was received from local research ethics committees, and informed consent was obtained at the time of the visit.

### Risk Factors

In each subject, a fasting venous blood sample was analyzed for insulin, glucose, total cholesterol, HDL, and triglycerides, and LDL was calculated.<sup>16–19</sup> Personal and family medical histories (including details of heart disease and risk factors) were gathered by questionnaire at interview. Current or past smoking was recorded, and for current smokers, the dose effect of smoking was measured by allocating a pack-years score, 1 pack-year being equivalent to smoking 20 cigarettes a day for a year. Subjects were coded into standard socioeconomic groups based on recent occupation and education level.<sup>20</sup> Birth weight and placental weight, as well as length and head circumference at birth, were obtained from maternity records. Weight at 1 year of age was obtained from school health records.

Blood pressure was measured as the average of the last 2 of 3 seated readings with an automated oscillometric device (Critikon Inc). Weight was recorded (to  $\pm 0.1$  kg) with scales (Soehnle Ltd), and height was recorded (to  $\pm 0.1$  mm) with a portable stadiometer. After the assessment of endothelial function, a physical step test was used to grade fitness level. The subject stepped onto and off a box (41.3 cm high) with both feet for 3 minutes, in time with a metronome (22 steps per minute for women, 24 steps per minute for men); their oxygen capacity was estimated from their recovery heart rate by sex-standardized tables.<sup>21</sup>

The distributions of risk factors in the study group were compared with risk factor data for the United Kingdom, birth weight data for Cambridge Maternity Hospital, and questionnaire data from a subgroup of adults who initially had declined to participate. This allowed any significant biases in cardiovascular risk profile or birth-related data to be identified.

### Ultrasound Investigation

Every subject underwent measurement of endothelium-dependent and -independent vascular responses of the brachial artery by high-resolution ultrasound imaging (Acuson 128XP/10, 7-MHz probe, and automated vessel-diameter measurements).<sup>22</sup> The subject lay supine on a couch, and after a 10-minute rest, brachial artery diameter and blood flow were recorded (baseline). A pneumatic cuff was then inflated to suprasystolic pressure on the forearm for 4.5 minutes. Cuff deflation resulted in reactive hyperemia, causing increased flow through the brachial artery and stimulating endothelium-dependent FMD. The change in brachial artery diameter at maximal response, 1 minute after cuff release, was measured. Arterial diameter measurements were then repeated after a 10-minute rest, between 3 and 4 minutes after a single sublingual spray (100 to 400  $\mu$ g) of nitroglycerin, which produces an endothelium-

independent dilation. Room temperature and time of day were recorded at the start of each scan. Hard-copy printouts of the diameter measures and videotape recordings of the vessel image and flow recordings were kept for quality analysis, as described previously.<sup>14,23</sup>

### Statistical Analysis

The primary outcomes were vascular function (measured by FMD and nitroglycerin-induced dilation [NTGD]) and the cardiovascular risk factors, including birth weight. All subjects were allocated a risk score based on their relative position within the study group for the individual risk factors (cholesterol, blood pressure, fasting insulin, body mass index, and exercise capacity). For each risk factor, those subjects in the third of the distribution that conventionally would be considered highest risk had 2 points added to their risk score, those in the middle third had 1 point added, and those in the lowest third had no points added. Ex-smokers were given an additional 1 point and current smokers 2 points.

FMD was represented by change in vessel diameter.<sup>14</sup> All variables were reasonably normally distributed except FMD, which was positively skewed owing to the proportional nature of the measure. Logarithmic transformation was therefore used before analysis. The group was analyzed as a whole, and linear regression was used to model the relations between continuous explanatory variables (risk factors) and vascular measures. Variables were added in a model that included vessel size, age, and sex.<sup>14</sup> Linearity of associations was assessed visually and by fitting cubic and quadratic terms in models.

The variation in the association between birth weight and endothelial function at different levels of risk was examined by dividing the sample into 3 layers (stratified according to the value of the appropriate variables) and observing the relation in each layer. Interaction between birth weight, risk factors, and vascular function was assumed when an interaction term (the product of birth weight and risk factor) added to the regression model including both factors was significant.<sup>24</sup>

## Results

### Population Characteristics

Invitations were sent to 1526 young adults. Of these, 420 (28%) agreed to take part, 229 (15%) declined, and 877 (57%) did not reply. There were 344 (23%) subjects who were able to attend for vascular investigations. Full details of vascular function, risk factors (including blood samples and questionnaires), and birth weight were available for 315 subjects (165 women and 150 men), 92% of those taking part. Weight at 1 year was obtained for 257 subjects (75%) (Table 1). The study group had similar characteristics to the general young adult population and to birth-weight measures for those born in Cambridge between 1969 and 1975, with no apparent biases in cardiovascular risk profile or birth measures (birth weight in study group was 3.27 [0.57 SD] kg, and in the sample born in Cambridge, it was 3.24 [0.60 SD] kg). Cardiovascular health profiles in subjects who declined to participate were also comparable (55 of 71 postal questionnaires distributed were returned).

### Vascular Studies

Resting vessel size, change in vessel size (FMD and NTGD), and brachial artery blood flow, at baseline and after reactive hyperemia, were not affected by room temperature, time of day, or month of visit. There were no relations between birth measures or cardiovascular risk factors and resting vessel size or brachial artery blood flow, except that women had smaller

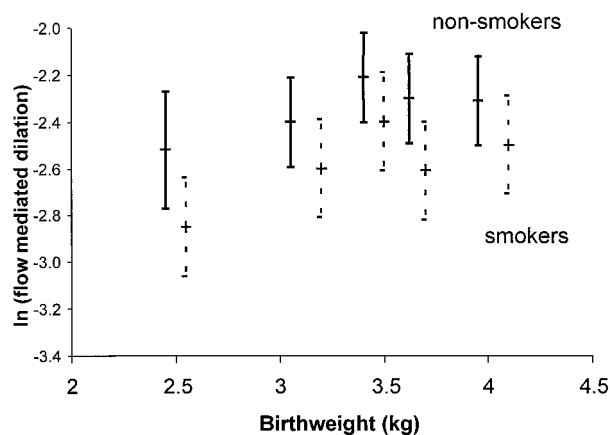
**TABLE 1. Main Characteristics of Study Population**

Variable	Mean	SD or Range
Age, y	23	20 to 28
Height, cm	169	10
Weight, kg	69.5	14
Body mass index, kg/m <sup>2</sup>	24.3	4.2
Birth weight, kg	3.27	0.51
Birth length, cm	50.1	2.8
Placental weight, kg	0.63	0.15
Weight at 1 year, kg	10.32	2.48
Total cholesterol, mmol/L	4.54	1.05
LDL cholesterol, mmol/L	2.81	0.92
HDL cholesterol, mmol/L	1.18	0.28
Insulin, mU/L	9.10	5.71
Glucose, mmol/L	5.03	0.51
Systolic blood pressure, mm Hg	127	14
Diastolic blood pressure, mm Hg	70	9
Smokers/ex-smokers/nonsmokers, %	34/15/51	...
Smoking pack-years	1.84	0 to 16
Alcohol, U/wk	11.3	0 to 56

brachial arteries ( $2.9 \pm 0.3$  mm) than men ( $3.7 \pm 0.5$  mm;  $P < 0.001$ ).

### Early Life Measures and Vascular Function

There was a significant relation between birth weight and FMD (coefficient  $0.18 \text{ kg}^{-1}$ , 95% CI 0.004 to 0.35,  $P = 0.04$ ) but not with NTGD response (coefficient  $-0.03 \text{ kg}^{-1}$ , 95% CI  $-0.09$  to 0.04,  $P = 0.41$ ) (Figure). Other measures of fetal growth, such as birth length, head circumference, placental weight, ponderal index at birth, and placenta-weight to birth-weight ratio, were unrelated to the 2 measures of vascular function. There was no relation between vascular function and weight at 1 year (coefficient  $-0.0013 \text{ kg}^{-1}$ , 95% CI  $-0.06$  to 0.04,  $P = 0.63$ ).



Relation between FMD and birth weight in smokers and non-smokers (see Statistical Analysis). There is similar strength of association in both groups, but smokers have lower FMD than nonsmokers across birth-weight range.

### Cardiovascular Risk Factors and Vascular Function

Smokers had lower FMD than nonsmokers (mean difference 0.29, 95% CI 0.07 to 0.51,  $P = 0.009$ ), and there was an inverse relation between FMD and number of smoking pack-years (coefficient  $-0.04 \text{ pack-year}^{-1}$ , 95% CI  $-0.004$  to  $-0.07$ ,  $P = 0.03$ ). Response to nitroglycerin did not differ between groups (mean difference 0.03, 95% CI  $-0.05$  to 0.11,  $P = 0.48$ ). In the whole group, neither FMD nor NTGD was related to serum cholesterol or LDL level. However, in the subgroup of smokers, there was a trend toward an inverse association between FMD and LDL (coefficient  $-0.02 \text{ mmol/L}$ , 95% CI  $-0.03$  to 0.00,  $P = 0.06$ ) and a positive association with HDL level (coefficient  $0.04 \text{ mmol/L}$ , 95% CI  $-0.01$  to 0.09,  $P = 0.08$ ). Vascular responses were not related to other cardiovascular risk factors such as fasting insulin, blood pressure, or exercise capacity in this study population (Table 1).

### Birth Weight, Vascular Function, and Risk Factors

There was a significant relation between birth weight and FMD in the lowest risk score third that was not found in the middle and highest thirds. This was also found for the individual risk factors of fasting insulin, body mass index, and exercise capacity (Table 2). The association between birth weight and endothelial function was of similar strength but at a lower level of significance in both smokers and nonsmokers, with smokers tending to have lower FMD than nonsmokers at all birth weights (Figure). Inclusion of an interaction term in the model indicated a significant effect of the risk score on the birth weight–endothelial function relation so that higher levels of acquired risk factors reduced the impact of birth weight on FMD (coefficient for interaction term [birth weight  $\times$  risk score] =  $-0.10$ , 95% CI  $-0.16$  to  $-0.04$ ,  $P = 0.001$ ).

### Birth Weight and Risk Factors

To determine whether birth weight was acting as a surrogate marker for risk factors, we investigated whether there were relations between blood pressure, fasting insulin, glucose, serum cholesterol level, tobacco use (measured by number of smoking pack-years), and birth weight in the study group. Birth weight was unrelated to blood pressure (birth weight as determinant of diastolic blood pressure: coefficient  $-0.001 \text{ mm Hg} \cdot \text{kg}^{-1}$ , 95% CI  $-0.008$  to 0.006,  $P = 0.75$ ) even when current body size was taken into account, nor were there associations between birth weight and any of the other risk factors (birth weight as determinant of: insulin, coefficient  $-0.006 \text{ mU} \cdot \text{L}^{-1} \cdot \text{kg}^{-1}$ , 95% CI  $-0.017$  to 0.005,  $P = 0.26$ ; glucose, coefficient  $0.011 \text{ mmol} \cdot \text{L}^{-1} \cdot \text{kg}^{-1}$ , 95% CI  $-0.088$  to 0.111,  $P = 0.82$ ; body mass index, coefficient  $0.024 \text{ m}^{-2}$ , 95% CI  $-0.056$  to 0.103,  $P = 0.55$ ; smoking pack-years, coefficient  $-0.187 \text{ years/kg}$ , 95% CI  $-0.738$  to 0.363,  $P = 0.50$ ).

### Discussion

This study shows that birth weight, a measure of growth in utero, is related to endothelial function in the systemic



**TABLE 2. Regression Coefficients (Plus 95% CI and P Values) for Association Between FMD and Birth Weight for Each Third in the Distribution of Key Risk Factors**

	Regression Coefficient	95% CI	P
Fasting insulin			
1	0.403	0.112 to 0.694	0.007
2	0.126	-0.142 to 0.422	0.40
3	-0.050	-0.366 to 0.262	0.74
Body mass index			
1	0.491	0.199 to 0.783	0.001
2	0.096	-0.169 to 0.360	0.50
3	-0.111	-0.466 to 0.244	0.54
LDL cholesterol			
1	0.251	-0.070 to 0.571	0.12
2	0.261	-0.038 to 0.360	0.09
3	0.048	-0.263 to 0.358	0.76
Systolic blood pressure			
1	0.176	-0.180 to 0.532	0.33
2	0.292	0.015 to 0.568	0.04
3	0.050	-0.256 to 0.342	0.76
Exercise capacity			
1	0.450	0.139 to 0.760	0.005
2	0.082	-0.215 to 0.380	0.58
3	0.052	-0.258 to 0.362	0.74
Nonsmokers	0.194	-0.049 to 0.436	0.10
Smokers	0.220	-0.078 to 0.578	0.10
Risk score			
1	0.393	0.141 to 0.644	0.003
2	0.176	-0.107 to 0.459	0.22
3	-0.226	-0.633 to 0.180	0.27

Row 1 indicates low risk; row 3, high risk. Coefficient units are in kg<sup>-1</sup>.

arteries of young adults in their third decade of life. However, in the presence of increased levels of classic risk factors, this influence of birth weight on endothelial function is overwhelmed rather than amplified. In individuals with favorable risk profiles, the birth-weight effect continues to have a significant adverse impact on vascular function, with a difference in endothelial function across the birth-weight range that was as great as the effect of smoking. A 1-kg difference in birth weight led to a change in FMD that was equivalent to 4.5 cigarette pack-years.

Coronary heart disease remains one of the leading causes of mortality in the world.<sup>1,25</sup> Interventions to decrease the prevalence of smoking, hypercholesterolemia, hypertension, and glucose intolerance in the population have led to improvements in cardiovascular mortality and morbidity. Further reduction in the disease burden, however, may require that other influences on atherogenesis be investigated both early and late in its development. Genetic factors and in utero growth are of particular interest because of their consistent associations with coronary heart disease mortality and risk factors.<sup>5-10</sup> There are several possible ways in which this association with intrauterine factors could be mediated, in-

cluding programming of risk factor development or by altered susceptibility to them in later life. Alternatively, there may be a primary influence of prenatal factors on the atherosclerotic process itself from early in the disease.

Endothelial function is central to atherogenesis; in particular, endothelium-derived nitric oxide affects vascular tone, antiplatelet activity, and smooth muscle cell activity.<sup>26-28</sup> Endothelial dysfunction is seen in subjects with coronary heart disease and its risk factors. We have developed a noninvasive ultrasound test to assess endothelial function in vivo<sup>22,23</sup> and have shown abnormal nitric oxide-dependent responses in conduit arteries from remarkably early in life, associated with factors known to increase risk of later vascular disease.<sup>29-31</sup> Using this method, we have also demonstrated a relation between birth weight and endothelial function in children in the first decade.<sup>14</sup> This finding was replicated in a small, independent study of subjects without risk factors in the second decade of life.<sup>32</sup> Because the risk factor burden in these populations was low, it is likely that the effect of birth weight on endothelial function was not dependent on the presence of adverse cardiovascular risk factors at this early stage.

Our present study extends these findings to a representative cohort who were 10 to 20 years older and enabled us to address the questions of whether aging influenced the strength of the association and how the relation would be affected by emerging classic risk factors. The findings show that the relation is still present in early adult life and is strongest in those with lower cardiovascular risk profiles. This suggests the continued association does not operate via increased risk factors, such as cholesterol and blood pressure, and that early growth may have a direct influence on the blood vessel wall. Birth weight had a relationship with endothelial function in the low-risk 20- to 28-year-olds that was comparable to that found in our previous study of 9- to 11-year-old children.

The relation between birth weight and vascular function may result from programming of the vascular wall by fetal nutrition or environment, or it may be a marker for a genetic association between early growth and endothelial function. Genes controlling insulin sensitivity have been proposed as a potential link,<sup>33</sup> and those directly involved in vascular endothelial physiology, such as the gene encoding endothelial nitric oxide synthase, may be of relevance.<sup>34</sup> The association between birth weight and endothelial function in our population was not trivial. Being in the lowest as opposed to the highest fifth for birth weight had an equivalent influence on the vascular endothelium as starting smoking. The narrow range of many acquired risk factors in this "healthy" population precluded a quantitative comparison of the influence of birth weight with other risk factors.

Birth weight is associated with subsequent growth, and it has been suggested that the association with cardiovascular disease may be due to factors that influence postnatal growth.<sup>24,35,36</sup> This did not explain the findings of the present study, because weight at 1 year did not have an effect on the relation between birth weight and endothelial function.

The noninvasive measure of conduit artery endothelial function developed by our group is accurate and reproduc-

ible,<sup>22,23</sup> enabling the study of the determinants of vascular function and their relation to the atherosclerotic process from a very early stage. Endothelial function in the brachial artery reflects that in the coronary circulation, is associated with risk factors for atherosclerosis, and can be modified by appropriate interventions.<sup>37</sup> Only a proportion of those invited were able to attend for full vascular investigations, which increases the chance that bias was introduced into the study group. The Cambridge Young Adults cohort, however, had a range of cardiovascular risk factors and birth-related measures comparable to those found in other populations in the third decade of life.<sup>38,39</sup>

Moderate to high levels of cardiovascular risk factors are associated with abnormal endothelial function irrespective of birth weight in this and other studies. Such findings appropriately place emphasis on reduction of classic risk factors, but this can only have a limited impact on cardiovascular outcome when introduced in adult life. Low birth weight has now been associated with reduced endothelium-dependent dilation both in childhood and at 20 to 30 years of age, in subjects who would be considered at low risk for developing atherosclerosis, with an effect equal to that of a major risk factor like smoking. These findings may provide insight into the basis of the association between birth weight and coronary heart disease and are relevant to the understanding of vascular disease development in populations.

### Acknowledgments

This research was funded by the Medical Research Council. Research at the Institute of Child Health and Great Ormond Street Hospital for Children NHS Trust benefits from funding received from the NHS Executive. We would like to thank Ian Merryweather, Sarah Jones, and Tristan Cope for their assistance with recruitment, data collection, and laboratory analysis, and Dr David Muller for coordinating the biochemical work.

### References

- WHO. *World Health Report*. Geneva, Switzerland: World Health Organization; 1997.
- Dawber TR. *The Framingham Study: The Epidemiology of Atherosclerotic Disease*. Cambridge, Mass: Harvard University Press; 1980.
- Gordon T, Kannel WB. Premature mortality from coronary heart disease: the Framingham Study. *JAMA*. 1971;21:1617-1625.
- Rose G. Sick individuals and sick populations. *Int J Epidemiol*. 1985;14:32-38.
- Barker DJP. *Fetal and Infant Origins of Adult Disease*. London, UK: BMJ; 1992.
- Barker DJP, Gluckman PD, Godfrey KM, et al. Fetal nutrition and cardiovascular disease in adult life. *Lancet*. 1993;341:938-941.
- Barker DJP, Osmond C, Simmonds SJ, et al. The relation of small head circumference and thinness at birth to death from cardiovascular disease in adult life. *BMJ*. 1993;306:422-426.
- Hales CN, Barker DJP. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia*. 1992;35:595-601.
- Barker DJP, Osmond C, Golding J, et al. Growth in utero, blood pressure in childhood and mortality from cardiovascular disease. *BMJ*. 1989;298:564-567.
- Martyn CN, Barker DJP, Jespersen S, et al. Growth in utero, adult blood pressure and arterial compliance. *Br Heart J*. 1995;73:116-121.
- Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990's. *Nature*. 1993;362:801-809.
- Harris KH, MacLeod KM. Influence of the endothelium on contractile responses of arteries from diabetic rats. *Eur J Pharmacol*. 1988;153:55-64.
- Panza JA, Quyyumi AA, Bush JE, et al. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med*. 1990;323:22-27.
- Leeson CPM, Whincup PH, Cook DG, et al. Flow mediated dilation in 9- to 11-year-old children: the influence of childhood and intrauterine factors. *Circulation*. 1997;96:2233-2238.
- Yano K, Reed DM, McGee DL. Ten year incidence of coronary heart disease in the Honolulu Heart Program: relationship to biological and lifestyle characteristics. *Am J Epidemiol*. 1984;119:653-666.
- Allain CC, Poon LS, Chon CSG, et al. Enzymatic determination of total serum cholesterol. *Clin Chem*. 1974;20:470-475.
- Bousserman LL, Saritelli AL, Milosavtjevic D. High density lipoprotein subfractions measured in stored serum. *Clin Chem*. 1994;40:1713-1716.
- Becker W, Rapp W, Schenk HG, et al. Methods for the quantitative determination of plasma proteins by immunoprecipitation [in German]. *Z Klin Chem Klin Biochem*. 1968;6:113-122.
- Friedewald WT, Levy RI, Friedrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without the use of the preparative ultracentrifuge. *Clin Chem*. 1972;18:499-502.
- OPCS. 1979-80;1982-83 Occupational Mortality Decennial Suppl. HMSO: London. 1986.
- McArdle WD, Pechar GS, Katch FI, et al. Percentile norms for a valid step test in college women. *Res Q*. 1973;44:498-500.
- Celermajer DS, Sorenson KE, Gooch VM, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet*. 1992;340:1111-1115.
- Leeson CPM, Thorne S, Donald AE, et al. Non-invasive measurement of endothelial function: effect on brachial artery dilation of graded endothelial dependent and independent stimuli. *Heart*. 1997;78:22-27.
- Lucas A, Fewtrell M, Cole TJ. Fetal origins of adult disease: the hypothesis revisited. *BMJ*. 1999;319:245-249.
- WHO. *Prevention in Childhood and Youth of Adult Cardiovascular Disease: Time for Action*. Geneva, Switzerland: World Health Organization; 1990.
- Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*. 1980;288:373-376.
- Furlong B, Henderson AH, Lewis MJ, et al. Endothelial derived relaxing factor inhibits platelet aggregation. *Br J Pharmacol*. 1987;90:687-692.
- Henderson AH. Endothelium in control. *Br Heart J*. 1991;65:116-128.
- Celermajer DS, Sorenson KE, Georgakopoulos D, et al. Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation*. 1993;88:2149-2155.
- Sorenson KE, Celermajer DS, Georgakopoulos D, et al. Impairment of endothelium-dependent dilation is an early event in children with familial hypercholesterolemia and is related to lipoprotein(a) level. *J Clin Invest*. 1994;93:50-55.
- Clarkson P, Celermajer DS, Donald AE, et al. Impaired vascular reactivity in insulin-dependent diabetes mellitus is related to disease duration and low-density lipoprotein cholesterol levels. *J Am Coll Cardiol*. 1996;28:573-579.
- Goodfellow J, Bellamy MF, Gorman ST, et al. Endothelial function is impaired in fit young adults of low birth weight. *Cardiovasc Res*. 1998;40:600-606.
- Hattersly AT, Tooke JE. The fetal insulin hypothesis: an alternative explanation of the association of low birthweight with diabetes and vascular disease. *Lancet*. 1999;353:1789-1792.
- Hingorani AD, Liang CF, Fatibene J, et al. A common variant of the endothelial nitric oxide synthase (Glu298→Asp) is a major risk factor for coronary heart disease in the UK. *Circulation*. 1999;100:1515-1520.
- Barker DJP, Winter PD, Osmond C, et al. Weight in infancy and death from ischaemic heart disease. *Lancet*. 1989;2:577-580.
- Taylor SJ, Whincup PH, Cook DG, et al. Size at birth and blood pressure: cross sectional study in 8- to 11-year-old children. *BMJ*. 1997;314:475-480.
- Clarkson P, Adams MR, Powe AJ, et al. Oral L-arginine improves endothelium-dependent dilation in hypercholesterolemic young adults. *J Clin Invest*. 1996;97:1989-1994.
- OPCS. *Health Survey of England 1991*. London, UK: HMSO; 1993.
- OPCS. *Health Survey of England 1995*. London, UK: HMSO; 1997.