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# Birth size, blood pressure and glucose tolerance in twins: testing the fetal origins hypothesis

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ABSTRACT

FACULTY OF MEDICINE
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# BIRTH SIZE, BLOOD PRESSURE AND GLUCOSE TOLERANCE IN TWINS: TESTING THE FETAL ORIGINS HYPOTHESIS

By Janis Baird

There is growing evidence that non-insulin dependent diabetes and hypertension are linked to impaired fetal growth as indicated by small size at birth. The fetal origins hypothesis suggests that these diseases result from the persistence of fetal adaptations to an adverse early environment. However, evidence from twin studies, showing higher concordance in monozygotic twins than dizygotic twins, has suggested that genetic factors play an important role in the aetiology of both non-insulin dependent diabetes and hypertension. Twins have a lower mean birth weight than singletons and two thirds of monozygotic twins are monochorionic: that is they share a placenta and therefore have to compete for nutrients. Further more, they are perfectly matched for many of the characteristics that are thought to confound the association between birth size and adult disease in singletons. Twins therefore provide a tool to explore the association between birth size and adult disease and to address some of the potential challenges to the fetal origins hypothesis. The aim of this study was to explore two main hypotheses. Firstly, that the greater similarity in glucose tolerance and blood pressure in monozygotic twins compared with dizygotic twins is due to their more similar prenatal environment. Secondly, that within pair differences in glucose tolerance and blood pressure would be determined by differences in size at birth.

A longitudinal study of births in the city of Birmingham from 1950 onwards provided the sampling frame for the study and allowed a population-based sample of twins to be studied. Twins born between 1950 and 1954 were identified and followed up. They were visited at home where their blood pressure was measured. They were then invited to attend a clinic for an oral glucose tolerance test.

Adult levels of glucose tolerance and blood pressure were more highly correlated in the monozygotic than dizygotic twins. These trends were not explained by size at birth. Examination of within pair differences in glucose tolerance revealed significant associations between size at birth and insulin resistance in the monozygotic twins. However, inconsistencies in these trends and the fact that they were based on small numbers of twin pairs weakened these findings. Further more, the well documented inverse associations between birth size and adult glucose tolerance and blood pressure in singleton populations were absent in the twins.

The findings of this study were not consistent with the fetal origins hypothesis. Although this may be partly explained by limitations of the study, it is possible that small size at birth has different implications in twins than in singletons. Twins may down-regulate their growth as a protective mechanism against fetal undernutrition. The association of within pair differences in birth size and insulin resistance in monozygotic twins does, however, suggest an effect of birth size that is independent of genetic factors.

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# 1 INTRODUCTION

There is accumulating evidence that coronary heart disease, non-insulin dependent diabetes and hypertension, three common diseases of adult life, are linked to impaired fetal growth. The evidence initially came from UK studies of men and women in middle-age in whom strong inverse association between size at birth and adult levels of glucose tolerance and blood pressure were observed. These findings have been widely replicated in studies of populations in other European and non-European countries. The accumulating evidence for an association between size at birth and disease in adult life led to the formulation of the 'fetal origins hypothesis' which states that undernourishment of the fetus in utero programmes disease in later life due to the persistence of fetal adaptations to an adverse early environment.[1] However, a number of alternative explanations for the birth size/adult disease association have been proposed. These include the role of factors that may confound the association including socio-economic factors and adult lifestyle.[2] It has also been proposed that genetic factors may account for the observed association in that genetic influences that first lead to growth failure in early life may also lead to degenerative diseases in later life.[3] The evidence from twin studies showing higher concordance for diabetes and hypertension in genetically identical monozyogtic twins than in dizygotic twins supports the importance of genetic factors in the aetiology of these diseases.[4;5] However two thirds of monozygotic twins are monochorionic: that is they share a placenta and have vascular connections between their circulations. This results in competition for nutrients and may expose them to a more adverse prenatal environment than dichorionic twins where each twin has a separate placenta. It has been proposed that previous twin studies may have been confounded by the effects of early environment and that the higher concordance levels for disease observed in monozygotic twins may be explained by their more adverse and similar prenatal environment.[6]

Twins have a lower mean birth weight than singletons being on average 900g lighter at birth.[7] Observational studies have shown that the rate of growth of twins is slower than that of singletons during the last trimester of pregnancy and there is some evidence from serial utrasound studies that they may down-regulate their growth as early as the first trimester. Further more, twins are perfectly matched for many

characteristics that are thought to confound the association between birth size and adult disease in singletons. Twins therefore provide a tool to explore the associations between prenatal environment and adult disease and to address some of the potential challenges to the fetal origins hypothesis. In particular, since monozygotic twins can be regarded as genetically identical, twin studies provide a mechanism for controlling for genetic factors. Exploration of within pair differences in glucose tolerance and blood pressure in monozygotic twins provides a means of establishing whether the effects of early environment are independent of genetic factors.

## 1.1 The fetal origins of adult disease

### 1.1.1 Geography of coronary heart disease

Geographical studies provided the first clues to the possible importance of early life in determining adult disease. These studies were undertaken in an attempt to gain a better understanding of the aetiology of coronary heart disease since the epidemiology of the disease is poorly explained by known risk factors such as cigarette smoking, obesity and raised cholesterol levels.

Coronary heart disease is a disease of affluence, with increasing rates of disease being associated with increasing prosperity and yet, paradoxically, its incidence is highest in poorer areas of the United Kingdom. Geographical studies revealed that mortality from coronary heart disease between 1968-78 was highest in areas that had high infant mortality in 1921-2.[8] One possible explanation for this is that events in early life determine an individual's risk of developing heart disease. More detailed analysis revealed that neonatal mortality (that is deaths in the first month of life) seemed to have a stronger association with coronary heart disease deaths than post-neonatal mortality (deaths from one month to one year of age).[9] This observation suggested that factors associated with prenatal life played a greater part in determining later risk of coronary heart disease than the postnatal environment.

### 1.1.2 Birth size and coronary heart disease

The geographical trends suggesting a link between prenatal environment and adult disease led to a systematic search of archives and hospital record departments for birth records of subjects in middle and old age. Three groups of records were identified in Hertfordshire, Preston and Sheffield. The Hertfordshire records were

maintained by health visitors who weighed all babies born in the county from 1911 onwards. All babies, whether born in hospital or at home, were weighed at birth and again at one year. The records identified in Preston and Sheffield had more detailed obstetric information that documented body proportions at birth but were only based on births within hospital.

A series of retrospective cohort studies were then undertaken in which birth measurements, used as a measure of fetal growth, were related to adult cardiovascular disease. In the first study in Hertfordshire, 6,500 men born in eight districts of the county between 1911 and 1930 were followed up.[10] Standardised mortality ratios fell steeply as birth weight and weight at one year increased. The trend was strongest in relation to weight at one year. Similar findings were seen in women. The combined mortality data for men and women is shown in table 1.1.

**Table 1.1: Death rates from coronary heart disease among 15,726 men and women according to birth weight**

| Birth weight (lbs) | Number of people | Standardised mortality ratio | Number of deaths |
|---|---|---|---|
| ≤5.5 | 765 | 100 | 57 |
| -6.5 | 2385 | 81 | 137 |
| -7.5 | 4947 | 80 | 298 |
| -8.5 | 4698 | 74 | 289 |
| -9.5 | 2056 | 55 | 103 |
| >9.5 | 875 | 65 | 57 |
| All | 15,726 | 74 | 941 |

A subsequent study in Sheffield, where gestational age had been recorded, showed that it was people who were small at birth because they failed to grow rather than because they were born prematurely, who were at increased risk of disease.[11]

Support for these findings from the Medical Research Council (MRC) in Southampton has come from the work of other groups. In an attempt to replicate the finding that lower birth weight was associated with increased morbidity and mortality from coronary heart disease, Frankel and colleagues explored the association in a large cohort of men based in the town of Caerphilly, South Wales.[12] Although ascertainment of birth weight was based on recall by the subjects' mother or close relative, the validity of reported weights was supported by strong graded associations between birth weight and anthropometric measures in adulthood. A strong inverse relationship was found between fatal and non-fatal coronary heart disease and birth weight ($p=0.01$) and this could not be explained by social or behavioural variables, or other risk factors operating in adult life.

The association between low birth weight and coronary heart disease was also shown in a large scale study of 70,000 female nurses in the United States aged between 46 and 71 years.[13] The observed associations in this study for non-fatal cardiovascular disease (coronary heart disease and stroke) were very similar to those seen in Hertfordshire women with respect to fatal disease. The association was largely determined by the 13% of women born at the extremes of birth weight. Relative risks of 1.49 (95% confidence intervals1.05 to 2.10) were seen for women with birth weights less than 2268g. Women who weighed more than 4536g at birth had a much lower relative risk of 0.68. Since the study relied on subjects' recall of their own birth weight there was a potential for bias but, as the authors point out, any misclassification of birth weight is likely to have been random with respect to disease and, if anything, would have led to underestimation of relative risks. The association was not weakened by controlling for childhood socio-economic group or adjusting for adult lifestyle.

The findings of the MRC studies together with those of other groups led to studies to explore the underlying mechanisms for the associations between low birth weight and weight at one year and risk of death from coronary heart disease. These studies

revealed that the trends in coronary heart disease and birth weight were paralleled by similar trends in two of its major risk factors: hypertension and non-insulin dependent diabetes.

### 1.1.3 Birth size and glucose tolerance

In a study of men aged 59 to 79 years, born and still living in East Hertfordshire, a strong association was found between low birth weight and the development of impaired glucose tolerance and non-insulin dependent diabetes in adult life.[14] A similar inverse association was seen with weight at one year. In the 370 men who had complete glucose tolerance tests, the percentage with impaired glucose tolerance (plasma glucose of 7.8 – 11.0 mmol/l at 2 hours) or non-insulin dependent diabetes (11.1mmol/l or greater at 2 hours) fell progressively with increasing birth weight and increasing weight at one year of age. The relative risk of impaired glucose tolerance or diabetes was six times higher in those who weighed 5.5lb (2.5kg) or less at birth than in those who weighed more than 9.5lb (4.3kg) as shown in table 1.2..

**Table 1.2: Percentage of men (mean age 64 years) with impaired glucose tolerance or diabetes according to birth weight**

| Birth weight (lb) | Number of men | Percentage of men with 2-hour glucose of: | | | Odds ratio adjusted for BMI (95%CI) |
|---|---|---|---|---|---|
| | | 7.8-11.0 | ≥11.1 | ≥7.8 | |
| ≤5.5 | 20 | 30 | 10 | 40 | 6.6 (1.5-28) |
| 5.6-6.5 | 47 | 21 | 13 | 34 | 4.8 (1.3-17) |
| 6.6-7.5 | 104 | 25 | 6 | 31 | 4.6 (1.4-16) |
| 7.6-8.5 | 117 | 15 | 7 | 22 | 2.6 (0.8-8.9) |
| 8.6-9.5 | 54 | 4 | 9 | 13 | 1.4 (0.3-5.6) |
| >9.5 | 28 | 14 | 0 | 14 | 1.0 |
| All | 370 | 18 | 7 | 25 | P value for trend <0.001 |

The associations between weight at birth and at one year and adult IGT and diabetes were independent of subjects' current body mass index and were seen in each social class. When subjects were divided according to weight at one year and current body mass index, two-hour plasma glucose was found to be highest in those who had the

lowest weight at one year but the highest current body mass. Conversely the lowest two-hour plasma glucose was observed in those subjects with the highest weight at one year and lowest current body mass. These findings suggest that greater fetal and infant growth protect against the effect of higher body mass in adult life while lower body mass protects against the adverse effects of reduced early growth.

A similar study of women aged 60 to 71 years in East Hertfordshire confirmed the association between birth weight and impaired glucose tolerance.[15] The findings in the women differed, however, in that the relation between low birth weight and raised plasma glucose and insulin was strongest in the fasting specimens rather than those at two hours. It is unclear why these differences were seen. However, the picture observed in women is more likely to be seen in the presence of insulin resistance. Fasting levels of insulin and its precursors (proinsulin and split proinsulin) have been shown to be correlated with insulin resistance both in normoglycaemic subjects and in those with impaired glucose tolerance.[16] Therefore, one possible explanation is that low birth weight is more strongly associated with insulin resistance in women than in men.

The Hertfordshire findings were substantiated by studies based on the records identified in Preston, Lancashire where 140 men and 126 women aged between 45 and 64 years were studied.[17] This study was important because information relating to gestational age and more detailed anthropometric measurements at birth (birth weight, length, head circumference and placental weight) were available. Evidence from animal experiments suggested that disproportionate relationships between head size, length and weight correspond to growth failure at different periods of gestation,[18] and the Preston study allowed the exploration of these associations in humans.

The prevalence of impaired glucose tolerance and non-insulin dependent diabetes in Preston fell from 27% in those who weighed 2.5kg or less at birth, to 6% in those who weighted 3.41kg or more. This trend was statistically significant and, as in Hertfordshire, remained so after adjusting for current body mass index. Two-hour plasma glucose and insulin decreased with increasing birth weight ($p=<0.004$ and $p=<0.001$ respectively). In addition, low ponderal index (birth weight/ length$^3$) which is a measure of thinness, and a high head circumference to length ratio were

independently associated with raised two-hour plasma glucose and insulin concentrations and individuals with impaired glucose tolerance and non-insulin dependent diabetes had a high ratio of placental weight to birth weight. This study confirmed the findings of the Hertfordshire study and demonstrated that the association between fetal growth and impaired glucose tolerance existed in both men and women. More importantly, by revealing that the association was independent of gestational age, the Preston study confirmed that it was mature growth retarded infants rather than premature infants in whom the birth weight / glucose tolerance association was seen. A new observation was the association of particular patterns of fetal growth: thinness and shortness at birth, with increased risk of impaired glucose tolerance in adult life revealing that it was not merely retarded growth but disproportionate growth that was associated with increased risk of diabetes.

One of the disadvantages of the studies in Hertfordshire and Preston was that they were unable to account for events occurring between infant life and adulthood that may have influenced any later predisposition to develop non-insulin dependent diabetes. In an early attempt to replicate the findings in Hertfordshire and Preston in a younger age group, a small-scale study was conducted on a sample of 40 men aged between 18 and 25 years in Southampton who underwent an abbreviated 30-minute glucose tolerance test.[19] A negative association between 30-minute glucose levels and birth weight was observed. A lkg increase in birth weight corresponded to a 1.5 mmol decrease in 30-minute plasma glucose (95% confidence intervals 0.4 to 2.6, p=0.01). This trend was independent of gestational age. The confidence intervals are wide and indicate that this association may have arisen by chance. Nevertheless this study suggests that an association exists at an early age and further work is needed in this age group to substantiate these findings.

The associations between birth size and glucose tolerance observed in Hertfordshire and Preston have been observed in many studies based on European and non-European populations. In Uppsala, Sweden, an inverse relationship between birth weight and prevalence of non-insulin dependent diabetes at 60 years of age (relative risk 1.4 for each standard deviation decrease in birth weight, p= <0.02) was observed in a cohort of men born between 1920 and 1924.[20] A stronger inverse relationship

was observed in relation to thinness and 60 minute insulin level (relative risk 1.9 for each standard deviation decrease in ponderal index, p= <0.001).

Valdez et al studied two groups of young adults: non-Hispanic Caucasians and Mexican Americans aged 31-32 years.[21] The latter group is known to have a high prevalence of non-insulin dependent diabetes. In both groups there was a strong inverse relationship between birth weight and fasting plasma insulin concentrations. Findings in relation to two-hour insulin showed a similar linear trend but did not achieve statistical significance. In addition, this study also revealed an inverse association between birth weight and insulin resistance as seen in Hertfordshire and Preston. This study was of particular note in that the group studied were young adults in whom the prevalence of chronic disease is low. These findings added more weight to the earlier findings of the small-scale study of young men in Southampton. The inverse association between birth weight and two-hour glucose has also been demonstrated in studies of Swedish middle-aged men and post-menopausal American women.[22; 23]

The association between birth weight and adult glucose tolerance was also explored in a large cohort of 1,179 Pima Indians who were participating in an established longitudinal study.[3] The Pima Indians have the highest reported prevalence (20-30%) of non-insulin dependent diabetes, which often begins at a relatively early age. Glucose tolerance was evaluated when participants in the cohort study were aged 20-39 years. A 'U'-shaped relationship between birth weight and glucose tolerance was observed. Age adjusted prevalence of non-insulin dependent diabetes at birth weights under 2.5kg was 30%, while at birth weights of 3.5–4.49 kg it dropped to 16%, increasing to 32% at birth weights of 4.5kg or more. In keeping with previous findings in Hertfordshire, the group with the highest prevalence of disease was that with the lowest birth weight but the highest current body mass index. The associations between higher birth weights and a raised prevalence of diabetes is not surprising since maternal gestational diabetes, which is associated with fetal macrosomia, is known to lead to increased prevalence of diabetes in successive generations. Exclusion of the subjects whose mothers may have suffered from gestational diabetes led to a significant reduction in prevalence of diabetes in the high birth weight group. The observation of a 'U' shaped relationship had not been noted

in UK studies probably because gestational diabetes is less prevalent. In addition, the survival of infants of diabetic pregnancies born more than 60 years ago is likely to have been poor.

Recent large-scale studies in the United States and Finland have demonstrated an inverse association between birth weight and risk of non-insulin dependent diabetes in adulthood. In a study based on a cohort of 22,846 American men, the men who weighed less than 5.5lb at birth had an odds ratio of developing diabetes of 1.75 (95% confidence intervals 1.21 to 2.54) when compared with men with birth weights in the range 7-8.4lb.[24] Although this study relied on self-reported data for birth weight and for diagnosis of diabetes, validation studies were conducted which demonstrated the reliability of this data. A similar inverse association was demonstrated in 69,526 American nurses aged between 46 and 71 years and in 7,086 men and women born in Finland between 1923 and 1933.[25;26] The association was also demonstrated in a recent study exploring the association between fetal undernutrition and the insulin resistance syndrome in 627 men and women aged 45 years who were born and still living in Bejing, China.[27] They represent a population who were born at a time of chronic malnutrition and maternal measurements indicated that many of the mothers were thin and malnourished. Glucose tolerance was measured using a standard oral glucose tolerance test. For every 1kg increase in birth weight two-hour glucose decreased by 5.1% (95% confidence intervals 0.7% to 9.3%). In addition, low maternal body mass index in early and late pregnancy was associated with higher levels of plasma glucose, insulin and triglyceride levels. These findings suggest that maternal undernutrition prior to and during pregnancy may lead to greater risk of the insulin resistance syndrome.

A further study from the MRC in Southampton explored the association between birth size and non-insulin dependent diabetes in 506 men and women aged 47 years born in a hospital in Mysore, South India. The epidemic of non-insulin dependent diabetes in urban Indians is well documented and was supported by the findings that prevalence of non-insulin dependent diabetes among the study population was 15%.[28] The birth weight/glucose tolerance association seen in Western populations was absent. However, higher rates of diabetes were seen in men and women who were short at birth ($p=0.07$) and who had a high ponderal index ($p=0.05$). Their mothers also

tended to be heavier during pregnancy (p=0.004). These findings appeared to suggest that babies born in South India were more likely to develop non-insulin dependent diabetes as adults if their mothers became obese during pregnancy leading to gestational glucose intolerance which in turn leads to macrosomia in the infant.

The association between low birth weight and impaired glucose tolerance has also been demonstrated in studies in children. Abbreviated 30-minute glucose tolerance tests were carried out in a study of 250 children, aged 7 years, in Salisbury, Wiltshire.[29] Children who were thinnest at birth (low ponderal index) had a higher level of plasma glucose at 30 minutes. The association was independent of gestation, gender, social class and current weight. Although this study was not based on a full two-hour glucose tolerance test, it is the rapid (within 30 minutes) response of insulin to an oral glucose load that is crucial in determining glucose tolerance. A further study was based on children aged 4 years in India who underwent a full two-hour glucose tolerance test.[30] The inverse association between 30-minute plasma glucose was observed and was independent of current size. No relation was found, however, between fasting and two-hour glucose and birth weight. In a study of 10-11 year old British school children based in 10 towns in England and Wales the relations between size at birth (birth weight and thinness) and levels of glucose and insulin were examined following a 30-minute glucose tolerance test.[31] The response rate in the study was relatively high at 64% and, although birth weight was based on maternal recall, ponderal index was established using birth records. No consistent relation was found between birth weight or ponderal index and fasting and post-load glucose. However birth weight did show an inverse relationship with fasting and post-load insulin, after adjustment for childhood height and ponderal index at birth, suggesting that low birth weight may be related to early development of insulin resistance. Although the findings in children differ from those in adults, these three studies suggest that the link between events in utero and non-insulin dependent diabetes can be observed from an early age and do not simply reflect the confounding influence of adverse environmental factors during childhood and adult life.

A recent systematic review of the evidence for the association between birth size and glucose tolerance has confirmed the weight of evidence in favour of an inverse

association in both adults and children. Of 33 studies exploring the association, the majority (27) have demonstrated an inverse association.[32]

### 1.1.4 Birth size and blood pressure

Initial studies of men and women in Hertfordshire revealed a negative association between birth weight and systolic blood pressure in adults aged 60 to 71 years.[33] Blood pressure fell progressively across the whole range of birth weights (table 1.3).

This trend was statistically significant in men but did not achieve statistical significance in women. Similar trends were seen for diastolic blood pressure although differences were stronger for systolic blood pressure. These findings were independent of current body mass index. A similar relation was also found in a large-scale national study of men and women aged 36 years.[34]

**Table 1.3: Mean systolic blood pressure in men and women aged 60 to 71 years according to birth weight**

| Birth weight (lbs) | Systolic blood pressure adjusted for sex (mm Hg) | Number of individuals |
|---|---|---|
| -5.5 | 168 | 54 |
| -6.5 | 165 | 174 |
| -7.5 | 165 | 403 |
| -8.5 | 164 | 342 |
| -9.5 | 160 | 183 |
| >9.5 | 163 | 72 |
| | 164 (mean) | 1228 (total) |
| | 25 (SD) | |

Similar studies were carried out in Sheffield and Preston.[35; 36] The association between low birth weight and raised blood pressure was demonstrated in both studies and, as for glucose tolerance, was dependent on babies who were small for dates rather than those who were premature. Strong inverse associations were demonstrated between

birth weight and shortness at birth and adult blood pressure in 337 men and women born in Sheffield. After adjustment for potential confounding factors (current body mass index, alcohol intake, sex and gestational age), systolic blood pressures were 17mm Hg higher and diastolic pressures 10mm Hg higher in those whose birth weight had been less than 5.5lb compared with those who had weighed more than 8.5lb. Similar trends were seen in relation to length at birth as determined by crown-heel length. In the earlier study in Preston the same inverse association between birth weight and blood pressure had been observed. In addition, thinness at birth and greater placental weight were also associated with higher blood pressure in adult life. Adult systolic blood pressure was related to thinness at birth, as indicated by a low ponderal index, in those babies with placental weights of 1.25lb (567.5g) or less. The highest blood pressures and highest risk of hypertension were among people who had been small babies with large placentas.

A study of 4 year old children born in Salisbury showed the same opposing association with birth weight and placental weight as was found in the 50 year old men and women in Preston.[37] These findings were thought to suggest that the undernourished fetus was placing increased demands on the placenta thus leading to increased placental weight. However, the findings of a systematic review of the evidence for associations between birth size and adult blood pressure have recently shown that there is no consistent trend for a relation between placental weight and adult blood pressure.[38]

Of the cardiovascular risk factors, blood pressure has been the most extensively investigated by the MRC and by other groups. Two systematic reviews of the published evidence have been conducted both of which confirm the negative relation between birth weight and systolic blood pressure in children and adults.[38; 39] The first review of publications up to 1996 included 44 papers describing the relation between birth weight and blood pressure. The majority (35) were cohort studies, while four were longitudinal and five were case-control or comparative studies. The second and more recent review included a further 34 studies published between March 1996 and August 1999.

Fourteen studies published before 1996 described the linear regression of blood pressure on birth weight and current size (body mass index in adults and current weight in children). Most studies in children reported a decrease of 2 to 3mm Hg in systolic blood pressure per 1kg increase in birth weight. Regression coefficients in adults were all negative and were commonly around 2 to 3mm Hg, the size of the coefficients tending to increase with increasing age of the subjects. Similar trends were seen in the 19 studies reporting regression coefficients in the later systematic review with all but one regression coefficient being negative after adjustment for current size. Reported regression coefficients were, however, smaller with a 1 to 2mmHg decrease in blood pressure per 1kg increase in birth weight. Two studies in adolescents in the 1996 review showed inconsistent findings. The first showed conflicting findings for boys and girls. The other showed a negative coefficient for both sexes combined. The majority of studies of adolescents performed since 1996 have shown that the inverse association between birth weight and blood pressure does exist but regression coefficients are often smaller than in pre-pubertal children or adults. It is suggested that this may be due to perturbed tracking of blood pressure during the adolescent growth phase.

In the 1996 review, 26 studies described the relation of birth weight and blood pressure as a quantitative difference in mean blood pressure between birth weight groups or in terms of the direction of the relationship. The adult studies confirmed the negative association although only one had adjusted for current body size. In children the association was also negative. In neonates, however, the majority of studies showed a positive relation between birth weight and blood pressure and in two out of three studies in adolescents the relation was positive. This positive association between birth weight and blood pressure in neonates was also confirmed in four studies published since 1996.

These findings suggest that the negative association between blood pressure and birth weight exists through childhood and in adult life other than in the neonatal period. Evidence from longitudinal studies confirms this pattern. Law et al found a positive relation between birth weight and blood pressure up to the age of six months in children of both sexes who were assessed at 13 different time-points. After the first six months the relation became negative.[33] These findings were supported by a

longitudinal study by Launer et al who reported a positive regression coefficient at one week of age and a negative coefficient at 13 to 25 weeks of age.[40] At four years they found a 'U' shaped relation with higher blood pressure at low and high birth weights. In the nine United Kingdom towns study, Whincup et al found negative coefficients at age five to seven years and nine to eleven years.[41;42] A further six longitudinal studies have been conducted since 1996. Three studies reported consistent inverse associations between birth weight and systolic blood pressure in later life. However, two reported no association and one reported a weak inverse association in men only.

The evidence for associations between other measures of birth size (length, ponderal index , head circumference and placental weight) was also recently reviewed.[38] Head circumference was consistently inversely associated with systolic blood pressure with a 0.5mm Hg decrease in blood pressure per cm increase in head circumference. However, there were no consistent trends for associations between length at birth, thinness at birth or placental weight and adult blood pressure.

### 1.1.5 Birth size and insulin resistance

There is increasing evidence that insulin resistance plays an important role in the link between low birth weight and diabetes. Insulin resistance is an early metabolic defect that precedes non-insulin dependent diabetes and impaired glucose tolerance. Low birth weight is associated with a higher prevalence of Syndrome X (the co-existence of raised blood pressure, glucose intolerance and dyslipidaemia) which in turn is known to be associated with insulin resistance.

In Hertfordshire, Syndrome X was found to be most prevalent in those with birth weights of 5.5lb (2.5kg) or less.[43] The percentage of men with Syndrome X (defined as two-hour glucose concentration of 7.8mmol/l or more, systolic blood pressure of 160mm Hg or more or currently receiving treatment for hypertension and serum triglyceride concentration of 1.4mmol/l or more) fell progressively from 30% in those who had birth weights of 5.5lb or less to 6% in those who weighed 9.5lb or more. The corresponding odds ratio, adjusted for body mass index, was increased 18-fold in the group with lowest birth weight. Similar trends were seen in Preston where subjects who had a low birth weight, or were thin at birth, had a high prevalence of

Syndrome X.[43] These findings have also been confirmed in a study of 30-year old Mexican-American and non-Hispanics in San Antonio, Texas.[21]

These three studies suggested a link between reduced fetal growth and insulin resistance. This led to a study to determine whether there was an association between body size at birth and adult levels of insulin resistance in a sample of men and women who took part in the Preston study.[44] Measurements of insulin resistance were carried out using insulin tolerance tests in 103 men and women aged 50 years, 81 of whom were normoglycaemic and 22 glucose intolerant. The study showed that people who were thin at birth were insulin resistant in adult life, independent of body mass index. Subjects who were thin at birth but obese as adults were the most resistant to insulin.

More recent studies have also suggested that birth size is associated with insulin resistance in both adults and children. The association was confirmed in a cohort of men with a mean age of 70 years who were born in Uppsala, Sweden.[45] Insulin sensitivity was measured in 696 of the men using a euglycaemic clamp. Men with the lowest birth weights were the most insulin resistant as adults. The association was strongest in the men who were obese at 70 years of age. A Danish study of 331 men and women aged 18 to 32 years revealed that the association between birth weight and insulin resistance existed in a younger age group.[46] Hofman et al carried out intravenous glucose tolerance tests on a group of short pre-pubertal children and compared those who were growth retarded at birth (less than the 10$^{th}$ centile) with those whose birth weights fell in the normal range.[47] They found that the group who were growth retarded at birth were significantly less insulin sensitive than the other group. Although the sample size in this study was small (15 and 12 subjects in the growth retarded and normal birth weight groups respectively), these findings suggests that fetal undernutrition is associated with insulin resistance even in childhood.

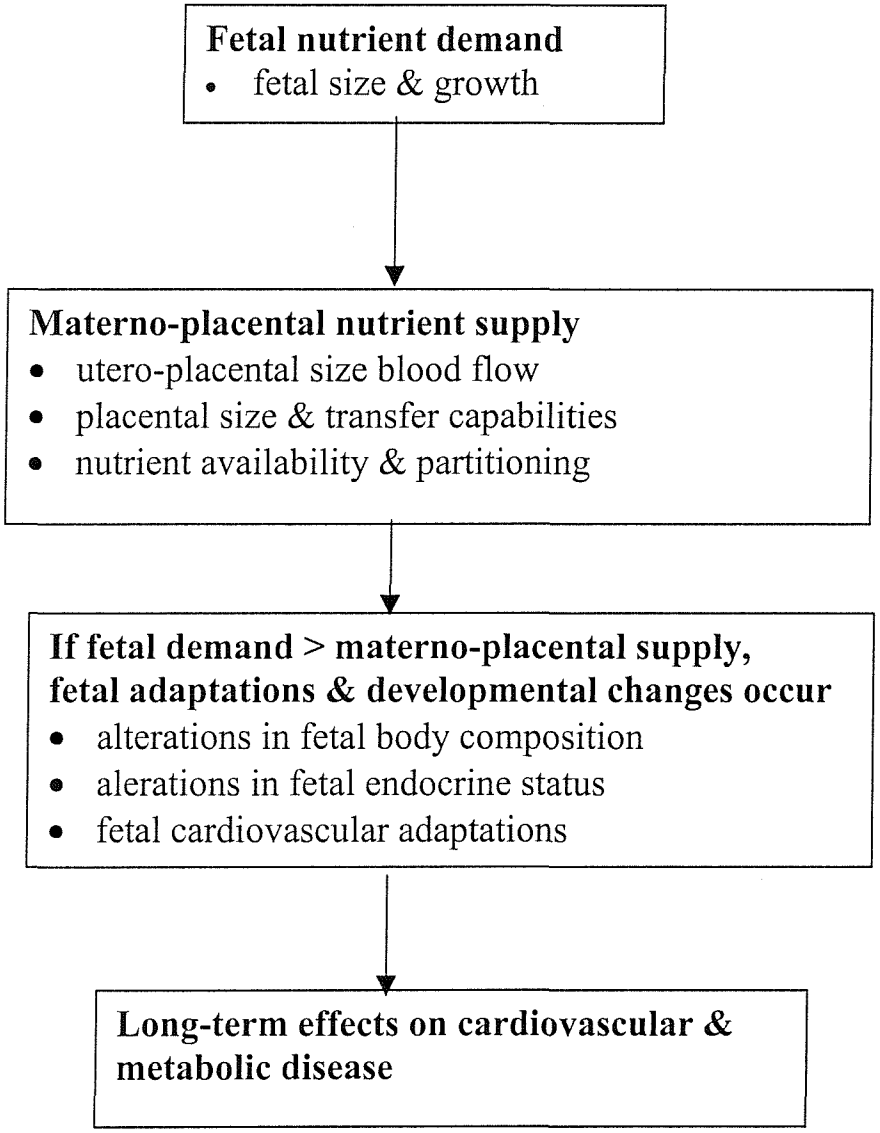
### 1.1.6 The fetal origins hypothesis

The accumulating evidence for an association between size at birth and disease in adult life led to the formulation of the fetal origins hypothesis which states that undernourishment of the fetus in utero programmes disease in later life.[1] The principle behind programming, as proposed by Lucas, is that a stimulus or insult at a critical period of development has lasting or lifelong significance.[48] During intra-

uterine life the tissues of the body grow during periods of rapid cell division or 'critical periods'. Growth depends on nutrients and oxygen and the fetus' main adaptation to lack of these is to slow its rate of cell division, especially in those tissues which are undergoing 'critical periods' at the time. Undernutrition slows cell division either as a direct effect or through altered concentrations of growth factors or hormones.

It is thought that the nutritional, hormonal and metabolic environment offered by the mother permanently affects the structure and function of the fetus, so programming it to develop disease in later life. The processes which could explain the link between reduced fetal growth and adult disease are not understood but are currently under intensive investigation. A number of factors can cause undernourishment of the fetus including poor maternal diet, poor nutritional reserves in the mother, inadequate uterine blood flow or defects in passage of nutrients across the placenta (figure 1.1). If fetal demand outstrips supply of nutrients then this could lead to fetal adaptations and developmental changes including alteration in body composition, alterations in fetal endocrine status and fetal cardiovascular adaptations which then have long-term effects on the likelihood of cardiovascular and metabolic disease.

**Figure 1.1: The framework of ideas underlying the fetal origins hypothesis**




### 1.1.7 Evidence to support programming of disease

Although the mechanisms linking low fetal growth with adult disease are still poorly understood, evidence to support the framework of ideas described above comes from experimental work in animals which offers numerous examples of programming of disease by undernutrition in utero. In addition, recent evidence from studies in humans populations subjected to famine during the World War Two has confirmed the importance of fetal undernutrition in determining adult disease.

### 1.1.7.1 Animal studies

The evidence for the role of early life in determining adult disease is reinforced by experimental studies in animals. Animal experiments offer numerous examples of programming. Young female rats injected with testosterone at a period shortly after birth develop normally until puberty but then fail to ovulate and exhibit abnormal sexual behaviour.[49] Similarly a critical period for development of sexual characteristics exists in humans where the secretion of testosterone by the fetal testis, at a critical period during gestation, leads to development of male internal genitalia. It has also been shown that periods of undernutrition during critical periods of development may permanently reduce the numbers of cells in particular organs.[50]

A number of experiments have shown that undernutrition for even brief periods in utero leads to persisting changes in blood pressure and insulin responses to glucose. Programming of blood pressure in response to early undernutrition has been demonstrated in female rats. Woodall et al subjected pregnant rats to severe dietary restriction. This led to raised blood pressure in the offspring (6mm Hg higher than the control group) when fully mature.[51] In another study pregnant rats fed an iron deficient diet gave birth to low birth weight rats who developed increased blood pressure in association with postnatal catch up growth.[52] Langley and Jackson fed pregnant rats suboptimal diets, with differing protein contents, before mating and throughout pregnancy. Normal feeding was restored after birth and the offspring were allowed to develop normally. Nine weeks after birth, the offspring of the groups fed on a low protein diet had significantly higher systolic blood pressure than the offspring of those on a normal diet. These differences persisted until the rats reached maturity.[53]

Nutritional programming of carbohydrate metabolism has been demonstrated in both small animal models such as the rat and in larger models, namely the sheep and pig. Protein energy undernutrition of the pregnant ewe in mid to late gestation leads to intra-uterine growth retardation and reduced insulin secretion in response to glucose in offspring close to term.[54] In one experiment young rats were exposed to a low protein diet.[55] This resulted in transient impaired glucose tolerance and persistent blunting of the insulin secretory response despite a return to a normal diet later. A later experiment showed that the offspring of rats fed on a low protein diet during

pregnancy showed reduction in pancreatic cell proliferation, reduction in islet size and vascularisation, and reduced pancreatic insulin content.[56] Female rats fed with a low protein diet developed subnormal insulin response and reduced β-cell mass.[57] These changes were maintained despite the resumption of normal diet and when these rats became pregnant their offspring were macrosomic with increased β-cell mass and increased pancreatic insulin content. Furthermore, Van Assche and Aerts were able to demonstrate that the effects of a low protein diet on female rats not only influenced carbohydrate metabolism in their offspring but also in future generations.[58] It has also been shown experimentally that pregnant sows, deprived of protein, give birth to viable but growth retarded offspring.[59] In addition, piglets placed on a low protein diet at weaning, become glucose intolerant and have abnormalities in pancreatic islet structure later in life.[60]

### 1.1.7.2 Studies of human famine

A unique opportunity to study the potential link between maternal nutrition during specific periods of gestation and glucose tolerance in later life was provided by a study based on 702 adults aged around 50 years who were born at the time of the Dutch winter famine of 1944.[61] It occurred when the occupying German army blockaded the western part of the Netherlands at the end of World War Two resulting in acute famine. The calorie intake of pregnant women fell at times to 700 kilocalories per day which is about 30% of the recommended daily intake in pregnancy.

Glucose and insulin responses were investigated in men and women who had been exposed to famine at any point during gestation and in those who were born in the year before or conceived in the year after the famine. Significantly higher two-hour plasma glucose levels were seen among the group exposed to famine. Levels were highest in men and women who were exposed to famine during mid and late gestation. The mean two-hour glucose in non-exposed participants was 5.8mmol/l. In those exposed to famine in late gestation, mean levels were 0.5mmol/l higher (95% confidence intervals 0.1 to 0.9) and in those exposed in mid gestation they were 0.4mmol/l higher (95% confidence intervals 0 to 0.8). Adults who were born as thin babies (with low ponderal index) to mothers with low body weights had the highest

two-hour glucose concentrations which were especially high among those who were exposed to famine and then became obese as adults.

The importance of this study is that it provided more direct evidence to support the theory that poor maternal nutrition during pregnancy leads to permanent changes to the fetal metabolic control systems. It suggested that undernutrition at any stage of gestation is linked with reduced glucose tolerance and evidence of insulin resistance in the offspring.

An earlier study based on the more severe World War Two siege of Leningrad failed to show an association between maternal malnutrition during pregnancy and the development of cardiovascular disease, glucose intolerance or hypertension in adult life.[62] However the sample studied was predominantly female and no birth weight data was available. In addition, the famine in Leningrad was of much longer duration, lasting from 1941 to 1944, compared with the five month duration of the Dutch hunger winter, and it preceded the siege. The situations in the two studies are therefore different in that the Leningrad population were chronically malnourished compared with the Dutch who were a well-fed population subjected to acute malnutrition.

### 1.1.8 Underlying mechanisms

Current work to explore the underlying mechanisms for the association between birth size and adult disease is focusing on the role of hormonal programming. Evidence from animal experiments and preliminary findings in humans suggest that impaired fetal nutrition may lead to alterations in the neuroendocrine development of the fetus which may result in long term changes in the hormonal systems which regulate metabolism. The hypothalamic-pituitary-adrenal axis (HPAA) may be particularly important since raised levels of corticosteroids are known to affect carbohydrate metabolism and cause raised blood pressure. In rats fetal growth retardation induced by dexamethasone leads to permanently increased HPAA activity resulting in raised levels of circulating corticosteroids. This in turn results in glucose intolerance and hypertension in the rats. Preliminary findings in humans seem to support the importance of the HPAA in linking fetal undernutrition and adult disease. Fasting plasma cortisol levels were measured in 370 men born in Hertfordshire.[63] The mean

age of the subjects was 64 years. An inverse relationship between birth weight and fasting cortisol was found which was independent of age and adult body mass index. The highest cortisol levels were seen in the group with birth weights of 5.5lb or less and the lowest in those with birth weights of 9lb or more (p for trend 0.02 after adjusting for body mass index). However, the low birth weight group was small with only 20 subjects and these findings were based on men only.

One study in children has also explored the association of size at birth with the HPAA.[64] A cohort of children born in Salisbury with a mean age of 9 years collected 24 hour urine samples in order to assess the relationship between size at birth and urinary excretion of adrenal androgens and glucocorticoid metabolites. Higher levels of metabolite excretion were seen in the lighter children: a 1kg increase in birth weight was associated with a 40% increase in metabolite excretion (95% confidence intervals 9 to 79). The studies in Hertfordshire and Salisbury both suggest that the HPAA plays an important role in programming of adult disease although further studies will be needed to substantiate these findings.

## 1.2 Challenges to the fetal origins hypothesis

### 1.2.1 Confounding

A number of authors have suggested that the associations between birth size and adult disease may be due to the influence of confounding factors.[2; 65; 66] It is suggested that people exposed to an adverse environment in utero also go on to adopt a more unhealthy lifestyle as adults and that it is this adverse environment in adult life that is the main influence on later health rather than the adverse fetal environment. The fact that the associations between birth size and adult disease are independent of adult lifestyle factors such as smoking argues against this. Adult lifestyle does, however, add to the effects of intra-uterine life. The highest prevalence of impaired glucose tolerance and diabetes, for example, is seen in people who were small at birth and who become obese as adults.

Another criticism of the fetal origins hypothesis is that other unknown confounding factors related to socio-economic status in childhood and adult life are responsible for the observed associations. Although it is not possible to account for environment between birth and adulthood due to the retrospective nature of the MRC studies, the

fact that the associations are seen across all social classes would seem to argue against this. It is still possible, however, that the MRC studies do not adequately control for socio-economic deprivation. A large scale ecological study based on the population of England and Wales, carried out in 1991, suggested that socio-economic deprivation was an important factor in determining the association between infant mortality and adult coronary heart disease mortality 70 or more years later.[67] Deprivation was defined by the Carstairs index, which is based on routinely collected census variables and has been shown to be strongly related to mortality in ecological studies.[68] In the MRC studies the Registrar General's social class classification was used, which has been shown to be a relatively insensitive measure of socio-economic status.[69] As the authors of the 1991 study point out, however, the lack of a significant correlation after adjustment for the deprivation index may not necessarily indicate that factors related to infant mortality are not aetiologically important, but merely reflect the relative imprecision of the proxy measure of infant mortality compared with the deprivation score.

### 1.2.2 Genetics

An alternative explanation for many of the observed associations between body proportions at birth and later disease is that genetic influences, that first show themselves in early life as growth failure, also lead to degenerative disease in later life. It has been suggested that selective survival in infancy of those genetically predisposed to insulin resistance and diabetes provides an explanation for the observed relation between low birth weight and diabetes.[3] Insulin has a central role in fetal growth ensuring that growth rates are commensurate with the nutrient supply and insulin resistance would therefore impair fetal growth. This has been demonstrated in transgenic mice who lack key intermediates in insulin receptor signalling.[70]

The fetal genome determines growth potential in utero. However, there is evidence to suggest that genetic factors are not the most important influence in determining the growth that is actually achieved in utero. This evidence comes from animal cross-breeding experiments, from studies of half-siblings related either through the mother or father, and from embryo transfer studies.[71-73] Animal cross-breeding experiments show that it is the body proportions of the mother that determine the size of the

offspring. Experimental cross-breeding of Shire horses and Shetland ponies revealed that the foals were smaller at birth when the Shetland pony was the mother than when the Shire horse was the mother. The genetic composition of the two cross-breeds was similar and so these findings implied that the Shetland mother had constrained the growth of the fetus. Similar findings were observed in cross-breeding experiments in cattle. Studies of half-siblings related through only one parent show that half-siblings with the same mother had similar birth weights with a correlation coefficient of 0.58. However, the birth weights of half-siblings with the same father were dissimilar with a correlation coefficient of 0.1.[72] In an analysis of the familial aggregation of birth weight, 62% of the variation in birth weight was attributed to environment factors (32% to maternal health and nutritional state and 30% to unknown uterine environmental factors) and only 20% and 18% to maternal and fetal genes respectively.[74] Embryo transfer studies also confirm that it is the recipient mother rather than the donor mother that more strongly influences the growth of the fetus. A fetus transferred to a larger uterus will achieve a larger birth size as a result.[73] This evidence suggests that it is the nutritional and hormonal environment of the fetus in utero that is the dominant determinant of fetal growth.

## 1.3 Twins as a tool to explore the fetal origins hypothesis

The study of twins allows a number of challenges to the fetal origins hypothesis to be explored:

### 1.3.1 Genetic factors

The study of twins provides a potential mechanism for exploring the role of genetic factors in the observed associations between fetal growth and adult disease. Since Galton first studied twins more than a century ago, they have been used to investigate the role of genetic factors in the aetiology of many diseases.[75] The basic premise of twin studies is that monozygotic twins are genetically identical whereas dizygotic twins share the same genes as siblings and therefore differences within monozygotic pairs can be attributed to environmental factors. In fact, although the majority of cytogenetic studies in twins show that monozygotic twins are identical, there are a small number showing chromosomal differences which are thought to be due to mutations or non-disjunction occurring around the time of the twinning event.[4; 75; 76] Such mutations are nevertheless rare and are unlikely to influence the outcome of large-scale studies of twins.

Assuming that monozygotic twins are genetically identical, if associations between within pair differences in birth size and adult outcomes could be demonstrated in monozygotic twins these could be attributed to differences in prenatal environment rather than to genetic factors.

### 1.3.2 Control of potential confounding factors

The co-twin control method where co-twins are used as controls was first described by Gesell in 1942 who advocated its use in behavioural science studies.[77] Its use was later extended to observational studies where one twin but not the other has the disease or exposure of interest.[4; 78] Co-twins are used as controls because they allow matching of many factors which might potentially confound the relationship of interest.

This co-twin control method allows many of the variables which potentially confound the association between birth size and adult disease to be controlled for since twins share the same mother and, if reared together, the same family environment during

childhood. They are therefore matched for age, for maternal factors that may influence the outcome of pregnancy such as maternal nutrition and maternal smoking, for gestational age and for socio-economic factors in childhood.

### 1.3.3 Fetal growth in twins

The growth of twins in utero is retarded in comparison with that of singletons. They have a lower mean birth weight than singletons and observational studies suggest that this is due to a slower rate of growth than is seen in singletons. Wilson undertook a longitudinal study of 900 twin pairs, following them up from birth and conducting serial measurements of weight and height in order to construct growth curves. The twins were 30% lighter than singletons at birth being on average 900g lighter.[7] Naeye et al studied over 2,000 infants and found that after 33 weeks' gestation the mean weights of the twins was 10% lower than that of singletons.[79] McKeown and Record, in an earlier study of all births in the City of Birmingham in 1947 which included 22,454 singleton and 352 twin pregnancies, found the rate of growth was slower in twins from 30 weeks' gestation.[80] Similar trends were observed in a cohort of 365 twin pairs born in one American hospital over an eight year period: deviation from normal growth by singleton standards began early in the third trimester.[81] Twins also have a shorter mean gestation but this does not account for the difference in birth weight.[80; 82] In the Birmingham study, the mean birth weight of the singletons was 7.43lb compared with 5.27lb in the twins and the twins remained lighter than the singletons even when singleton weights were standardised for gestational age.[80] Although these observational studies suggest that the rate of growth in twins deviates from that of singletons from the third trimester, there is some evidence to suggest that the down-regulation of growth may begin even earlier in gestation. Over a six year period, serial ultrasound studies were carried out on 123 twin pregnancies at a teaching hospital in the United States.[83] The twin pregnancies studied were uncomplicated other than for the fact that they were multiple pregnancies. Serial ultrasound revealed that biparietal diameter measurements on the twins were consistently lower than those of singletons throughout gestation. The early down-regulation of fetal growth in twins is supported by observations in animals which also indicate that the prenatal growth of twins differs from that of singletons. Experiments in lambs suggest that twin growth is down regulated in early pregnancy to protect the fetus from the effects of later undernutrition.[84]

Twins also have more adverse perinatal outcomes than singletons having higher mortality rates and higher frequency of congenital malformations.[4; 82; 85] The higher mortality rates are largely a consequence of the fact that twins are more likely to be born prematurely. This is backed up by evidence from observational studies. In one large-scale study, 1,655 pairs of twins born in two clinical centres between 1931 and 1975 were followed up.[86] The twins had shorter gestation than singletons and were born on average three weeks earlier. Mortality in the twins was also higher and was largely due to the higher incidence of prematurity. In a more recent longitudinal study of 60,000 pregnant women in the United States data was collected in relation to 615 twin pairs. Deaths due to prematurity and congenital malformations were the most frequent causes of death in twins who died in the neonatal period.[87]

The fact that twins are growth retarded at birth in comparison with singletons appears to make them an ideal group in which to study the associations between impaired fetal growth and adult disease. In addition, since low average birth weight among twins is not a consequence of lower socio-economic status, using twins to study the fetal origins hypothesis avoids one of the concerns expressed about studies in singletons in which low birth weight is likely to be associated with social disadvantage. However, the differences between twins and singletons in terms of their intra-uterine development may limit the generalisability of findings in twins.[4; 75] This makes it potentially difficult to extrapolate findings in relation to fetal growth and adult disease to singletons.

## 1.4 Twin study evidence for genetic determinants of diabetes and hypertension

Support for a strong genetic component in the aetiology of non-insulin dependent diabetes and hypertension comes largely from twin studies which adopt a classical design. Studies of non-insulin dependent diabetes showed that concordance rates in monozygotic twins were high compared to those in dizygotic twins. Likewise, studies of blood pressure showed higher intra-class correlations for systolic blood pressure in monozygotic than in dizygotic pairs.

A small scale American study of 47 twin pairs first established that concordance rates for non-insulin dependent diabetes were higher in monozygotic than dizygotic twins.[88] This was confirmed in a British study by Pyke et al.[89] They studied a sample of 53 monozygotic pairs and reported concordance rates as high as 90% in monozygotic twins. However, this study was based on twin pairs who were selected on the basis of disease in one or both twins and this ascertainment bias is likely to have led to over-estimation of concordance rates for diabetes. In addition dizygotic twins were not studied and so no comparison of concordance rates according to zygosity could be carried out. An American study showing high concordance rates for diabetes in identical twin pairs was subject to similar ascertainment bias.[90]

More recent population based studies of twin registers in Finland and the United States, whilst confirming the higher concordance rates in monozygotic twins, found rates to be lower than previously reported, suggesting that environmental factors must also be important determinants of non-insulin dependent diabetes.[91;92] In a large study based on 13,888 same sex twin pairs born before 1958 and sampled from the Finnish twin register, the concordance rate for non-insulin dependent diabetes in monozygotic twins was 34% compared with 16% in dizygotic twins.[91] Cases were identified from medical records, disease registers and death certificates, however, thus potentially excluding mild cases of disease. In the American study, which included 250 monozygotic and 264 dizygotic twin pairs, subjects were examined twice at mean age 47 and 57 years.[92] At first examination the prevalence of diabetes was 5.7% and there was no difference in concordance rates between monozygotic and dizygotic twins. Ten years later the prevalence was 13% and the probandwise concordance rate for monozygotic twins was 58.3% compared with 17.4% in dizygotic twins. Of the 15 pairs who had been discordant at first examination all but one had become concordant 10 years later. In addition, 65% of the non-diabetic monozygotic co-twins of diabetic twins were found to be glucose intolerant. This study, therefore, appeared to provide strong evidence for genetic determinants of non-insulin dependent diabetes. However, it was restricted to male army veterans. This raises some doubts about the generalisability of the findings since this group did not include women and, being army recruits, are likely to be more healthy than the general population.

A number of the twin studies have shown greater similarity between the blood pressure of monozygotic compared with dizygotic twins. In one of the earliest studies, Stocks measured the blood pressure of a group of English school children comprising 93 monozygotic twin pairs, 101 unlike-sexed dizygotic twin pairs and 85 like-sexed dizygotic pairs.[93] He also recorded blood pressure in their siblings. The intraclass correlation coefficients for systolic blood pressure were 0.81 for monozygotic twins compared with 0.44 in like-sexed and 0.45 in unlike-sexed dizygotic twins. These results have been confirmed in two further studies in children. McIlhany et al studied 200 twin pairs with a mean age of 14 years and reported intraclass correlations of 0.85 in monozygotic twins compared with 0.39 in dizygotic twins.[94] The second study based on 197 twin pairs aged 7 years also reported greater correlation in the monozygotic twins.[95] However, monozygotic twin pairs were over-represented in this study making up nearly two-thirds of the twin pairs taking part.

Two studies in adults have also shown higher intraclass correlations in monozygotic twins. A large-scale study of 514 pairs of male like-sexed twins aged 42 to 56 years, suggested that as much as 82% of the systolic and 64% of the diastolic pressure differences in middle-aged twins were genetically determined. This study was based on a twin register of male army veterans thus limiting the generalisability of the findings.[96] The most recent study based on middle-aged and elderly twins sampled from the Swedish twin register was based on a comparison of twins reared together and apart. This allows the influence of environmental factors to be assessed since those reared together will be matched for many aspects of their upbringing, such as diet and exercise, that will differ in those reared apart. The intraclass correlation coefficients in monozygotic compared to dizygotic twins were 0.40 and 0.20 in elderly twins and 0.64 compared to 0.01 in middle-aged twins suggesting that genetic influences on blood pressure were more apparent in middle-aged than elderly twins.[97]

Population based studies of twins have also suggested that there are significant genetic influences on the insulin resistance syndrome. A large scale American study of female twins sampled from the Kaiser Permanente twin register revealed strong genetic influences on insulin resistance syndrome although environmental factors were also found to be important. The study included 165 monozygotic and 113 dizygotic twin pairs with a mean age of 59 years.[98] The researchers found significant

genetic influences on fasting insulin concentrations with a heritability estimate of 0.53 after adjustment for body mass index and other potential confounding factors. They also found that environmental factors played an important role since, in a monozygotic intra-pair analysis, body mass index was independently associated with fasting insulin levels. A later analysis on the same sample of female twins revealed high heritability estimates of fasting and post-load insulin and glucose, and moderate heritability for body mass index and lipids.[99]

These findings were supported by a similar but smaller scale study in men and women aged 20 years.[100] Within-pair variance of fasting insulin was greater in dizygotic than monozygotic twins with correspondingly higher intraclass correlation in the monozygotic twin. However, this study was based on only 34 twin pairs with female twins pairs being over-represented in the sample. In addition, details of ascertainment of twin pairs are not given and so these findings must be interpreted with caution. A recent study based on the Swedish Adoption/Twin Study of Ageing was carried out in an attempt to explore the extent to which the various components of the insulin resistance syndrome are determined by a common set of genetic and environmental factors.[101] A sample of 289 male and female same sex twin pairs with a mean age of 65 years were included in the study and 140 of them had been reared apart. A combination of model fitting and intraclass correlations was used to evaluate the relative importance of genetic and environmental influences. All of the five principal metabolic components were found to be influenced to some degree by a common genetic factor. Genetic influences were of particular importance to body mass index and insulin resistance. Only three of the components (triglycerides, insulin resistance and HDL cholesterol) were influenced by a common environmental factor.

## 1.5 Challenges to the genetic interpretation of twin studies

### 1.5.1 Assumption of equal environmental variance

The genetic interpretation of twin studies is dependent on the underlying assumption of equal environmental variances in monozygotic and dizygotic twins.[102] The assumption is made that members of a monozygotic twin pair share environmental factors to a similar extent as members of a dizygotic twin pair. It is widely acknowledged, however, that there are marked differences in prenatal and postnatal environment in the two groups. Postnatally monozygotic twins tend to be more

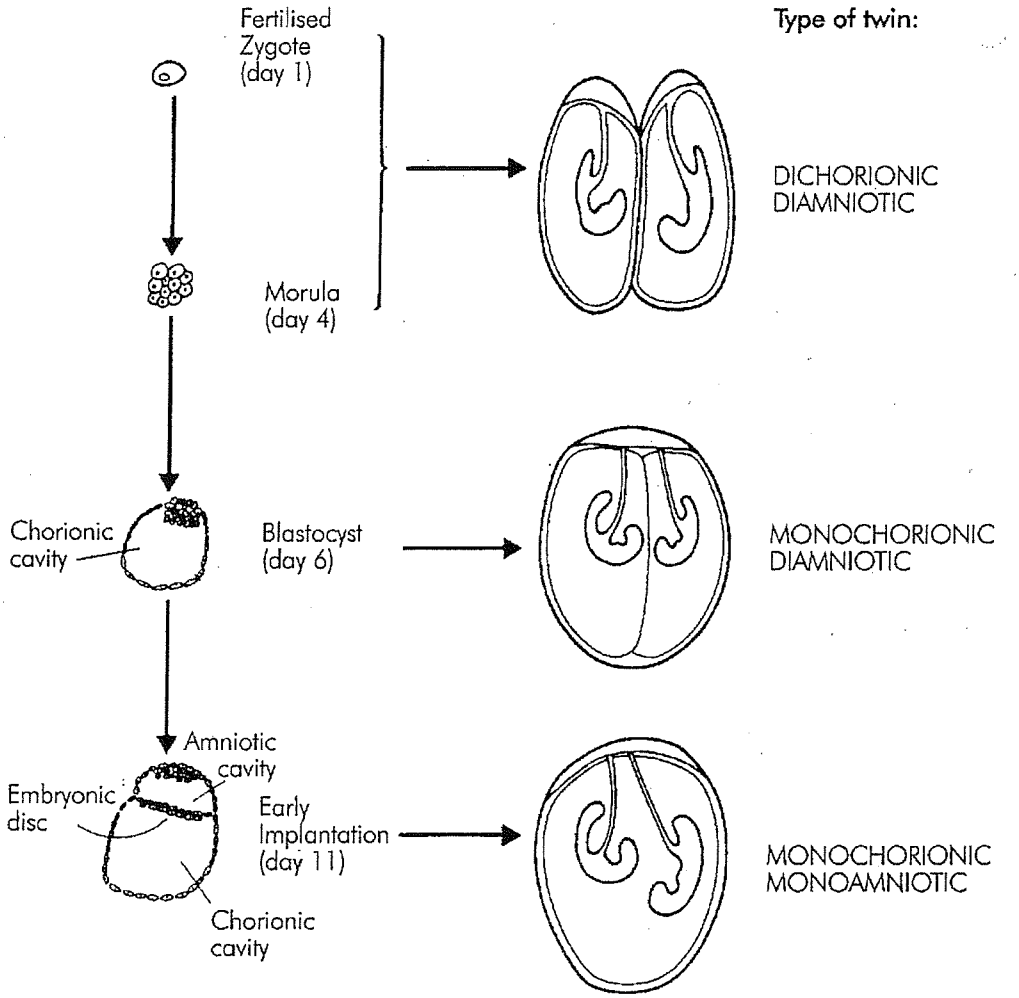
similar both psychologically and behaviourally than dizygotic twins leading to increased concordance for many lifestyle factors that may influence risk of disease.[102; 103] Prenatally, monozygotic twins also share a more similar environment than dizygotic twins since two thirds of monozygotic twins share a common circulation in utero (see section 1.5.2 below).

The design of many twin studies takes account of postnatal environmental differences between monozygotic and dizygotic twins. For example, adoption studies are particularly valuable since they allow the comparison of twins reared together and twins reared apart and thus are able to take account of postnatal influences. The study of monozygotic twins who have been reared apart allows any non-genetic familial effects to be accounted for.[104] Few studies of twins, however, have attempted to take account of prenatal environment despite well documented evidence from a number of studies demonstrating differences between the prenatal environment of monozygotic and dizygotic twins as described below.

### 1.5.2 Prenatal bias in twin studies

An understanding of the importance of prenatal factors in the interpretation of twin studies can be obtained by studying the embryological development of twins (figure 1.2).

**Figure 1.2: The embryology of monozygotic twinning**




In general, monozygotic twins are genetically identical as they arise from a single zygote, which divides into two embryos during the first 13 days of gestation before the primitive streak forms. Dizygotic twins develop from the fertilisation of two ova by two spematozoa and each fertilised ovum develops in its own set of fetal membranes. Therefore dizygotic twins have, on average, one half of their genes in common and are genetically equivalent to siblings.[85]

Monozygotic twins arise from a single fertilised ovum which divides at some stage between zygote and formation of the embryonic disc to give rise to two complete ova. The timing of division of the fertilised zygote determines whether monozygotic twins will share their membranes (chorion and amnion). In around a third, division occurs up to the morula phase (day 4) and this results in monozygotic twins with separate amniotic and chorionic membranes. In the remaining two thirds, division occurs later after the formation of the blastocyst, and this usually results in monozygotic twins who share a chorion but have separate amniotic membranes. Since the placenta develops from the chorion, these twins will share a placenta. Rarely, division occurs after formation of the amniotic cavity resulting in twins with shared chorionic and amniotic membranes; few such twins survive because of the tendency of their umbilical cords to knot together.[82]

The importance of these differences in early embryological development lies in the effects they have on the development of the placenta. Since the placenta is formed from chorionic tissue, dizygotic twins and monozygotic dichorionic twins will have separate placentas. Monochorionic twins, however, will share a placenta. This not only results in competition for nutrients but also enables vascular anastomoses to form between the circulations of the two twins. In a review of studies exploring the nature of these anastomoses, Strong and Corney found that the commonest form of anastomoses was between two arteries.[105] Anastomoses between two veins were less commonly observed. Rarely, a placental cotyledon may be supplied by an artery from one fetus and be drained by a vein to the other. In this case, blood will leak from one twin to the other – the twin transfusion syndrome – resulting in the birth of a large plethoric twin and a smaller anaemic one. Mortality in this situation can be as high as 70%. In a review of the effects of the twin transfusion syndrome, Bernischke suggests that the adverse outcome for the donor twin resembles the abnormalities observed in singletons who have been subjected to intra-uterine deprivation.[85]

Price attributed the first account of the mutual circulation in monochorionic twins to Weil in the 17th century.[106] Weil proposed that the common prenatal circulation of monochorionic twins accounted for many of their postnatal similarities. However, it was not until 200 years later, following a review of the evidence by Huter, that the

existence of the shared circulation of monochorionic twins was accepted. Huter suggested that it may account for the fact that monochorionic twins shared the same susceptibility for disease in adult life. Support for this idea came from detailed work by Shatz later that century who conducted detailed studies of the placental vessels by the use of dyes. He suggested that the shared circulation in monochorionic twins commonly resulted in an imbalance of blood flow due to chance factors such as position and growth of placental vessels and torsions of cord vessels. Price went further and proposed that the imbalance in mutual circulation may be an important influence on later health mediated by its effects on hormonal mechanisms in the twins.

Evidence from a number of large-scale studies suggests that the differences in prenatal environment in monochorionic and dichorionic twins lead to differences in size at birth and perinatal outcome. Four studies, summarised in table 1.4, have shown that monozygotic monochorionic twins are substantially lighter at birth than dizygotic or monozygotic dichorionic twins.[79; 81; 107; 108]

**Table 1.4: Birth weight differences between monochorionic and dichorionic twins**

| | Mean birth weight (g) | | |
|---|---|---|---|
| **Study** | **Monochorionic** | **Dichorionic** | **Mean difference in birth weight (g)** |
| Naeye 1966 | 2453 (396) | 2560 (1110) | -107 |
| Grunewald 1970 | 2290 (198) | 2494 (522) | -204 |
| Corey 1970 | 2862 (236) | 2990 (500) | -128 |
| Ramos-Arroyo 1987 | 2338 (73) | 2567 (109) | -229 |

Number of individual twins is shown in parentheses.

Naeye et al studied over 2,000 twins in whom chorionicity was established by gross and microscopic examination of the placenta.[79] Complete data on birth weight and chorionicity was obtained in over 1,500 twins. At nearly all gestational ages monochorionic twins were lighter than dichorionic twins with a mean difference in birth weight of 107g between the two groups. Greater differences in mean birth weight were demonstrated in the other three studies ranging up to 229g. Although

these differences are relatively small, they are potentially important in light of the lower mean birth weight of twins. In two of the studies chorionicity alone was assessed and the zygosity of dichorionic twins was not established.[79; 81] However, in the other two studies both zygosity and chorionicity were established.[107; 108] In the most recent study, Ramos-Arroyo et al used genetic markers to establish zygosity while chorionicity was determined by gross and microscopic examination of the placenta.[108] When monochorionic and dichorionic subsets of monozygotic twins were examined, the mean birth weight of the monochorionic group was significantly lower than the dichorionic group. No significant difference in birth weight was found, however, between the dichorionic monozygotic or dizygotic groups. These findings suggested that chorionicity was more important than zygosity in determining birth weight.

The reason for the differences in birth weight between monochorionic and dichorionic twins is unclear. Mean duration of gestation was between 6.3 and 10.6 days shorter in monochorionic twins than dichorionic twins. However, adjusting for gestation did not reduce the differences in birth weight between the different types of twin. The finding that chorionicity was more important than zygosity in determining birth weight difference suggests that competition for nutrients in the monochorionic twins by virtue of their shared placentae may have been the cause.

Monochorionic and dichorionic twins also have well documented differences in perinatal outcome with evidence of higher perinatal mortality rates in monochorionic twins. Over a period of eight years, Grunewald studied a series of 365 twins born in one hospital.[81] Chorionicity was established both by macroscopic and histological examination of placentae. He compared perinatal mortality in the 198 monochorionic and 522 dichorionic twins and found rates of 7.1% and 4.1% respectively.[81] In a larger scale study Bleker et al studied a series of 1,655 sets of twins born between 1931 and 1975 in two clinical centres. They found rates of 15% in monochorionic twins compared with 8.4% in dichorionic twins.[86] In a longitudinal study of 60,000 pregnant women across the United States, there were 213 deaths in 615 twin pregnancies.[87] Perinatal mortality was higher in the 102 pairs of diamniotic monochorionic twins (8.8%) compared with the dichorionic

twins (3.9%). However, chorionicity and zygosity were not established for a substantial proportion (85) of the twins pregnancies studied.

There is also some evidence to suggest that monochorionic twins are more discordant for size at birth than dizygotic twins and that these differences in size may persist into childhood. Naeye et al found that monochorionic twins were both smaller than dichorionic twins and had greater within pair variation in birth weight.[79] In Wilson's study of 900 twin pairs, a group of the most birth weight discordant pairs who had birth weight differences greater than 750g were followed up at six years of age.[7] The smaller twin was still significantly lighter than the larger twin. However these observations were based on only 10 twin pairs. Babson and Phillips studied nine monozygotic twin pairs who differed in birth weight by an average of 36%.[109] The twins were followed up at ages eight and thirteen years. The smaller twin at birth remained significantly lighter and shorter than their co-twin at both ages. However, chorionicity was only confirmed in 5 of the pairs, only three of which were monochorionic.

## 1.6 Twin studies exploring the role of prenatal factors

Since the emergence of evidence of an association between early environment and adult disease, a number of twin studies have investigated the role of prenatal factors in determining levels of disease in later life.

Two studies have explored the risk of disease in twins compared with singletons. The basic premise of these studies is that, according to the fetal origins hypothesis, since twins have a lower mean birth weight than singletons, they should also have more disease in adult life. Vagero and Leon investigated whether ischaemic heart disease mortality was higher in twins than in the general population.[110] They based their study on male and female twins sampled from the Swedish twin register and compared them with the general Swedish population. Although mortality was not higher among the twins, a nested case control study revealed that the shorter twin in a twin pair, who was also the smaller twin at birth, was more likely to die of heart disease. These findings suggest that birth size influences adult disease independent of genetic factors. The second study by Christiansen et al investigated mortality rates in

a cohort of Danish twins born before 1900 and found no difference in mortality compared with the general population.[111]

Both of these studies of twins were based on historical cohorts born at a time when twin pregnancy was associated with a relatively high perinatal mortality. As the two studies only included twin pairs who had survived infancy or reached middle-age respectively, a significant number of twins who were low birth weight and died in early life would have been excluded from the analyses. The extent of this underascertainment is particularly evident in the Danish study where the analysis was based on only a third of possible twin pairs. In addition, the absence of higher mortality amongst the twins is difficult to interpret: twins are heterogeneous and are a mixture of proportionately and disproportionately small babies.[112] A group of twins might have low or high rates of coronary heart disease depending on whether they had predominantly been proportionately or disproportionately small babies.

More recently, a New Zealand-based study compared blood pressure in a cohort of twins and singletons at ages 9 and 18 years.[113] The 22 twins included in the study had lower blood pressure than singletons at both ages after adjusting for potential confounding factors including maternal smoking and maternal height. The authors interpret these findings as contrary to the fetal origins hypothesis. However, this interpretation does not take account of the differences in fetal growth between twins and singletons as outlined above. Their findings may reflect the fact that low birth weight for twins has different consequences than for singletons.

Other studies have examined within pair differences in size at birth and adult disease. This method is particularly powerful since twins are matched for maternal factors such as smoking or diet as well as for genetic factors. Study of within pair differences therefore allows exploration of the influence of fetal nutrient supply and placentation. A recent study based on randomly recruited twin pairs from the Danish twin register adds weight to the evidence for a link between fetal undernutrition and later development of diabetes.[114] In this study of like-sexed twin pairs aged 55 to 74 years, 45 twin pairs were discordant for non-insulin dependent diabetes. Birth records were traced for 28 (14 monozygotic and 14 dizygotic) of the disease

discordant pairs. The birth weight of both monozygotic and dizygotic twins with diabetes was significantly lower than that of their non-diseased or glucose intolerant co-twin: mean birth weight of diabetic monozygotic twins was 2634g compared with 2829g in their non-diabetic co-twin. The corresponding weights for dizygotic twins were 2509g and 2854g. These findings confirm that the birth weight/glucose intolerance association is not due to gestational age or to maternal factors such as height since these are controlled for in studies of twins. In addition, because of the genetic similarity of monozygotic twins, they also suggest that the effects of early environment are independent of genetic factors.

An American study of 166 pairs of infant twins suggested the positive birth weight/ blood pressure relation was also present in twins since the smallest twins (birth weight less than 1500g) showed a significantly higher mean rate of rise of blood pressure at one year than twins weighing more than 1500g.[115] In addition, large differences in birth weight in monozygotic twin pairs were associated with small differences in systolic blood pressure at one year of age. It is likely that the more birth weight discordant twins were monochorionic since observational studies where chorionicity is established have found the greatest degree of discordance in the monochorionic group.[81] The findings of the study, therefore, suggest that the influence of shared circulation in utero may lead to more similar blood pressure in infancy.

Two studies have examined within pair differences in blood pressure in cohorts of twins aged 8 and 50 years respectively. The first study was based on a relatively small sample of eight year old Australian twins.[116] Although the lightest twin in a monozygotic pair had a higher systolic blood pressure at eight years, after adjustment for age and body mass index, this finding was based on only 16 pairs of twins and was not statistically significant. The second study was based on a sample of 50 year old female twins taken from the St Thomas's UK adult twin register.[117] The lighter monozygotic twin had higher blood pressure at 50 years of age . Mean birth weights for the heavier and lighter twins were 2.51kg (standard deviation 0.61) and 2.12kg (standard deviation 0.59) respectively. Although these findings were based on 167 monozygotic pairs, there were relatively few pairs showing substantial discordance in birth weight. The birth weight data in this study was self-reported and this may have

led to underestimation of the size of within pair differences. In addition this study was based on women recruited from a volunteer twin register, giving rise to the potential for selection bias.

The findings of these studies suggest that prenatal environment has an influence on later risk of disease that is independent of genetic factors. If substantiated in larger scale studies, these findings suggest that the role of fetal nutrient supply and placentation may be more important in determining later risk of disease than the nutritional status and body composition of young women.

### 1.7 Hypotheses

The evidence outlined above leads to a number of questions:

1. Since twins have a lower birth weight than singletons, does this make them more susceptible to non-insulin dependent diabetes and raised blood pressure as adults? Further more, since monochorionic monozygotic twins have a lower mean birth weight than dizygotic twins, are monozygotic twins more susceptible to these diseases than dizygotic twins?
2. Are the greater similarities within monozygotic twin pairs compared with dizygotic twin pairs explained by the more adverse and more similar intra-uterine environment of monochorionic twins?
3. Can differences in adult glucose tolerance and blood pressure in monozygotic twins be explained by differences in size at birth? Because monozygotic twins are genetically identical this will provide important evidence as to whether the influence of early growth on glucose tolerance and blood pressure is independent of genetic effects.
4. Is mortality in twins higher than that of the general population? If mortality rates are higher, is adverse prenatal environment as indicated by low birth weight, associated with a greater risk of death?

# 2 METHODS

## 2.1 Study design

The study was a retrospective cohort study of adult twins for whom birth records were available. The target population included both like-sexed and unlike-sexed twin pairs in order to allow comparisons between monozygotic and dizygotic twins.

The majority of twin studies in the UK have been based on volunteers or twins identified due to disease and, as a result, have been subject to ascertainment bias.[78] We based our study on a register of all births in the city of Birmingham, which was established in the 1950s, and were therefore able to study a population-based sample of twins.

## 2.2 Study population

### 2.2.1 The Birmingham birth study

The sampling frame was an existing population-based birth register of all births within the City of Birmingham. From 1950 onwards details of all births within the City, including those delivered at home, were recorded.[118] Data from maternity and child welfare records, that was routinely collected by midwives and health visitors, was coded and recorded on punched cards. Surname was recorded in full but a Hogben code was used to represent forename. Data from obstetric records included birth weight, length at birth, gestational age, birth order and details of maternal health during pregnancy. Birth weight was recorded in quarter pounds and length was recorded in inches. The study included all live births within the City but details of stillbirths were also recorded.

The methods used to form the birth register and completeness of the records are described in a detailed report published in 1951.[118] Birth weight, length at birth and gestational age were recorded by the doctor or midwife at delivery. Paternal occupation was recorded by the health visitor. The standard of housing was recorded as 'good' or 'poor' according to the ward of residence.

There was complete ascertainment of births to Birmingham residents although detailed records were not made for residents who gave birth outside the city. There were high levels of completeness for much of the birth data (table 2.1).

**Table 2.1: Completeness of the birth register data**

| Birth variable | Level of completeness in first year of birth study (1950)* | Completeness of twin records (1950-54) |
|---|---|---|
| Sex | 99.5% | 99.9% (sex unknown for 1 individual) |
| **Birth weight** | 99.7% | 99.6% |
| **Paternal occupation** | 98.6% | 77% |
| **Quality of housing** | 98% | 99.8% |
| **Period of gestation** | 73% | 87% |
| **Length at birth** | No data | 79% |

*The level of completeness in the first year is taken from a description of the study published in 1951.

Gestational age was only recorded when it could be confirmed by definite data relating to the first day of the last menstrual period before the birth of the baby and so it had lower levels of completeness (87%). The data relating to maternal health during pregnancy and to congenital anomalies and abnormalities following birth has much lower levels of completeness. There is no reference to completeness of data on length at birth but completeness was 79% for the twins born between 1950 and 1954.

We selected all births registered from 1950 to 1954 as the sampling frame for the study.

### 2.2.2 Identification of twins from the birth register

Between 1950 and 1954 there were 94,474 births within the City of Birmingham. Details of date of birth, surname, Hogben code, maiden name of mother and address at birth were abstracted from the records and used to select pairs of individuals with the same date of birth in the indexes of registered births in Birmingham held by the Office of Population Censuses and Surveys (now the Office of National Statistics).

Twin status was assigned to pairs of individuals with matching date of birth, surname, maiden name of mother and address at birth. When doubt still existed regarding twin status, copies of birth certificates were obtained to check that address and date and time of birth were consistent with those recorded on the record cards. Using this technique 2,562 twins (1,281 pairs) born from 1950 to 1954 were identified, of which 796 pairs were like-sexed and 484 unlike-sexed. We were unable to determine sex for one twin pair. Of the like-sexed pairs, 400 were male and 396 female.

### 2.3 Tracing subjects

Lists of names and dates of birth of twins were sent to the Office of Population Censuses and Surveys (OPCS) and the twins were traced via the National Health Service Central Register (NHSCR). Pairs of twins where one or both had died or emigrated were excluded from the study. Full details of exclusions are given in section 3.2.

Mortality data were flagged up until the end of 1994. Subjects were tagged at the NHSCR and we obtained copies of death certificates for those who died in the period of follow up. Underlying cause of death was coded to the ninth revision of the International Classification of Diseases (ICD).

More than half of the twin pairs eligible to participate in the study were still resident in Birmingham or the surrounding health authority areas of Solihull, Sandwell, Dudley, Staffordshire, Coventry, Warwickshire, and Hereford and Worcester and these pairs were selected as the target population. Individuals living outside these areas were excluded from the study because of the practical difficulties involved in covering a more extensive geographical area. With permission from their general practitioners, 486 twin pairs were invited by letter to take part in the study. Of these, 312 were like-sexed and 174 unlike-sexed. Of the like-sexed pairs, roughly equal numbers were male and female (157 male pairs and 156 female pairs).

Weinberg's method was used to give an estimate of the numbers of monozygotic and dizygotic twin pairs that could be expected in the 486 pairs.[4] This method relies on the fact that all monozygotic pairs are identical and in the dizygotic pairs the sexes are combined at random so that half should be unlike-sexed (male/female) and a half like-sexed (a quarter male/male and a quarter female/female). It was estimated that 138 pairs would be monozygotic and 348 dizygotic.

## 2.4 Estimation of appropriate sample size

The following equations were used to estimate the sample size required to give adequate statistical power to detect the regression coefficient for the 'twin effect' in a paired analysis. The variance of the 'twin' terms assesses the strength of the genetic effects. The regression coefficient for birth weight assesses the strength of the early life effects.

$$y_{i1}=a+bsex_{i1}+cbirth\ weight_{i1}+dbmi_{i1}+twin_i+error_{i1}$$

$$y_{i2}=a+bsex_{i2}+cbirth\ weight_{i2}+dbmi_{i2}+twin_i+error_{i2}$$

We ran a simulation study to assess the power of our study. Data from the NHANES study, a large population-based American study of 6,000 adult males and females aged 20 to 74 years, was used to represent the value ranges of plasma levels of glucose and insulin which we could expect on glucose tolerance testing.[119] Data relating to size at birth was obtained for the 486 twin pairs from the birth register.

The power of the study to detect a change of 0.1 logmmol/l in log two-hour glucose concentration per kilogram of birthweight (i.e. the regression coefficient c=0.1) was assessed. The standard error of c was estimated as 0.006, indicating that, with a sample size of 486 twin pairs, the power was over 99%. Even with a 50% response rate, the power to detect this effect size would sill exceed 90%.

## 2.5 Ethical approval

Ethical approval was obtained from the OPCS central ethical committee prior to tracing the subjects. In addition, 13 ethical committees covering the geographical area in which the intended subjects lived gave ethical permission for the study to proceed.

## 2.6 Data collection

A team of four research nurses and one doctor were responsible for data collection. Family Health Service Authorities (FHSAs) in the relevant geographical areas were given details of subjects' names, date of birth and NHS number as obtained from the NHS central register. FHSAs were then asked to give details of subjects' current general practitioner. Permission was sought from general practitioners before contacting subjects and they were asked to provide addresses for contacting subjects.

Subjects were invited to participate by letter. They were offered the opportunity to discuss the study with one of the research nurses at a home visit. If they agreed to take part the nurse arranged to visit them at home in order to administer a questionnaire and take measurements of blood pressure, height, weight and waist and hip circumference. They were then invited to attend a clinic at which glucose tolerance tests were performed.

### 2.6.1 Interviews with subjects

Subjects were interviewed in their homes by the fieldworkers with the help of a questionnaire (appendix A). They were asked about their smoking habits, level of physical activity and alcohol intake. They were also asked about their medical and drug history and, in particular whether they had a history of, or were being treated for diabetes or high blood pressure. Likewise, they were asked whether there was any family history of diabetes or high blood pressure. Current social class was derived from the subject or husband's occupation using the Registrar General's classification.

The questions relating to social class, subjects' medical and family history were based on questions used in previous MRC studies. The questions relating to alcohol intake and current physical activity were based on the National Health Survey for England & Wales 1991.[120]

### 2.6.2 Determination of zygosity

Like-sexed twin pairs may be monozygotic or dizygotic. The zygosity of like-sexed twins pairs in our study was determined using a well validated questionnaire. It has been used to assign zygosity in a number of population-based twin registers where

other methods are inappropriate due to the size of the cohort. Comparisons with blood typing and analysis of genetic markers carried out on the Finnish and Norwegian twin registers of twins revealed that the questionnaire method correctly assigns zygosity in 99% of monozygotic and 91% of dizygotic pairs.[121-123] The analysis of genetic markers is the most reliable method of determining zygosity.[4] It is used as the standard by which to measure other methods. The disadvantages of this method are its high cost and requirement for a blood test. The questionnaire method was selected for our study partly on the basis of cost but also because not all twins participating in the blood pressure study would necessarily go on to attend the clinic for a blood test thus making analysis of genetic markers impossible for a proportion of subjects.

Subjects who were part of a like-sexed pair were asked the following questions:

- During childhood, were you and your twin described as:
  Alike as two peas in a pod; Of ordinary family likeness; Quite different
- Were you and your twin mistaken for each other:
  Very often; Now and then; Never
- Who mixed you up:
  Parents; Other relatives; Teachers; Strangers; Others; Nobody

They were also asked to grade how alike they and their twin were in relation to:

- eye colour
- natural hair colour
- height
- weight
- facial appearance
- complexion.

There were four possible options: very similar; somewhat similar; different and don't know.

Note: The zygosity questions are shown in full in the questionnaire in appendix A, questions 16 to 20.

### 2.6.3 Analysis of zygosity data

The analysis of these questions was based on the calculation of two scores originally described by Torgesen and Magnus who compared zygosity questionnaire data with the results of blood typing.[122; 124] The score described by Torgesen was assigned as follows:

| | |
|---|---|
| As alike as two peas in a pod;<br>Mixed up very often;<br>Mixed up by parents. | Each given a score of 1 |
| Usual sibling similarity (ordinary family likeness)<br>Mixed up now and then;<br>Mixed up by teachers. | Each given a score of 2. |
| Quite different in appearance;<br>Never mixed up;<br>Mixed up by others. | Each given a score of 3. |
| Mixed up by nobody. | Given a score of 4. |

The sum of the score for each question was then calculated. Twin pairs with scores of 12 or less were defined as monozygotic and twin pairs with a score of 14 or more as dizygotic. When twins scored 13, half were monozygotic and half dizygotic.

The score described by Magnus was calculated as follows:

Answers indicating likeness received a score of +1:
- Alike as two peas in a pod;
- Often mixed up;
- Mixed up by parents;
- Mixed up by teachers;
- Same physical features (eye colour etc.).

Inconclusive answers received a score of 0:
- Don't know;
- Can't remember;
- Occassionally confused;
- Somewhat similar features.

Answers indicating dissimilarity received a score of -1:
- Quite different;
- Never mixed up;
- Different physical features.

The intra-pair mean of the score for each question was then calculated. Positive scores indicated monozygosity and negative scores dizygosity. Scores of '0' left twin pairs unclassified.

Both scores described by Torgesen and Magnus were calculated for like-sexed twin pairs in our study. The scores were highly correlated ($r=-0.96$, $p<0.0001$). A comparison of the scores with answers to the peas in the pod question revealed that this question correctly predicted zygosity in all pairs where the responses of one twin were the same as those of the co-twin.

Within pair responses to the zygosity questions were the same in all but 4 pairs. In these four cases the twins' mother was asked a series of questions which have previously been shown to correctly indicate zygosity when information was obtained from mothers. In one study, 74 teenage twin pairs and their mothers were asked questions relating to similarities between the twins and whether they were mixed up as children. The researchers had determined zygosity beforehand using genetic markers. Over 90% of the twins had their zygosity correctly identified whether questions were asked of the twins themselves or their mothers.[125] In a second study of 125 pairs of twins aged between 6 months and 6 years, a postal questionnaire was used to ask mothers about similarities between the twins and the findings were compared with zygosity diagnosed by blood grouping. The frequency of misclassification was around 4%.[126] Both of these studies were based on younger age groups than the subjects in this study. Nevertheless, the first study suggests that maternal recall relating to similarities in childhood will be as reliable as that of the twins.

The responses of mothers to zygosity questions are summarised in table 2.2.

**Table 2.2: Summary of maternal responses to zygosity questions for 4 twin pairs of unknown zygosity**

| **Question to mother:** **Each question began: 'When the twins were children...'** | Twin pair 1 | Twin pair 2 | Twin pair 3 | Twin pair 4 |
|---|---|---|---|---|
| Would you describe them as: 1=Alike as 2 peas in a pod 2=Of ordinary family likeness 3=Quite different | 2 | 1 | 1 | 2 |
| Were they mixed up: 1=Very often 2=Now and then 3=Never 4=Can't remember | 3 | 2 | 1 | 3 |
| Who mixed them up: 1=Parents 2=Relatives 3=Teachers 4=Strangers 5=Others 6=Nobody | 6 | 3,4 and 5 | 3 and 4 | 6 |
| Did you yourself think they were: 1=Identical 2=Non-identical | 2 | 1 | 1 | 2 |
| **Zygosity assigned on basis of maternal responses** **monozygotic (MZ)** **dizyogtic (DZ)** | DZ | MZ | MZ | DZ |

Data relating to placentation was not available for the twins and so it was not possible to determine chorion status. Comparison of fingerprints of co-twins has been proposed as a method of determining chorionicity.[127; 128] However, we decided against their use due to the difficulties of interpretation suggested by studies evaluating their use. Fingerprints start to form in the first trimester and are completed before the second trimester. Reed et al found that within-pair differences were related to placental type in monozygotic twins.[127] They derived an index which was correlated with placental type in a sample of 370 twin pairs. A later evaluation of the index on two groups of monozygotic twins (one French and one American) of known placental type, confirmed that monochorionic twins had higher index scores than dichorionic twins. However, mean scores in both monochorionic and dichorionic twins were higher than in the original study suggesting that the index had limited use in that a cut off point for identifying zygosity could not be identified.[129]

### 2.6.4 Blood pressure measurement

Blood pressure and pulse rate were measured using an automated recorder – the Dinamap 8100 (Critikon). Subjects were seated while their blood pressure was measured. Readings were taken on the left arm using the cuff of the size recommended for the subject's arm circumference. Two readings were taken and the average was used in the analysis. Room temperature was also measured.

Subjects' blood pressure was measured by three observers. An automated recorder was used in preference to a sphygnomanometer in order to minimise the potential for bias introduced by observer error. There are, however, some potential limitations in using such a device. A number of studies have revealed that there are inaccuracies in blood pressure measurement when using the dinamap 8100. A recent review of its accuracy revealed that the dinamap 8100 underestimated systolic blood pressure by 0.71mmHg and diastolic blood pressure by 7.6mmHg.[130] However we were most interested in the relationships between birth size and systolic blood pressure in the twins and the inaccuracies in relation to systolic blood pressure are relatively small. In addition, all subjects in a study are likely to be affected uniformly by such inaccuracy. Further more, the use of the dinamap 8100 is consistent with the methods

of many other studies which have reported the inverse association between birth size and adult blood pressure.

### 2.6.5 Anthropometric measurements

**Height**

Height was measured using a portable stadiometer (CMS Weighing equipment Ltd, Camden, London). Fieldworkers recorded measurements to the nearest millimetre.

**Weight**

Weight was measured in kilograms using a SECA scale (SECA Ltd, Birmingham, UK). Subjects were asked to remove their shoes and any heavy items of clothing or jewellery. Weight was recorded to the nearest 0.1 kg.

**Waist and hip circumference**

Waist and hip circumference were measured using a fibre glass tape measure. Subjects were measured over one thin layer of clothing. Waist circumference was measured midway between the costal margin and the iliac crest at the end of normal expiration. Hip circumference was measured at the maximum circumference around the buttocks. The tape was checked to ensure it was level before a reading was taken. Measurements were taken to the nearest 0.1 cm.

### 2.6.6 Glucose tolerance testing

Glucose tolerance tests were carried out by a doctor and nurse following a standard protocol. Clinics were held during the morning following an overnight fast by the subjects. Initially the clinic was held at a general practice surgery in central Birmingham. In the last eight months of the fieldwork, the location was moved to a health centre in Worcestershire in order to improve access for the large proportion of subjects living outside Birmingham.

Subjects attended the clinic between 8-30 and 9-30 a.m. following a 12-hour overnight fast. Prior to the test they were asked to confirm that they had not eaten or drunk (other than water) during this period. The doctor explained the test and each subject signed a consent form.

A fasting blood sample was taken immediately before the administration of the glucose load. Each subject was then asked to drink a 75g drink of anhydrous glucose over the course of a few minutes. The time was recorded half way through the drink and the 30-minute and two-hour samples were timed from this point. Two blood samples were collected at 30 minutes and two hours: blood for assaying insulin levels was collected in a heparinised tube and blood for assaying glucose in a fluoride/oxalate tube.

The specimens were centrifuged and the plasma was pipetted into small plastic tubes. The insulin specimens had been placed on ice immediately after venesection and they were centrifuged in batches at the earliest opportunity and always within an hour of being taken. These steps are taken to avoid enzymatic degradation of the specimen. The glucose samples remained at room temperature until they were centrifuged.

The tubes containing plasma were labelled with the subject's name, date of birth, serial number and a code to indicate the assay that was to be performed on that specimen. The tubes were stored in Sarstedt boxes which were kept in a –80°C degree freezer in Birmingham. Every fortnight the specimens were transported, in dry ice, back to the MRC in Southampton where they were stored in –80°C degree freezers which were linked by a central alarm system.

### 2.6.7 Biochemical analysis of specimens

At the end of the fieldwork the specimens were transported in dry ice to the Department of Clinical Biochemistry at Addenbrooke's Hospital, Cambridge. The following assays were performed for each subject:

Glucose at 0, 30 and 120 minutes;
Insulin at 0, 30 and 120 minutes;
Fasting 32-33 Split Proinsulin;
Fasting Proinsulin.

Plasma glucose was measured by the hexokinase method and plasma insulin, proinsulin and 32-33 split proinsulin by two-site immunometric assays with either iodine-125 or alkaline phosphatase as labels. The insulin assay was standardised

against the first international reference preparation coded 66/304 and the intact and split proinsulin assays against standards obtained from Lilly Research Laboratories (Indianapolis USA).

Fasting proinsulin and 32-33 split proinsulin are precursors of insulin. The object of assaying insulin and its precursors in this study was to use them as a measure of insulin resistance as has been done in previous studies.[131; 132] A recent study, validating their use in this context, showed that plasma concentrations of fasting insulin and its precursors derived from a standard glucose tolerance test were highly correlated with insulin resistance as determined by an insulin tolerance test in both normoglycaemic subjects and in those with impaired glucose tolerance.[16]

## 2.7 Training and observer variability studies

A two-week training period preceded the start of the study. During this time the fieldworkers received training in all aspects of the fieldwork. They were able to practice administering the questionnaires, performing blood pressure and anthropometric measurements and glucose tolerance tests.

A number of inter-observer and intra-observer variability studies were held before the fieldwork could commence in order to determine the repeatability of the blood pressure and anthropometric measurements. Inter-observer and intra-observer studies were carried out approximately every three months during the 18-month period of fieldwork and showed that the inter-observer and intra-observer differences remained relatively constant and small when compared to the standard deviation (SD) of each measurement in the study population (tables 2.3 and 2.4).

**Table 2.3: Intra-observer variability – average difference between 3 repeated measurements on 9 subjects for 3 observers in 5 studies**

| | Observer 1 | Observer 2 | Observer 3 | SD in main study |
|---|---|---|---|---|
| Intra-observer study | 1 / 2 / 3 / 4 / 5 | 1 / 2 / 3 / 4 / 5 | 1 / 2 / 3 / 4 / * | |
| Height (cm) | 0.2/ 0.2 / 0.2 / 0.2 / 0.2 | 0.4/ 0.4 / 0.8 / 0.5 / 0.2 | 0.3/ 0.3 / 0.4 / 0.4 | 9.0 |
| Waist circumference (cm) | 0.5/ 0.5 / 1.0 / 0.3 / 0.3 | 0.9/ 1.3/ 1.6 / 0.7 / 0.7 | 1.5/ 1.6 / 0.9 / 1.7 | 13.3 |
| Hip circumference (cm) | 0.9/ 0.5 / 1.1 / 0.2 / 0.3 | 0.5/ 0.5 / 2.0/ 0.5 / 0.3 | 1.3/ 0.6 / 1.0 / 0.6 | 8.6 |
| Systolic blood pressure (mm Hg) | 4.9/ 3.7 / 5.0 / 4.2 / 8.5 | 5.0/ 4.5/ 3.6 / 5.8 / 7.3 | 5.9/ 4.8 / 3.3 / 5.4 | 15.7 |
| Diastolic blood pressure (mm Hg) | 5.7/ 2.3 / 3.9 / 5.1 / 6.5 | 6.0/ 3.4/ 2.4 / 4.1 / 7.2 | 4.9/ 3.5 / 3.5 / 3.0 | 12.1 |

*Observer 3 left the study approximately six months before the end of the fieldwork and so did not participate in the final intra and inter-observer studies.

**Table 2.4: Inter-observer variability – average difference from overall mean for each observer in 5 inter-observer studies (IOV)**

| | IOV Study 1 | IOV Study 2 | IOV Study 3 | IOV Study 4 | IOV Study 5 | SD in main study |
|---|---|---|---|---|---|---|
| Observer | 1 / 2 / 3 | 1 / 2 / 3 | 1 / 2 / 3 | 1 / 2 / 3 | 1 / 2 | |
| Height (cm) | -0.1/0/0.1 | 0.2/-0.3/0.1 | 0.2/-0.4/0.2 | 0.3/-0.7/0.3 | -0.1/0.1 | 9.0 |
| Waist circ. (cm) | 0.4/0.1/ -0.5 | -0.4/0.2/0.2 | -0.4/-0.9/1.1 | -0.3/0.2/0.6 | -0.1/0.1 | 13.3 |
| Hip circ. (cm) | 0/0.5/0.5 | -0.4/0.7/-0.3 | -.03/1/0/- 1/0 | 0.2/0.6/-0.8 | 0.3/-0.3 | 8.6 |
| Systolic BP (mm Hg) | 1.3/-0.6/ -0.7 | -0.9/0.4/0.5 | 1.1/-2.3/1.1 | 0/-1.0/1.0 | -0.6/0.6 | 15.7 |
| Diastolic BP (mm Hg) | 0.8/-0.1/ -0.8 | -0.5/-0.5/1.0 | -0.8/0.3/0.6 | 1.7/-.1/-1.6 | -1.0/1.0 | 12.1 |

The dinamaps were calibrated prior to the start of the study and again half way through the fieldwork as recommended by the manufacturers.

### 2.8 Data set preparation

The questionnaires were collected at regular intervals during the fieldwork. They were checked for legibility and omissions before they were batched and taken to the MRC Environmental Epidemiology Unit for double data entry by clerical staff. The measurement data were recorded on standard results sheets. These too were periodically batched and returned to the MRC for data entry.

Range and consistency checks were performed to clean the data prior to analysis. Frequency distributions of data were examined to look for outliers. Statistical analysis was performed using the SPSS statistical software package.

## 2.9 Statistical analysis

Because plasma measurements of glucose, insulin, proinsulin and 32-33 split proinsulin have skewed distributions the data were log-transformed to normality.

### 2.9.1 Mortality analysis

Mortality ratios, standardised for age, sex and calendar period of death were calculated using rates for England and Wales as the standard. Confidence intervals for mortality ratios were based on the Poisson distribution. Mortality ratios were also calculated according to cause of death. Chapter headings from the 9th revision of the International Classification of Disease (ICD) were used to group causes of death. Survival analysis using the Cox proportional hazards regression model was performed to examine factors that might influence risk of death. This model allows exploration of the simultaneous effects of several variables on survival. It produces a hazard ratio as a measure of risk, which can be considered as equivalent to a relative risk.

### 2.9.2 Similarities within twin pairs

Probandwise concordance rates were calculated. They indicate the proportion of affected co-twins among individuals independently ascertained. This is preferable to the pairwise concordance rate which does not take account of ascertainment. When all cases have been ascertained independently, the rate is given by the formula:

$$C_p=2C/(2C+D)$$

Where $C_p$ is the probandwise concordance rate, C is the number of concordant pairs and D is the number of discordant pairs.[133]

Intraclass correlation coefficients were calculated in order to measure the resemblance in glucose tolerance and systolic blood pressure within monozygotic and dizygotic twin pairs.

The intra-class correlation coefficient (r) is derived from the within pair (Vw) and between pair (Vb) variances, as described by Soafer[133].

$r = Vb^2-Vw^2/Vb^2+Vw^2$.

The within and between pair variances are derived from the mean of the differences within pairs ($\delta^2$) and the standard deviation of the mean difference between pairs ($\delta b^2$).

$\delta^2 = 2Vw$ and $\delta b^2 = Vb-Vw$.

Calculations were confirmed using non-parametric methods to adjust for the potential influence of outliers. Intra-class correlation coefficients were used to provide an upper-limit estimate of heritability of the outcome variables. Estimates of heritability provide a means of expressing the proportion of overall variance that can be attributed to genetic factors.[133] It is similar to population attributable risk and does not directly reflect genetic risk to individuals. Heritability was calculated using the formula $h=2(r_{MZ}-r_{DZ})$.

This method of estimating genetic variance makes the assumption that environmental variance in the monozygotic and dizygotic pairs is equal. As outlined in section 1.5.1 there are well documented differences in the prenatal and postnatal environments of monozygotic and dizygotic twins. Therefore a multi-level modelling approach was used to estimate the relative contributions of prenatal environment (as determined by birth weight and length at birth), postnatal environmental factors (specifically adult body mass index) and genetic factors in determining the variance in the outcome variables.

### 2.9.3 Differences within and between twin pairs

We used multiple regression to analyse the relations of within pair difference in size at birth with the within pair difference in concentrations of the outcome variables of interest: plasma glucose and insulin at 120 minutes; fasting insulin, proinsulin, 32-33 split proinsulin and systolic blood pressure. The predictor variables in the regression analyses were birth weight, length at birth and ponderal index, which is a measure of thinness at birth. Analyses were adjusted for current body mass index and sex. Adjustments for sex in the regression analyses enabled inclusion of the unlike-sexed dizygotic pairs. This retained statistical power that would have been lost if the

analysis had been restricted to like-sexed pairs only. The adjustment for sex in the regression analysis effectively represents a stratification of the analysis by sex as shown by considering the regression equations for the analysis in relation to blood pressure:

The regression equation for the blood pressure analysis is:
$\Delta bp = a\Delta bwt + b\Delta bmi + c\Delta sex$, where $\Delta$ means twin2-twin1

Among dizygotic twin pairs:
If $\Delta sex=0$ (i.e. $sex_1=sex_2$ as in like-sexed pairs), then $\Delta bp = a\,\Delta bwt + b\,\Delta bmi$
In the unlike-sexed pairs:
If $sex_2=2$ and $sex_1=1$, then $bp_2-bp_1=a\,(bwt_2-bwt_1)+b(bmi_2-bmi_1)+c$
If $sex_2=1$ and $sex_1=2$, then $bp_2-bp_1=a\,(bwt_2-bwt_1)+b(bmi_2-bmi_1)-c$

c represents the amount by which blood pressure in females (sex=2) exceeds that in males (sex=1).
The analyses for like-sexed and unlike-sexed dizygotic twins are pooled into one regression and this gives the most efficient estimates of a and b.

Waist/hip ratio was also calculated since it gives a measure of abdominal obesity. Abdominal obesity is associated with a higher risk of non-insulin dependent diabetes.[134] However, body mass index was a stronger predictor of adult glucose tolerance in the twins and so analyses were adjusted for body mass index only.

As stated above, a multi-level modelling approach was used to further explore the between and within pair variations in the outcome variables in monozygotic and dizygotic twins. We fitted two-level models using the programme MLn. This programme allows the variation structure to be modelled flexibly and appropriately at both level one (subjects) and level two (twin pairs). Estimates and standard errors are obtained for the regression coefficients as well as for the variance components and their contrasts.

### 2.9.4 Analysis in individuals

The size at birth, gestation and socio-economic status at birth of participants in the study was compared with that of non-participants using t-tests for comparison of means and chi-squared tests to compare categorical data.

Similarly, the birth size, gestational age and socio-economic status at birth of participants in the glucose tolerance and blood pressure studies were compared with non-participants within the target population.

One-way analysis of variance was used to compare mean values of the predictor variables (birth weight, length at birth) and outcome variables (blood pressure and glucose tolerance) in the monozygotic, dizygotic like-sexed and dizygotic unlike-sexed twins who participated in the study.

Multiple linear regression was used to analyse the relations size at birth (birth weight, length and ponderal index at birth) with adult glucose tolerance and blood pressure in twin individuals who participated in the study. The regression analyses were adjusted for current body mass index and for sex, as in the paired analysis.

### 2.9.5 Influence of potential confounding variables

Obesity, excess alcohol consumption, physical inactivity and social deprivation are well established risk factors for diabetes and hypertension. In addition, past physical activity is known to have a protective effect against the development of impaired glucose tolerance and diabetes.[135] The simultaneous effects of eight potential confounding variables (body mass index, sex, current social class, social class at birth, smoking status, alcohol intake, current and past physical activity) was explored in both the analyses in individuals and in the paired twin analysis using multiple regression analysis.

- Body mass index (in $kg/m^2$) was calculated from subjects' weight and height.
- Current social class and social class at birth were derived from current (or husband's) occupation and father's occupation using a software package

which assigns social class according to Registrar General social class groupings.

- Subjects' smoking status was defined according to whether they were current smokers, ex-smokers or had never smoked.
- Alcohol intake was defined as the number of units of alcohol that a subject drank on average each week. This was derived from questionnaire data relating to the types of alcoholic drink subjects had drunk in the last 12 months and the frequency with which they had drunk them using the methods employed in the National Health Survey for England.[136]
- Physical activity was defined according to the frequency-intensity activity level, also using the methods employed in the National Health Survey for England.[136] The frequency-intensity activity level was based on the number of occasions of moderate or vigorous activity (in relation to occupation; housework, DIY and gardening; sports activity) performed during the four weeks prior to completion of the questionnaire. It takes account of frequency, duration and intensity of activity.
- Past physical activity was defined according to the level of physical activity undertaken at work and at home during two periods of adult life: age 15 to 25 years and 26 to 40 years. Past physical activity was defined according to the level of physical activity at home and work and the frequency of physical exercise sufficient to produce sweating or breathlessness.

Social class at birth was not considered in the paired analyses since this was the same for co-twins. Sex was not adjusted for in the paired analyses in monozygotic twins since all monozygotic pairs are like-sexed.

# 3 CHARACTERISTICS OF THE TARGET POPULATION

This chapter describes the selection of subjects for the study and outlines criteria for exclusion. The characteristics of the target population are compared with twins born in Birmingham in the same period who were not invited to participate in the study.

## 3.1 Ascertainment of twin pairs from the birth register

A total of 1,281 twin pairs (2,562 individuals), born between 1950 and 1954, were identified from the birth register of which 796 pairs were like-sexed and 484 unlike-sexed. Sex could not be determined for one pair of twins as it was not listed in the birth register and could not be ascertained from the names of the twins. Of the like-sexed pairs, 400 were male and 396 female.

## 3.2 Losses to follow up and exclusions

Of the 1,281 pairs of twins, the following pairs were excluded from the study:

- 336 pairs where one or both twins had died
- 49 pairs where one or both twins had emigrated
- 93 pairs where the twins could not be found in the birth index
- 9 pairs where one or both twins were not registered with GPs
- 29 pairs where one or both twins were untraced
- 1 pair where one twin was a psychiatric in-patient
- 3 pairs where one twin was in the armed forces

A total of 761 alive twin pairs were successfully traced through the NHS central register.

There were a total of 140 stillbirths in the population. In addition a total of 297 deaths occurred up until the end of 1994. A detailed analysis of mortality in the twins is described in chapter 4.

### 3.3 Twin pairs traced through the NHS central register

A total of 761 twin pairs were successfully traced via the NHS central register. Because of the logistical difficulties of studying a group with a wide geographical basis, only those twin pairs who were still resident in the Birmingham area were invited to take part. There were 210 twin pairs still resident within the City of Birmingham but statistical power calculations suggested that, unless response rates were very high, there would be insufficient power to estimate the size of effect expected. When the target area was extended to the counties surrounding Birmingham the number of potential twin pairs rose to 486. This area included the Health Authority areas of Solihull, Sandwell, Dudley, Staffordshire, Coventry, Warwickshire and Hereford and Worcester. These 486 pairs were invited to take part in the study.

### 3.4 Comparison of twins in the target population with twins excluded from the study

Twins in the target population were compared with those in the remainder of the cohort who were living but not eligible to take part due to the geographical area in which they lived or due to exclusion criteria as listed above. Data from the birth records was used to compare size at birth (weight and length) and gestational age. Data from the birth records relating to occupational status of father and quality of housing were used as proxy measures for socio-economic status at birth (table 3.1).

**Table 3.1: Comparison of the target population with twins excluded from the study**

| | Live twin individuals not eligible to participate (n=1151) | Twin individuals in target population (n=972) | Difference (95% CI) |
|---|---|---|---|
| **Birth weight (lbs)** | 5.48 (1.19) | 5.50 (1.07) | 0.02 (-0.31, 0.46) |
| **Length at birth (inches)** | 18.65 (1.44) | 18.60 (1.14) | -0.05 (-0.17, 0.08) |
| **Gestational age (weeks)** | 37.5 (3.0) | 37.7 (2.5) | 0.26 (-0.02, 0.49) |
| **Sex:** | | | |
| **Male** | 47.8% | 50.2% | $\chi^2$=0.884 |
| **Female** | 52.2% | 49.8% | (p=0.015) |
| **Year of birth:** | | | |
| **1950** | 21.9% | 20.2% | $\chi^2$ for trend=0.289 |
| **1951** | 20.4% | 21.4% | (p=0.591) |
| **1952** | 18.4% | 19.8% | |
| **1953** | 19.2% | 18.1% | |
| **1954** | 20.1% | 20.6% | |
| **Occupational status of father:** | | | |
| **Professional & executive;** | 13.7% | 4.8% | $\chi^2$ for trend=0.289 (p=0.591) |
| **Skilled worker;** | 51.5% | 60.5% | |
| **Unskilled worker;** | 10.9% | 12.1% | |
| **Unknown.** | 23.9% | 22.6% | |
| **Quality of housing:** | | | |
| **Good** | 54.9% | 47.3% | $\chi^2$=25.67 |
| **Poor** | 44.6% | 52.7% | (p<0.001) |
| **Unknown** | 0.5% | 0 | |

There were no significant differences in birth size or gestational age between the target population and the twins who were not eligible to participate. Mean birth weight for the target population was 5.50lb compared with 5.48lb in the remainder of

the cohort who were living but not eligible to take part in the study. Similar mean values of length at birth were also seen in the two groups (18.60 and 18.65 inches respectively).

Mean gestational age was also similar in the two groups: 37.7 weeks in the target population and 37.5 weeks in the twins not eligible to participate Males and females were equally represented in the target population and the remainder of the cohort but males accounted for only 48% of the remainder of the cohort. Since twin pairs where one or both had died are not included in this analysis, this may be a reflection of the fact that male twins are more likely to have died than female twins (see chapter 4).

The birth register data relating to occupational status of father and quality of housing suggested that there may be systematic differences in socio-economic status of the target and non-target populations. The proportion in the non-target population whose fathers' occupation was classed as 'professional and executive' was 13.7% compared with 4.8% in the target population. In addition a higher proportion of the non-target population were classed as having 'good quality' housing.

### 3.5 Conclusions

There were no significant differences in the birth size and gestational age of the target population and the remainder of the cohort. There were, however, systematic differences in relation to socio-economic status in that individuals whose father's occupation was classified as 'professional and executive' were more highly represented among the non-target population. One possible explanation for this is that families of higher socio-economic status may have been more likely to migrate to other parts of the country, since the non-target population includes those who have moved away from the Birmingham area. The quality of housing was more likely to be poor in target population and this seems to support the findings in relation to occupational status.

# 4 MORTALITY STUDY

## 4.1 Perinatal and infant mortality rates

In this population of 2,562 twins born from 1950 to 1954 there were a total of 140 stillbirths, and 227 deaths during the first year of life of which 158 were in the first week. The perinatal mortality rate, which is defined as stillbirths and deaths in the first week of life per 1000 total births, was 113 per 1000 total births. The infant mortality rate, which is defined as deaths in the first year of life per 1000 live births, was 93 per 1000 live births. According to the Registrar General's Statistical Review of England and Wales, comparable rates for perinatal and infant mortality in England and Wales during the same period were 30 and 25 per 1000 live births respectively.

The study of mortality was based on deaths in the twins up until the end of 1994. A further seventy deaths occurred between the end of the first year of life and the end of 1994.

## 4.2 Standardised mortality ratios

Based on an analysis of the total 297 deaths, overall mortality for the twins was high, with a standardised mortality ratio of 259 (95% confidence intervals 221 to 300), when compared with mortality in the general population. Table 4.1 shows that the standardised mortality ratio was highest in the first year of life at 415 but declined progressively with increasing age during the period of follow up. Standardised mortality ratios remained significantly higher than those of the general population until the age of five years. The numbers of deaths on which ratios beyond the 1 to 4-year age group are based were small and the 95% confidence intervals are wide. It was therefore not possible to draw any firm conclusions as to whether there was excess mortality in the twins in comparison with the general population in age groups beyond 1 to 4 years.

**Table 4.1: Observed and expected number of deaths by age in 2,562 twins**

| Age | Observed no. of deaths | Expected no. of deaths | SMR* | 95% CI |
|---|---|---|---|---|
| 0 | 227 | 54.7 | 415 | 363- 473 |
| 1 -4 | 18 | 8.0 | 224 | 133-355 |
| 5 -9 | 6 | 3.9 | 155 | 57-338 |
| 10-14 | 5 | 3.1 | 162 | 52-378 |
| 15-19 | 7 | 6.2 | 113 | 46-234 |
| 20-24 | 10 | 6.4 | 156 | 75-287 |
| 25-29 | 3 | 6.0 | 50 | 10-147 |
| 30-34 | 6 | 7.0 | 86 | 31-186 |
| 35-39 | 11 | 10.0 | 110 | 55-197 |
| 40-44 | 4 | 7.4 | 54 | 15-138 |
| All | 297 | 112.6 | 264 | 235-296 |

*Standardised mortality ratios with respect to overall mortality rates in England and Wales

## 4.3 Analysis of cause of death

Table 4.2 shows an analysis of the causes of death in the 297 twins who died during the period of follow up. Mortality due to 'conditions originating in the perinatal period' (ICD 760-779) was significantly raised in twins with an SMR of 541 (CI 460-632). This group of causes accounted for 159 (53.5%) of all deaths in the cohort and all but one of the deaths occurred in the 0-1 year age group. The most frequently recorded causes of death in this group were 'prematurity' (ICD 7650, 7651) accounting for 86 deaths, 'atelectasis' (ICD 7705) accounting for 22 deaths and 'multiple pregnancy'

(ICD 7615) accounting for 21 deaths. There was also significant excess mortality in the 0 to 1 year age group attributable to 'disease of the nervous and sense organs' (ICD 320-389) accounting for 11 deaths, 'respiratory disease' (ICD 460-519) accounting for 28 deaths, 'digestive disease' (ICD 520-579) accounting for 21 deaths, and 'congenital abnormalities' (ICD 740-759) accounting for 25 deaths.

Among the 70 twins who died after the first year of life the main causes of death were deaths due to 'injury and accident' (ICD 800-999) accounting for 25 deaths and 'neoplasms' (ICD 140-329) accounting for 11 deaths.

Eighteen deaths occurred in the 1 to 4-year age group. No one group of causes predominated in this age group with 'accident and injury', 'respiratory' and 'digestive' diseases being the most frequently recorded disease chapters accounting for 3,4 and 4 deaths respectively.

**Table 4.2: Analysis of causes of mortality in 2562 twins according to categories of the International Classification of Disease (9th version)**

| Cause of death (ICD codes) | Observed number of deaths | | | | Standardised Mortality Ratio (95% confidence intervals) | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 1-4 | 5+ | All | 0 | 1-4 | 5+ | All |
| All causes | 227[+] | 18 | 52 | 297 | 415(363-473) | 224(133-355) | 104(78-137) | 264(235-296) |
| Infectious and parasitic diseases (0-139) | 1 | 1 | 7 | 3 | 67(2-370) | 84(2-470) | 114(3-636) | 84(17-246) |
| Neoplasms (140-239) | 1 | 1 | 12 | 14 | 407(10-2266) | 111(3-616) | 103(53-179) | 109(60-183) |
| Endocrine, Nutritional and Metabolic diseases and Immunity disorders (240-279) | 0 | 0 | 1 | 1 | 0(0-1979) | 0(0-4741) | 95(2-528) | 76(2-422) |
| Diseases of the Nervous system and sense organs (320-389) | 3 | 1 | 7 | 11 | 317(65-927) | 201(5-112) | 306(123-631) | 295(147-528) |
| Diseases of the Circulatory system (390-459) | 1 | 1 | 2 | 4 | 756(19-4212) | 1119(28-6236) | 32(4-115) | 62(17-158) |
| Diseases of the Respiratory system (460-519) | 23 | 4 | 1 | 28 | 296(187-444) | 222(60-568) | 35(1-193) | 225(149-325) |
| Diseases of the Digestive system (520-579) | 13 | 4 | 4 | 21 | 445(237-760) | 686(187-1756) | 209(57-536) | 388(240-592) |
| Congenital anomalies (740-759) | 21 | 2 | 2 | 25 | 237(147-362) | 227(28-820) | 143(17-518) | 224(145-331) |
| Certain conditions originating in the perinatal period (760-779) | 158 | 1 | 0 | 159 | 538(457-629) | 519(132-289) | 0(0-0) | 541(460-632) |
| Injury and poisoning (800-999) | 4 | 3 | 22 | 29 | 206(56-526) | 178(37-520) | 114(72-173) | 127(85-182) |

## 4.4 Factors influencing mortality

A significantly higher percentage of the twins who had died were male (58.2%) compared with the remainder of the cohort (49%). The mean birth weight of the twins who had died was lower than that of the surviving twins at 4.1lb in the twins who died compared with 5.5lb in those who survived. Of the twins who had died, 82.5% had birthweights below 5.5lb with 12.7% weighing 4 lb or less. The mean gestational age of the twins who had died was lower than that of those who survived: 34.1 weeks in the twins who died compared with 37.1 weeks in those who survived. More than half (55.5%) of the twins who had died were preterm (i.e. born at less than 37 weeks gestation) with a substantial proportion (35.3%) born at 32 weeks or less.

Over half (58%) of the twins who died were male. This was largely explained by a greater number of deaths due to 'conditions originating in the perinatal period' accounting for 95 deaths in male twins compared with 65 in female twins. Further more, there were more than double the number of deaths due to 'congenital anomalies' in the male twins compared with the female twins at 17 and 8 deaths respectively. In addition, deaths due to 'injury and accident' were more common in the male twins accounting for 20 deaths in comparison with nine deaths in the female twins. In a survival analysis, using the Cox Proportional Hazards regression model, low birth weight, prematurity, male sex and death of a co-twin were all independently associated with an increased risk of death (table 4.3).

**Table 4.3: Analysis of the simultaneous effects of sex, death of a co-twin, birth weight and gestational age on twin survival**

| Variable | Hazard ratio (95%CI) | Significance |
|---|---|---|
| Being male | 1.46 (1.14-1.87) | 0.003 |
| Death of Co-twin | 2.26 (1.57-3.26) | <0.001 |
| Birth weight (for every 1lb lower) | 1.89 (1.65-2.17) | <0.001 |
| Gestational age (for every week less) | 1.11 (1.06-1.16) | <0.001 |

## 4.5 Summary of findings

The analyses presented in this chapter were based on relatively small numbers of deaths (297), the majority of which occurred in the first year of life. However ascertainment is likely to have been complete due to the use of the flagging system at the NHS central register. The findings are further strengthened by the fact that the sample is population-based.

Perinatal and infant mortality rates were 113 per 1000 and 93 per 1000 respectively. Calculation of standardised mortality ratios revealed that overall mortality for the twins was high with a standardised mortality ratio of 259 (95% confidence intervals 221 to 300). This was largely due to deaths in the first year of life: the standardised mortality ratio for this period being 415 (95% confidence intervals 363 to 473). This excess mortality extended into the 1 to 4-year age group. However the number of deaths in this age group was smaller and 95% confidence intervals around the mortality ratio were wide in this age group.

Analysis relating to cause of death revealed that the standardised mortality ratio for 'conditions originating in the perinatal period, were significantly raised and accounted for over half (53%) of the total deaths. Deaths due to prematurity and multiple pregnancy predominated in this group. Survival analysis revealed that low birth weight, prematurity, male sex and death of a co-twin were each independently associated with a higher risk of death. The findings of the mortality analysis are discussed in section 7.1.3.

# 5 GLUCOSE TOLERANCE

In this chapter the findings of the glucose tolerance study are presented. The characteristics of participants and non-participants are compared in order to assess the representativeness of the study group. Similarities within twin pairs are examined in order to examine the influence of size at birth and genetic factors in determining observed similarities. Within pair differences in adult glucose tolerance are examined in order to assess whether they are explained by differences in size at birth. The association of birth size with adult glucose tolerance is also examined in twin individuals in order to make comparisons with other fetal origins studies of singleton populations.

## 5.1 Characteristics of the study group

### 5.1.1 Response rate

A total of 376 individuals (39 % of the target population), comprising 136 complete and 104 incomplete twin pairs, agreed to have a glucose tolerance test. However, complete glucose tolerance data was obtained on 372 individuals (38.3% of target population) comprising 135 complete and 102 incomplete pairs. Two of the remaining four subjects did not complete the glucose tolerance test and two-hour specimens for two subjects could not be analysed due to errors in processing. The response rate for complete twin pairs was 28%.

### 5.1.2 Comparison of participants and non-participants

The birth weight, length at birth and socio-economic status at birth of participants in the glucose tolerance study was compared with that of non-participants using data from the birth records (table 5.1).

**Table 5.1: Comparison of characteristics of twins participating in the glucose tolerance study and non-participants**

| | Target population non-participants (n=596) | Twins participating in glucose tolerance study (n=376) | Mean difference (95% CI) |
|---|---|---|---|
| **Birth weight (lbs)** | 5.48 (1.08) | 5.52 (1.05) | 0.04 (-0.1, 0.17) |
| **Length at birth (inches)** | 18.64 (1.17) | 18.57 (1.09) | -0.07 (-0.22, 0.14) |
| **Sex:** | | | |
| **Male** | 54.4% | 43.6% | $\chi^2$=10.65 (p=.001) |
| **Female** | 45.6% | 56.4% | |
| **Gestational age** | 37.7 (2.6) | 37.7 (2.4) | 0.029 (-0.32, 0.38) |
| **Year of birth:** | | | |
| **1950** | 21.5% | 18.1% | $\chi^2$ for trend=6.886 |
| **1951** | 18.5% | 26.1% | (p=0.009) |
| **1952** | 16.9% | 24.2% | |
| **1953** | 17.8% | 18.6% | |
| **1954** | 25.3% | 13.0% | |
| **Occupational status of father:** | | | |
| **Professional & executive;** | 3.7% | 6.4% | $\chi^2$ for trend=1.268 (p=0.260) |
| **Skilled worker;** | 60.6% | 60.4% | |
| **Unskilled worker;** | 11.9% | 12.5% | |
| **Unknown** | 23.8% | 20.7% | |
| **Quality of housing:** | | | |
| **Good** | 46.0% | 49.5% | $\chi^2$=1.130 |
| **Poor** | 54.0% | 50.5% | (p=0.288) |

Figures in parentheses are standard deviations unless otherwise stated.

Mean birth weight and length at birth did not differ significantly in the twins participating in the glucose tolerance study and those who did not participate in the study. Females were over-represented in the glucose tolerance study group compared to the target population and cohort in general: they accounted for 56.4% of participants. In addition subjects who were born in 1951 or 1952 were significantly

more likely than those born in 1954 to participate. Socio-economic status as defined by the occupational status of the father and the quality of housing did not differ significantly in the two groups.

### 5.1.3 Characteristics of twin pairs who were tested for glucose tolerance

Mean birth weight, length at birth, gestational age and adult body mass index in monozygotic and dizygotic like-sexed and unlike-sexed twin pairs were compared using analysis of variance (table 5.2).

There were no statistically significant differences in birth weight, length at birth or adult body mass index in the three groups. Mean birth weight was similar in the monozygotic, dizyogtic like-sexed and dizygotic unlike-sexed twin pairs at 5.3, 5.4 and 5.6lb respectively. Similar mean length at birth was also seen in the three groups at 18.4, 18.4 and 18.5 inches respectively. Mean gestational age was greater in the dizygotic unlike-sexed pairs at 38.4 weeks compared with 37.5 and 37.3 weeks in the monozygotic and like-sexed dizygotic pairs. Mean adult body mass index was lower in the monozygotic than dizygotic twins (25.2kg/$m^2$ compared with 25.8 kg/$m^2$ in both the like-sexed and unlike-sexed dizygotic twins) but these differences were not statistically significant.

The mean values of two-hour plasma glucose and insulin concentrations, fasting insulin, proinsulin and 32-33 split proinsulin concentrations were also compared and no significant differences were seen in monozygotic and dizygotic twins. Among the complete twin pairs, only 4 twin individuals had non-insulin dependent diabetes (NIDDM) and 14 had impaired glucose tolerance (IGT). There were two additional cases of diabetes and 11 of impaired glucose tolerance occurring in individuals who were part of incomplete twin pairs.

**Table 5.2: Characteristics of monozygotic and dizygotic twin pairs participating in the glucose tolerance study**

| | Monozygotic twins | Dizygotic twins Like-sexed | Dizygotic twins Unlike-sexed | p value |
|---|---|---|---|---|
| No. of pairs (men/women) | 44 (19/25) | 50 (19/31) | 41 | |
| Age (years) | 43.5 (1.4) | 43.6 (1.5) | 43.8 (1.3) | 0.726 |
| Birth weight (lbs) | 5.3 (1.0) | 5.4 (1.1) | 5.6 (1.0) | 0.258 |
| Length at birth (inches) | 18.4 (1.1) | 18.4 (1.1) | 18.5 (1.1) | 0.812 |
| Gestational age (weeks) | 37.5 (2.6) | 37.3 (2.5) | 38.4 (2.2) | 0.02 |
| Body mass index ($kg/m^2$) | 25.2 (3.6) | 25.8 (4.2) | 25.8 (3.9) | 0.416 |
| Fasting plasma insulin (pmol/l) | 35.0 (1.8) | 34.4 (1.5) | 37.3 (1.6) | 0.901 |
| Fasting plasma split proinsulin (pmol/l) | 4.2 (2.1) | 4.4 (1.9) | 4.8 (2.1) | 0.478 |
| 2-hour plasma glucose (mmol/l) | 5.3 (1.3) | 5.3 (1.3) | 5.5 (1.3) | 0.636 |
| 2-hour plasma insulin (pmol/l) | 161.6 (2.3) | 155.6 (2.0) | 162.4 (2.1) | 0.901 |
| No. (%) with NIDDM | 2 (2.3) | 0 | 2 (2.4) | |
| No. (%) with IGT | 3 (3.4) | 7 (6.8) | 4 (4.8) | |

Mean (SD) are shown except for insulin and glucose measurements where geometric mean and SD are shown.

## 5.2 Similarities between twins

Probandwise concordance rates and intraclass correlation coefficients were calculated in order to explore the role of genetic factors in determining adult glucose tolerance.

### 5.2.1 Probandwise concordance rates

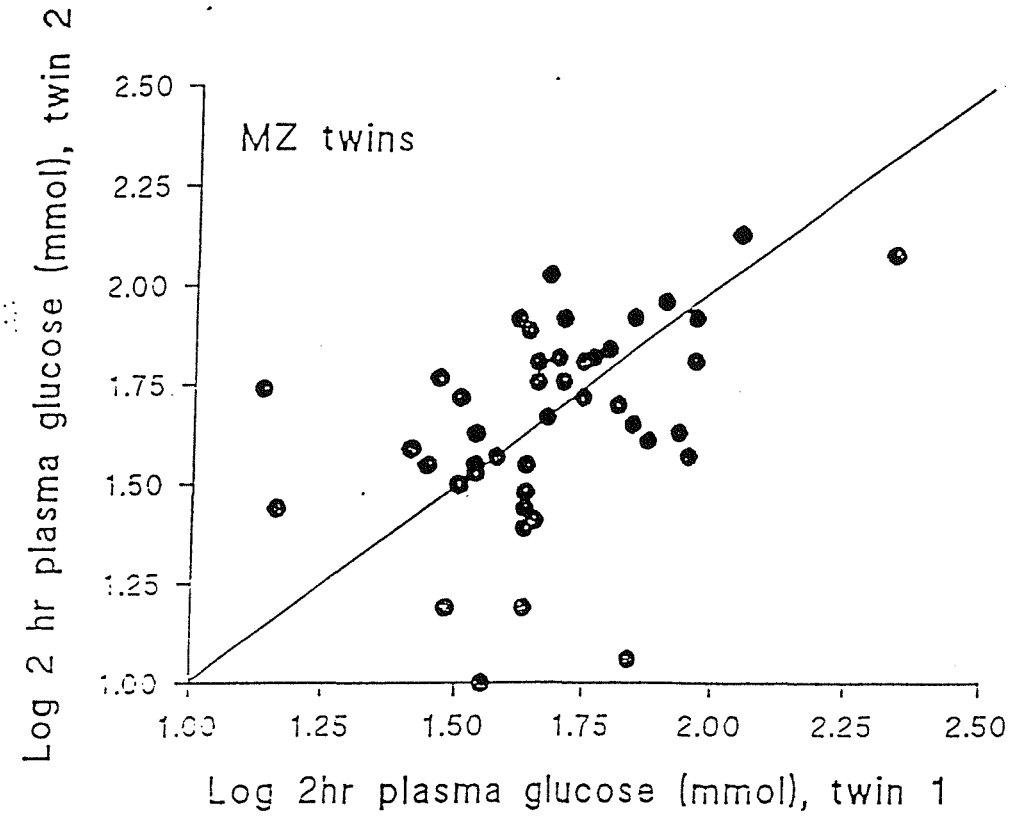
A total of six individuals (1.6%) had non-insulin dependent diabetes (four newly diagnosed and two known cases) and 24 (6.3%) had impaired glucose tolerance. However, very few twin pairs were concordant for either non-insulin dependent diabetes or impaired glucose tolerance: four twins pairs were concordant for impaired glucose tolerance or diabetes (two monozygotic and two dizygotic), while the co-twins of 13 other twins with diabetes or impaired glucose tolerance had normal glucose tolerance. Eleven of the diabetes/impaired glucose tolerance discordant pairs were dizygotic while the remaining two were monozygotic.

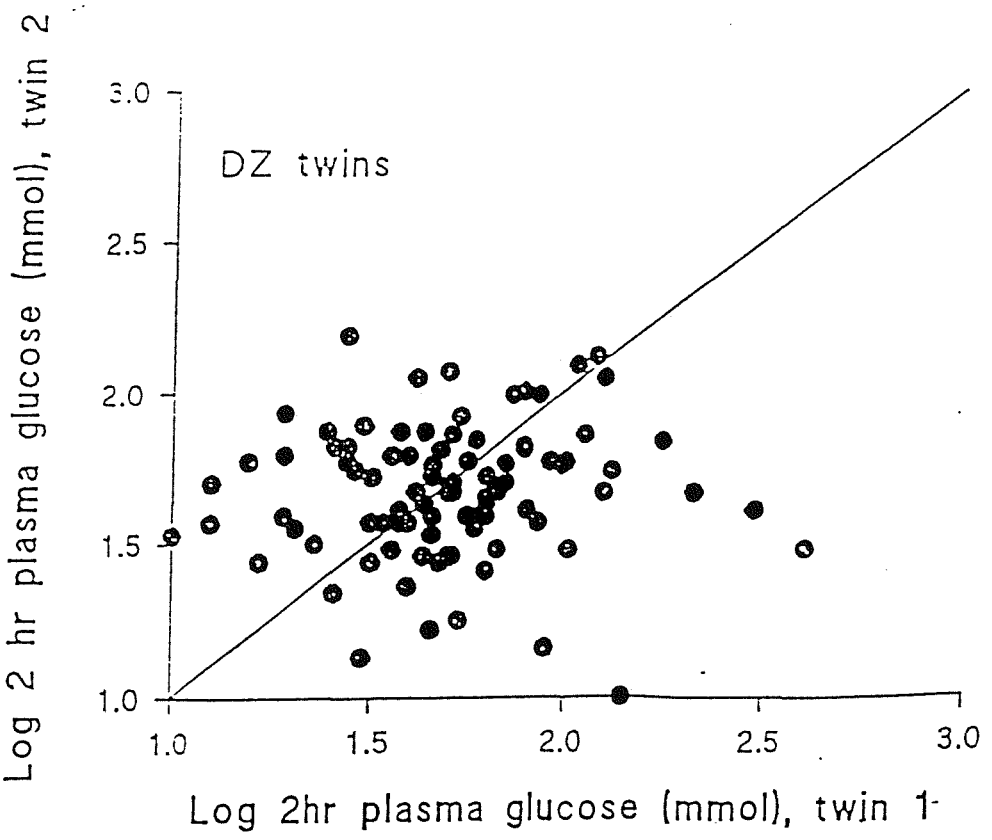
The combined (impaired glucose tolerance/diabetes) probandwise concordance rates were 0.80 for monozygotic twins and 0.27 for dizygotic twins.

### 5.2.2 Intra-class correlation coefficients

The similarities within twin pairs were explored by plotting the two-hour glucose concentration in one twin against that of their co-twin (figure 5.1). Each point on the graph represents a twin pair. The twins were plotted according to birth order. The line is the line of identity and points falling on this line represent twin pairs whose glucose levels correlate exactly. Two-hour glucose levels were more highly correlated in the monozygotic twins than in the dizygotic twins. Similar trends were seen for two-hour insulin, fasting insulin, proinsulin and split proinsulin.

**Figure 5.1: Correlations between two-hour plasma glucose concentrations in pairs of monozygotic (MZ) and dizygotic (DZ) twins**



Intra-class correlation coefficients were calculated from the within and between pair variances in the outcome variables. The plots displayed in figure 5.1 may have been biased by the labelling of twin 1 and 2. Calculation of intra-class correlation coefficients allows for this in that it is the within pair difference that is examined rather than absolute levels of glucose tolerance. Table 5.3 shows the within and between pair variances ($\delta^2$,$\delta b^2$) in log values of two-hour plasma glucose and insulin concentrations, and in fasting insulin, proinsulin and 32-33 split proinsulin according to zygosity. The corresponding intra-class correlation coefficients (r) are also shown. Within pair standard deviations in monozygotic twin pairs were lower than those in the dizygotic pairs for all outcome variaibles. This fits with the greater correlation in levels of two-hour glucose concentration in monozygotic twins compared with dizygotic twins as shown in figure 5.1.

**Table 5.3: Within pair ($\delta^2$) and between pair ($\delta b^2$) standard deviations and intraclass correlation coefficients (r) according to zygosity**

| | Monozygotic twins | | | Dizygotic twins | | |
|---|---|---|---|---|---|---|
| | $\delta^2$ | $\delta b^2$ | r | $\delta^2$ | $\delta b^2$ | r |
| **Two-hour glucose concentration (mmol/l)** | 1.12 | 0.79 | 0.40 | 1.88 | 0.15 | 0.06 |
| **Two-hour insulin concentration (pmol/l)** | 1.27 | 1.04 | 0.43 | 1.76 | 0.08 | 0.03 |
| **Fasting insulin concentration (pmol/l)** | 1.11 | 1.17 | 0.50 | 1.81 | 0.04 | -0.03 |
| **Fasting proinsulin concentration (pmol/l** | 0.76 | 1.09 | 0.61 | 1.60 | 0.45 | 0.18 |
| **Fasting 32-33 split prosinulin concentration (pmol/l)** | 0.82 | 1.53 | 0.63 | 1.63 | 0.19 | 0.08 |

Heritability estimates (h), derived from intraclass correlation coefficients, suggested a strong contribution of genetic factors to the variance in levels of fasting insulin, proinsulin, 32-33 split proinsulin and two-hour insulin with levels of 1.08, 0.86, 1.32 and 0.80 respectively. A non-parametric analysis was also used to derive heritability estimates in order to allow for the possible influence of outliers. The corresponding

heritability estimates derived using non-parametric methods were 0.98, 0.74, 1.08 and 0.82 suggesting that outliers had a minor influence on estimates for fasting insulin and its precursors but nevertheless confirming high heritability estimates for these variables. The heritability estimate for two-hour glucose was lower at 0.66 suggesting that genetic factors were less important in determining the variance of glucose tolerance. This was confirmed by the non-parametric estimate of 0.68.

## 5.3 Differences within and between twin pairs

### 5.3.1 Within pair regression analysis

Inspection of the relation between within pair difference in birth weight, length and ponderal index at birth and within pair difference in adult two-hour glucose level using scatter plots did not suggest that within pair differences in size at birth were predictive of within pair difference in adult glucose tolerance (figure 5.2 to 5.4).

**Figure 5.2: Relation between within pair difference in birth weight and within pair difference in two-hour glucose in monozygotic and dizygotic twins**

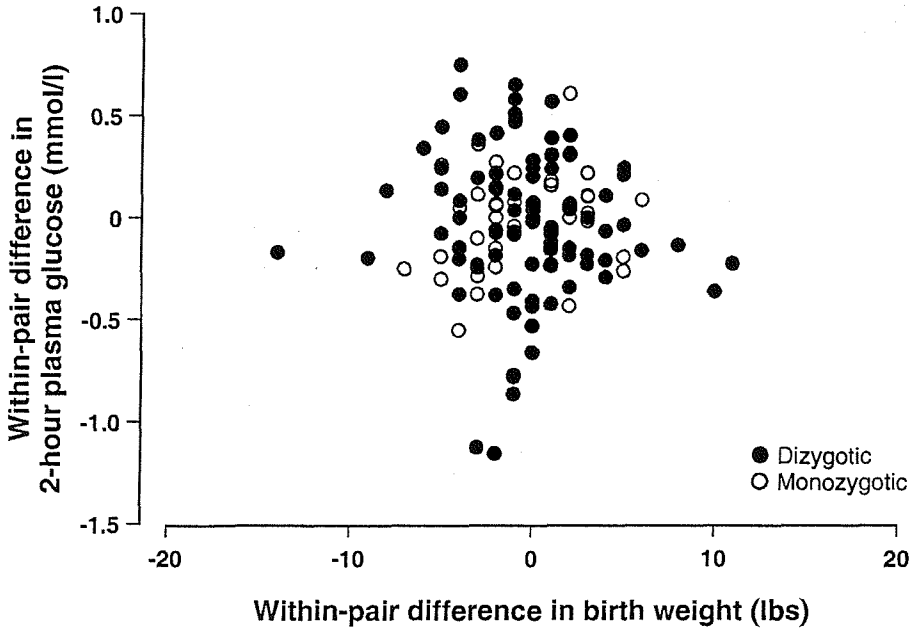


Figure 5.3: Relation between within pair difference in length at birth and within pair difference in two-hour glucose in monozygotic and dizygotic twins

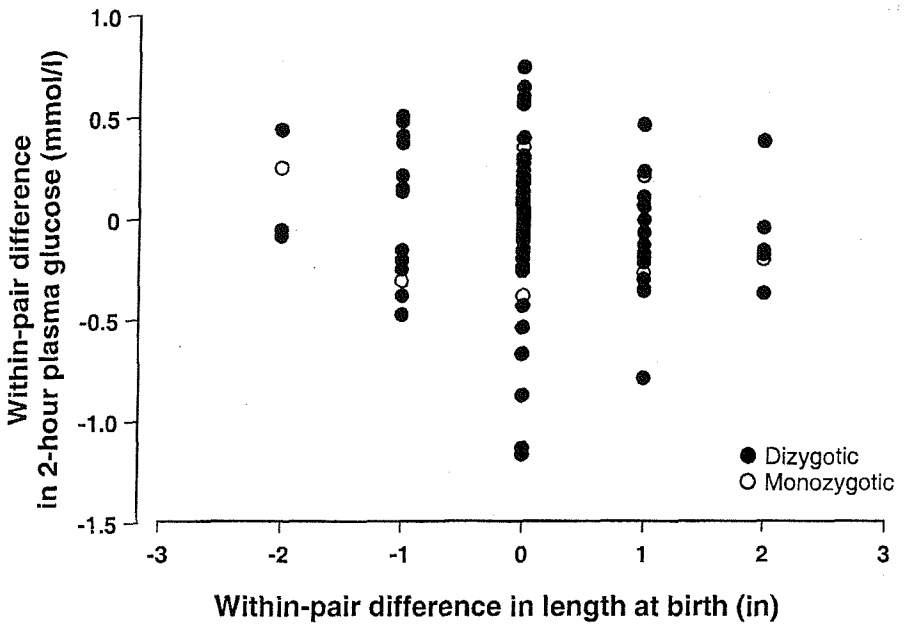
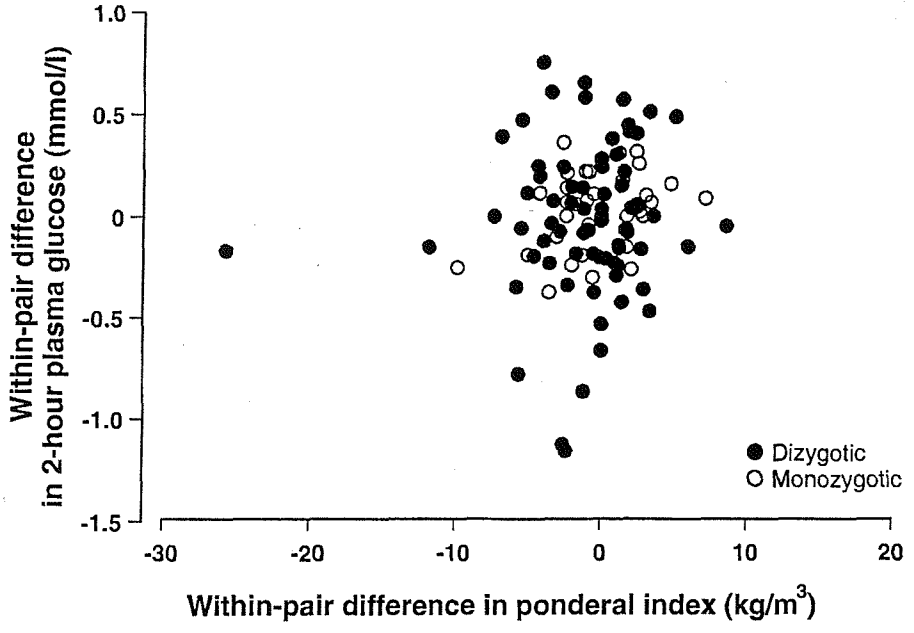



Figure 5.4: Relation between within pair difference in ponderal index and within pair difference in two-hour glucose in monozygotic and dizygotic twins



Multiple regression was used to explore the influence of within pair difference in the levels of eight potential confounding variables on the within pair difference in glucose tolerance (table 5.4). Adult body mass index showed a positive association with adult glucose tolerance. All regression analyses in twin pairs were adjusted for body mass index and sex, where appropriate.

**Table 5.4: Simultaneous effect of within pair difference in eight potential confounding variables on within pair difference in adult glucose tolerance**

| Change in glucose tolerance (mmol/l) | | | |
|---|---|---|---|
| | **All twins** | **Monozygotic twins** | **Dizygotic twins** |
| **Body mass index ($kg/m^2$)** | 0.03 (0.01, 0.04) | 0.03 (-0.02, 0.07) | 0.03 (0.02, 0.04) |
| **Sex** | -0.06 (-0.20, 0.08) | Not applicable | -0.11 (-0.29, 0.07) |
| **Current social class** | -0.03 (-0.07, 0.01) | 0.01 (-0.06, 0.09) | -0.03 (-0.09, 0.03) |
| **Current level of physical activity** | 0.01 (-0.02, 0.05) | -0.07 (-0.13, -0.01) | 0.04 (-0.004, 0.08) |
| **Level of physical activity (15-25 years)** | 0.03 (-0.03, 0.09) | 0.01 (-0.09, 0.12) | 0.04 (-0.04, 0.11) |
| **Level of physical activity (26-40 years)** | -0.03 (-0.09, 0.03) | 0.01 (-0.09, 0.11) | -0.04 (-0.12, 0.04) |
| **Smoking status** | -0.02 (-0.08, 0.04) | -0.05 (-0.19, 0.09) | -0.02 (-0.09, 0.05) |
| **Alcohol consumption** | 0.04 (-0.01, 0.09) | 0.05 (-0.04, 0.13) | 0.03 (-0.03, 0.09) |

Figures represent the change in two-hour glucose concentration for every unit change in the relevant confounding variable. 95% confidence intervals are shown in parentheses.
Definitions of confounding variables are given in section 2.9.5.

Table 5.5 shows the relation between within pair differences in the three measures of size at birth and the within pair variation in glucose and insulin at two hours and fasting insulin, proinsulin and 32-33 split proinsulin. All analyses were adjusted for sex and current body mass index. The regression line was constrained to pass through the origin to ensure that the results were independent of the labelling of the twins.

Differences in birth weight, length and ponderal index were not predictive of within pair differences in two-hour plasma glucose in either monozygotic or dizygotic twins. Length at birth was, however, inversely related to within pair differences in two-hour plasma insulin and fasting split proinsulin concentrations in monozygotic twins: two-hour insulin decreased by 0.415pmol/l and split proinsulin decreased by 0.403pmol/l for every 1 inch increase in length at birth. A similar trend was seen in relation to birth weight and split proinsulin: fasting split proinsulin decreased by 0.232pmol/l for every 1lb increase in birth weight. None of these trends were observed in the dizygotic twins. Although within pair differences in birth weight and length at birth were related to within pair differences in fasting split proinsulin, no significant associations were observed in relation to fasting insulin or proinsulin.

**Table 5.5: Within pair regression analyses of birth weight, length at birth and ponderal index on measures of adult glucose tolerance and insulin resistance in monozygotic and dizygotic twins**

| | Change in two-hour glucose conc. (mmol/l) | Change in two-hour insulin conc. (pmol/l) | Change in fasting insulin conc. (pmol/l) | Change in fasting proinsulin conc. (pmol/l) | Change in fasting 32-33 split proinsulin conc. (pmol/l) |
|---|---|---|---|---|---|
| **Mono-zygotic twins** | | | | | |
| **Birth weight (difference of 1 lb)** | 0.056 (-0.044, 0.16) | 0.054 (-0.289, 0.397) | 0.052 (-0.161, 0.266) | -0.035 (-0.177, 0.108) | -0.232[a] (-0.444, -0.018) |
| **Length at birth (difference of 1 inch)** | -0.038 (-0.141, 0.066) | -0.415[a] (-0.775, -0.056) | -0.064 (-0.314, 0.186) | -0.071 (-0.263, 0.122) | -0.403[a] (-0.641, -0.165) |
| **Ponderal index (difference of 1 kg/m$^3$)** | 0.016 (-0.005, 0.037) | 0.034 (-0.043, 0.112) | -0.004 (-0.056, 0.048) | -0.014 (-0.054, 0.027) | -0.028 (-0.086, 0.030) |
| **Dizygotic twins** | | | | | |
| **Birth weight (difference of 1 lb)** | -0.046 (-0.122, 0.028) | -0.160 (-0.371, 0.052) | 0.032 (-0.108, 0.172) | 0.026 (-0.104, 0.156) | 0.058 (-0.116, 0.232) |
| **Length at birth (difference of 1 inch)** | -0.054 (-0.140, 0.032) | -0.157 (-0.405, 0.091) | 0.046 (-0.127, 0.219) | 0.064 (-0.094, 0.222) | -0.004 (-0.214, 0.206) |
| **Ponderal index (difference of 1 kg/m$^3$)** | 0.005 (-0.014, 0.023) | -0.005 (-0.058, 0.048) | -0.000002 (-0.036, 0.036) | 0.0003 (-0.034, 0.033) | 0.003 (-0.040, 0.047) |

Figures represent the change in the outcome variable for every unit change in birth weight, length or ponderal index with 95% confidence intervals in parentheses.

[a] $p<0.05$

The birth weight analysis relates to 44 monozygotic and 91 dizygotic twin pairs.

The length and ponderal index analyses relate to 33 monozygotic and 74 dizygotic twin pairs

### 5.3.2 Differences between heavier and lighter twins

Another approach to examining the association between differences in birth size and adult levels of disease that has been employed in other twin studies is to examine differences in levels of outcome variables in the heavier and lighter twins in each twin pair. Using this approach, we examined levels of two-hour glucose in the heavier twins compared with the lighter twins. Mean (standard deviation) log 2-hour glucose in the heavier twins was 1.69mmol/l (0.23) compared with 1.67mmol/l (0.24) in the lighter twins and 1.74mmol/l in the twins of equal size, after adjustment for potential confounding variables.

This analysis did not show higher levels of glucose intolerance in the lighter twins. Further more, this analysis is weak since information is lost about the size of the birth weight differences in twin pairs and because it breaks the natural pairing of the twins. Although this type of analysis has benefits in overcoming the potential biases of incorrect coding of twin 1 and twin 2, we addressed this potential bias by examining differences within twin pairs and by forcing the regression line through the origins as described above.

### 5.3.3 Multi-level modelling analysis

Multi-level modelling analysis was used to further explore the between and within pair variance in glucose tolerance in order to assess whether size at birth was predictive of the observed patterns of variance. Table 5.6 shows the relationship of length at birth to within and between pair variances in two-hour glucose, two-hour insulin, fasting insulin and 32-33 split proinsulin for monozygotic and dizygotic twins. Within pair variances in the four outcome variables were significantly greater in dizygotic than in monozygotic twin pairs. In addition for two-hour insulin, fasting insulin and split proinsulin between pair variances were significantly greater in monozygotic than dizygotic twin pairs. Length at birth was not predictive of this pattern of variance for two-hour glucose, fasting insulin and two-hour insulin. Length at birth was, however, predictive of the observed patterns of variance in relation to 32-33 split proinsulin (B=-0.0987, p=0.01).

When birth weight replaced length at birth in the model, similar patterns of variance were observed (table 5.7). Within pair variance was greater in the dizygotic than

monozyogtic twins although the differences were not statistically significant in relation to two-hour or fasting insulin. Between pair variance was significantly greater in the monozygotic twins in all outcome variables examined other than two-hour glucose. Birth weight was not, however, predictive of these trends for any of the outcome variables.

The findings of this analysis suggest that size at birth is not predictive of observed within and between pair variance in glucose tolerance and insulin resistance in either the monozygotic or dizygotic pairs.

**Table 5.6: Multi-level modelling analyses: within and between pair variance in glucose tolerance and insulin resistance according to length at birth**

| Response variable | Between pair variances | | | Within pair variance | | | Fixed predictor | B | SE (B) | p value |
|---|---|---|---|---|---|---|---|---|---|---|
| | MZ | DZ | p for diff | MZ | DZ | p for diff | | | | |
| **Log (glucose at 120 minutes).log mmol/l** | 0.0175 | 0.0097 | 0.2 | 0.0171 | 0.0380 | <0.0005 | None | _ | _ | _ |
| | 0.0175 | 0.0093 | 0.1 | 0.0172 | 0.0383 | <0.0005 | length | 0.007 | 0.015 | NS |
| | 0.0177 | 0.0091 | 0.1 | 0.0170 | 0.0385 | <0.0005 | length x zygosity | 0.0255 | 0.0301 | NS |
| **Log (insulin at 120 minutes).log mmol/l** | 0.352 | 0.141 | 0.005 | 0.225 | 0.361 | 0.01 | none | _ | _ | _ |
| | 0.352 | 0.145 | 0.005 | 0.218 | 0.355 | 0.01 | length | -0.043 | 0.044 | NS |
| | 0.371 | 0.153 | 0.0041 | 0.205 | 0.349 | 0.005 | length x zygosity | 0.133 | 0.106 | NS |
| **Log (insulin at 0 minutes).log pmol/l** | 0.173 | 0.071 | 0.001 | 0.106 | 0.166 | 0.01 | none | _ | _ | _ |
| | 0.176 | 0.070 | 0.001 | 0.106 | 0.166 | 0.01 | length | -0.035 | 0.029 | NS |
| | 0.174 | 0.070 | 0.001 | 0.106 | 0.167 | 0.01 | length x zygosity | -0.030 | 0.072 | NS |
| **Log (32-33 split proinsulin).log pmol/l** | 0.279 | 0.153 | 0.01 | 0.125 | 0.226 | 0.001 | none | _ | _ | _ |
| | 0.286 | 0.153 | 0.01 | 0.110 | 0.220 | <0.001 | length | -0.0987 | 0.0405 | 0.01 |
| | 0.311 | 0.166 | 0.004 | 0.099 | 0.214 | <0.001 | length x zygosity | 0.1226 | 0.0888 | 0.17 |

**Table 5.7: Multi-level modelling analyses: within and between pair variance in glucose tolerance and insulin resistance according to birth weight**

| Response variable | Between pair variances | | | Within pair variance | | | Fixed predictor | B | SE (B) | p value |
|---|---|---|---|---|---|---|---|---|---|---|
| | MZ | DZ | p for diff | MZ | DZ | p for diff | | | | |
| **Log (glucose at 120 minutes).log mmol/l** | 0.0208 | 0.0127 | NS | 0.0313 | 0.0432 | 0.025 | None | – | – | – |
| | 0.0206 | 0.0126 | NS | 0.0312 | 0.0432 | 0.025 | birth weight | 0.0009 | 0.0037 | NS |
| | 0.0204 | 0.0126 | NS | 0.0304 | 0.0426 | 0.025 | birth weight x zygosity | -0.0130 | 0.0077 | NS |
| **Log (insulin at 120 minutes).log mmol/l** | 0.256 | 0.133 | 0.025 | 0.343 | 0.392 | NS | none | – | – | – |
| | 0.259 | 0.135 | 0.005 | 0.344 | 0.392 | NS | birth weight | -0.00555 | 0.01096 | NS |
| | 0.254 | 0.133 | 0.005 | 0.342 | 0.390 | NS | birth weight x zygosity | -0.0288 | 0.0247 | NS |
| **Log (insulin at 0 minutes).log pmol/l** | 0.130 | 0.060 | 0.005 | 0.135 | 0.165 | NS | none | – | – | – |
| | 0.127 | 0.059 | 0.005 | 0.135 | 0.163 | NS | birth weight | 0.0061 | 0.0068 | NS |
| | 0.122 | 0.056 | 0.01 | 0.135 | 0.164 | NS | birth weight x zygosity | -0.0178 | 0.0158 | NS |
| **Log (32-33 split proinsulin).log pmol/l** | 0.244 | 0.136 | 0.01 | 0.151 | 0.227 | 0.01 | none | – | – | – |
| | 0.250 | 0.137 | 0.005 | 0.147 | 0.227 | 0.005 | birth weight | -0.0078 | 0.0091 | 0.4 |
| | 0.256 | 0.141 | 0.005 | 0.144 | 0.225 | 0.005 | birth weight x zygosity | 0.0107 | 0.0203 | 0.4 |

### 5.3.4 Glucose tolerance in length discordant pairs

The within pair regression analysis showed a significant negative association between length at birth and adult levels of fasting split proinsulin and two-hour insulin, although this was statistically significant only in the monozygotic twin sub-group. We therefore examined the difference in mean two-hour plasma glucose and insulin and split proinsulin concentrations in three sub-groups divided according to the within pair difference in length at birth. Almost a half of twins pairs (n=46) had differences of one inch or more in length although only a small number had differences of 2 inches (n=10).

Table 5.8 shows that the shorter member of a twin pair had a higher mean two-hour plasma glucose, two-hour insulin and fasting 32-33 split proinsulin concentration where length differed by one inch or more. The differences were larger the greater the length discordance between the twins.

**Table 5.8: Mean two-hour glucose, two-hour insulin and fasting split proinsulin concentrations according to within pair length difference in all twin pairs**

| Within pair difference in length (inches) | Geometric mean plasma 2-hour glucose concentration (mmol/l) | Geometric mean plasma 2-hour insulin concentration (pmol/l) | Geometric mean plasma fasting 32-33 split proinsulin |
|---|---|---|---|
| **no difference** | 5.52 (61) | 151.15 (60) | 4.32 (59) |
| **1-inch difference** | | | |
| **shorter twin** | 5.50 (36) | 164.63 (35) | 5.39 (36) |
| **longer twin** | 5.20 | 134.75 | 4.33 |
| **2-inch difference** | | | |
| **shorter twin** | 6.07 (10) | 217.24 (10) | 4.34 (10) |
| **longer twin** | 5.43 | 145.04 | 4.68 |

Figures in parentheses represent numbers of twin pairs.

This analysis was repeated in the monozygotic twins only, allowing control for genetic factors. Similar trends were observed as for all twins. The shorter twin at birth had higher adult levels of mean two-hour glucose, two-hour insulin and fasting

32-33 split proinsulin. The exception was in the 1 inch difference group where the longer twin at birth had higher adult glucose tolerance. The numbers of length discordant pairs were small, however, with only six pairs having differences of one inch and two pairs having length differences of two inches. (table 5.9).

**Table 5.9: Mean two-hour glucose, two-hour insulin and fasting split proinsulin concentrations according to within pair length difference in monozygotic twin pairs**

| Within pair difference in length at birth (inches) | Geometric mean plasma 2-hour glucose concentration (mmol/l) | Geometric mean plasma 2-hour insulin concentration (pmol/l) | Geometric mean plasma fasting 32-33 split proinsulin |
|---|---|---|---|
| **no difference** | 5.68 (25) | 173.26 (25) | 4.30 (24) |
| **1-inch difference:** | | | |
| **shorter twin** | 5.66 (6) | 197.95 (5) | 5.88 (6) |
| **longer twin** | 5.99 | 195.98 | 4.24 |
| **2-inch difference:** | | | |
| **shorter twin** | 5.90 (2) | 285.72 (2) | 5.21 (2) |
| **longer twin** | 4.74 | 75.19 | 2.08 |

Figures in parentheses represent numbers of twin pairs.

### 5.3.5 Glucose tolerance in birth weight discordant pairs

We carried out a similar analysis in relation to birth weight in monozygotic twins since the within pair regression analysis had suggested that within pair differences in birth weight were predictive of within pair differences in adult levels of 32-33 split proinsulin in monozygotic twins. Levels of two-hour glucose, two-hour insulin, fasting 32-33 split proinsulin and body mass index were examined according to the size of the birth weight difference (table 5.10).

**Table 5.10: Mean two-hour glucose, two-hour insulin and fasting split proinsulin concentrations and body mass index according to within pair difference in birth weight in monozygotic twin pairs**

| Difference in birth weight (lb) | Body mass index (kg/m$^2$) | Geometric mean two-hour plasma glucose concentration (mmol/l) | Geometric mean two-hour insulin concentration (pmol/l) | Fasting plasma 23-33 split proinsulin (pmol/l) |
|---|---|---|---|---|
| <0.25 | | | | |
| lighter twin | 24.2 (10) | 5.02 | 115.3 | 2.85 |
| heavier twin | 24.2 | 5.47 | 179.8 | 2.77 |
| 0.25-0.5 | | | | |
| lighter twin | 25.4 (14) | 5.10 | 146.7 | 4.05 |
| heavier twin | 24.7 | 5.17 | 137.9 | 3.99 |
| 0.5-1.0 | | | | |
| lighter twin | 24.2 (13) | 4.76 | 139.4 | 4.60 |
| heavier twin | 25.2 | 5.31 | 162.1 | 5.59 |
| >1.0 | | | | |
| lighter twin | 27.6 (7) | 6.22 | 277.0 | 6.59 |
| heavier twin | 28.3 | 6.32 | 268.5 | 3.67 |
| All (SD) n=44 | | | | |
| lighter twin | 25.2 (3.7) | 5.14 (1.28) | 159.7 (2.27) | 4.20 (2.00) |
| heavier twin | 25.3 (3.4) | 5.45 (1.25) | 170.7 (2.27) | 4.01 (2.23) |

Figures in parentheses represent numbers of twin pairs unless otherwise stated.

Levels of two-hour glucose, two-hour insulin and fasting split proinsulin tended to increase with increasing levels of birth weight discordance. In contrast to the findings in relation to length at birth where the smaller twin had higher levels of the outcome variables, these increases were seen in both members of the twin pair. Similarly, the body mass index of the twins also increased with increasing birth weight discordance. These analyses are based on a small numbers of twin pairs, however, particularly in relation to the most discordant pairs: only 7 twin pairs had differences in birth weight of greater than 1lb.

## 5.4 Associations between birth size and glucose tolerance in twin individuals

### 5.4.1 Characteristics of individuals participating in glucose tolerance study

The birth size, adult body mass index and adult glucose tolerance of twin individuals was compared according to sex and zygosity (table 5.11).

**Table 5.11: Characteristics of twin individuals participating in the glucose tolerance study according to sex and zygosity**

| | All (n=376) | Male (n=164) | Female (n=212) | Mean difference (95% CI) | MZ (n=94 41M/ 53F) | DZ (n=246 104M/ 142F) | Mean difference (95% CI) |
|---|---|---|---|---|---|---|---|
| **Age (years)** | 43.6 (1.4) | 43.6 (1.3) | 43.6 (1.5) | 0.0003 (-.28, .28) | 43.7 (1.43) | 43.7 (1.4) | 0.02 (-.32, .35) |
| **Birth weight (lbs)** | 5.51 (1.04) | 5.52 (1.11) | 5.27 (0.94) | 0.25 (.19, 0.62) p=<0.001 | 5.33 (1.06) | 5.55 (1.04) | -0.22 (-0.47, 0.07) |
| **Length at birth (inches)** | 18.57 (1.09) | 18.68 (1.15) | 18.47 (1.03) | 0.21 (-.04, .46) | 18.44 (1.14) | 18.55 (1.10) | -0.11 (-.42, .19) |
| **Gestation (weeks)** | 37.7 (2.4) | 37.7 (2.5) | 37.8 (2.3) | -0.1 (-.61, .43) | 37.5 (2.5) | 37.8 (2.4) | -0.33 (-.95, .30) |
| **Body mass index ($kg/m^2$)** | 25.97 (4.32) | 26.63 (3.94) | 25.46 (4.53) | 1.17 (0.3, 2.0) p=0.008 | 25.25 (3.69) | 25.97 (4.43) | -0.72 (-1.73, 0.29) |
| **Log fasting insulin (pmol/l)** | 3.63 (0.52) | 3.66 (0.54) | 3.61 (0.50) | 0.05 (-0.06, 0.15) | 3.56 (0.56) | 3.63 (0.48) | -0.07 (-.19,0.05) |
| **Log fasting split proinsulin (pmol/l)** | 1.52 (0.70) | 1.69 (0.75) | 1.39 (0.63) | 0.30 (0.16, 0.44) p=<0.001 | 1.46 (0.78) | 1.54 (0.68) | -0.08 (-0.26,.09) |
| **Log 2-hour plasma glucose (mmol/l)** | 1.69 (0.25) | 1.68 (0.30) | 1.70 (0.22) | -0.02 (-0.07,0.04) | 1.68 (0.25) | 1.69 (0.26) | -0.01 (-.07,.05) |
| **Log 2-hour plasma insulin (mmol/l)** | 5.09 (0.76) | 4.98 (0.54) | 5.17 (0.63) | -0.19 (-0.4,-0.03) p=0.02 | 5.09 (0.81) | 5.06 (0.73) | 0.03 (-.16,0.20) |

Monozyogtic twins were lighter than dizygotic twins with mean birth weights of 5.33 compared to 5.55lb, but the difference was not statistically significant. The

monozygotic twins were also marginally shorter, with a mean length at birth of 18.44 compared to 18.55 inches in the dizygotic twins, and of slightly shorter gestation although these differences were not statistically significant. Adult body mass index was lower in the monozygotic twins but, as for size at birth, the difference was not statistically significant. There were no significant differences in adult levels of fasting insulin and its precursors or of two-hour glucose or insulin in the monozygotic and dizygotic twins.

When the subjects were subdivided according to sex, men had significantly higher birth weights and adult body mass indices than women. Mean levels of two-hour glucose, and fasting insulin were not significantly different in males and females. However, levels of fasting split proinsulin were significantly higher in the male twins.

### 5.4.2 Association between birth size and adult glucose tolerance

Inspection of the relation between two-hour glucose and birth weight, length and ponderal index using scatter plots (figure 5.5 to 5.7) did not suggest that size at birth was predictive of adult glucose tolerance in the subjects studied.

**Figure 5.5: Relation between birth weight and two-hour glucose in monozygotic and dizygotic twins**

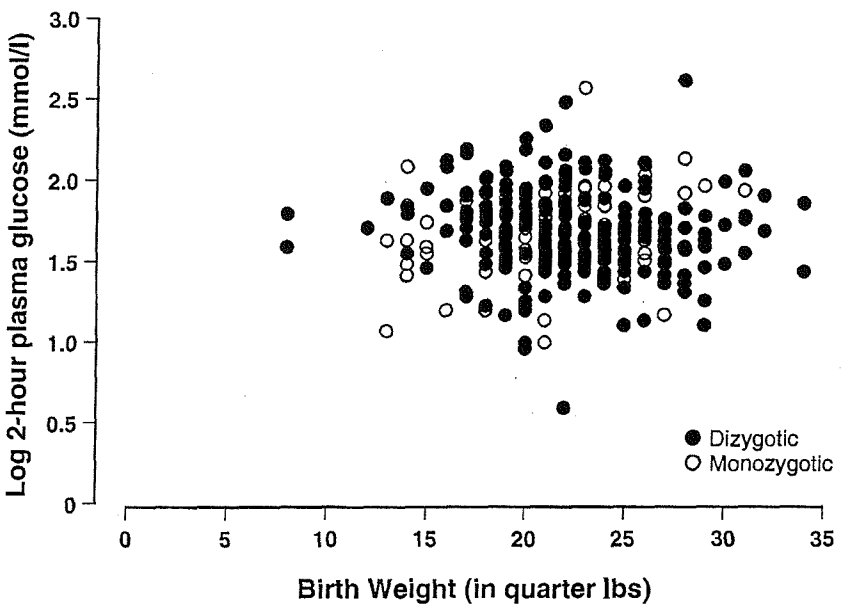


Figure 5.6: Relation between length at birth and two-hour glucose in monozygotic and dizygotic twins

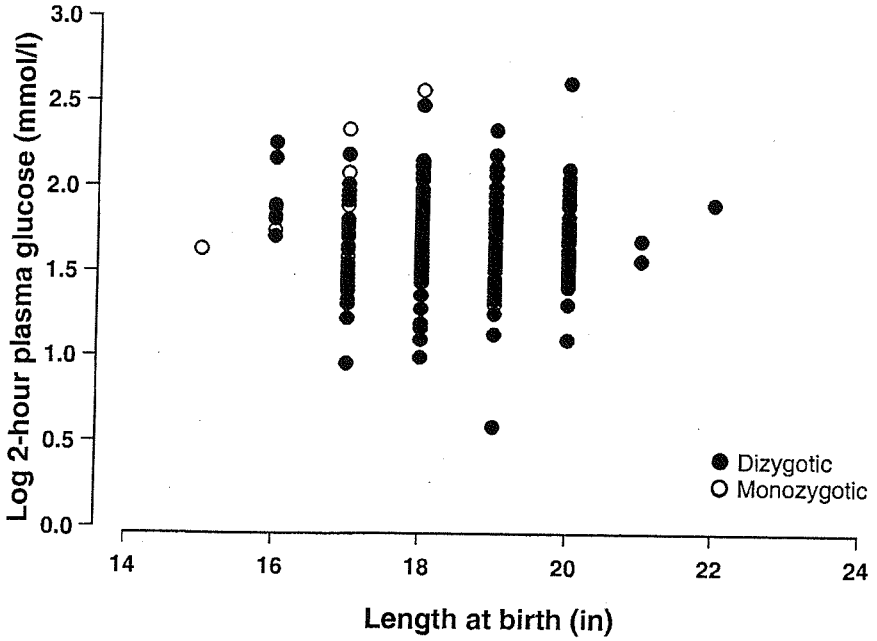
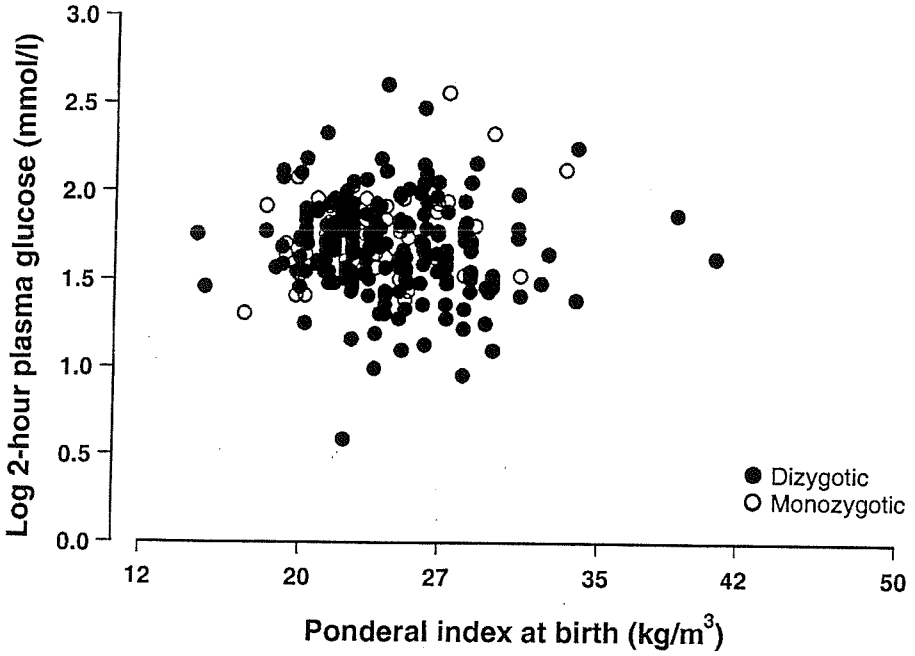



Figure 5.7: Relation between ponderal index at birth and two-hour glucose in monozygotic and dizygotic twins



The influence of eight potential confounding variables on two-hour glucose levels was explored (table 5.12) in order that appropriate adjustments could be made in a multiple regression analysis. Adult body mass index showed a positive association with adult glucose tolerance. All regression analyses in table 5.13 were adjusted for body mass index and sex.

**Table 5.12: Simultaneous effect of eight potential confounding variables on adult glucose tolerance in twin individuals**

| | Change in glucose tolerance (mmol/l) | | |
|---|---|---|---|
| | **All twins** | **Monozygotic twins** | **Dizygotic twins** |
| **Body mass index ($kg/m^2$)** | 0.02 (0.01, 0.03) | 0.02 (0.01, 0.03) | 0.02 (0.002, 0.04) |
| **Sex** | 0.03 (-0.03, 0.10) | 0.04 (-0.04, 0.12) | 0.05 (-0.08, 0.18) |
| **Social class at birth** | -0.01 (-0.03, 0.02) | -0.009 (-0.04, 0.02) | 0.02 (-0.04, 0.08) |
| **Current social class** | -0.01 (-0.03, 0.01) | 0.001 (-0.03, 0.03) | -0.05 (-0.08, -0.003) |
| **Current level of physical activity** | -0.009 (-0.03, 0.02) | -0.007 (-0.04, 0.02) | -0.05 (-0.10, 0.008) |
| **Level of physical activity (15-25 years)** | 0.004 (-0.03, 0.04) | -0.002 (-0.05, 0.04) | 0.02 (-0.06, 0.09) |
| **Level of physical activity (26-40 years)** | 0.01 (-0.03, 0.05) | -0.006 (-0.06, 0.04) | -0.06 (-0.008, 0.14) |
| **Smoking status** | 0.001 (-0.03, 0.03) | 0.02 (-0.03, 0.06) | -0.03 (-0.10, 0.04) |
| **Alcohol consumption** | -0.0009 (-0.002, 0.00) | -0.001 (-0.002. 0.001) | -0.0001 (-0.005, 0.005) |

Figures represent the change in two-hour glucose concentration for every unit change in the relevant confounding variable. 95% confidence intervals are shown in parentheses. Definitions of confounding variables are given in section 2.9.5.

Multiple logistic regression analysis was used to explore the relation between birth weight, length at birth and ponderal index and glucose tolerance in the 376 twin

individuals (table 5.13). Complete data was available for 372 twin individuals. There was no association between birth weight and glucose tolerance, after adjusting for body mass index and sex. Analysis of males and females separately and of monozygotic and dizygotic individuals did not strengthen these findings. Likewise, length at birth and ponderal index were not predictive of adult glucose tolerance.

In the sub-group of monozygotic individuals there was a statistically significant positive association between ponderal index and glucose tolerance. However, this was not seen in all twins, in the dizygotic individuals nor in the males or females alone.

**Table 5.13: Relation between birth weight, length and ponderal index at birth and glucose tolerance in 372 twin individuals**

| | Change in glucose tolerance (mmol/l) | | | | |
|---|---|---|---|---|---|
| | **all twins** | **males** | **females** | **monozygotic individuals** | **dizygotic individuals** |
| **birth weight (lbs)** | -0.076 (-0.326, 0.161) | 0.0048 (-0.036, 0.044) | -0.022 (-0.052, 0.008) | 0.044 (-0.008, 0.092) | -0.026 (-0.056, 0.004) |
| **length at birth (inches)** | -0.013 (-0.038, 0.013) | 0.003 (-0.040, 0.045) | -0.0270 (-0.058, 0.004) | -0.006 (-0.053, 0.040) | -0.008 (-0.039, 0.24) |
| **ponderal index (kg/m3)** | -0.0007 (-0.009, 0.008) | -0.0004 (-0.013, 0.014) | -0.022 (-0128, 0.008) | 0.018 (0.002, 0.035) | -0.006 (-0.016, 0.004) |

Figures represent the change in two-hour glucose for every unit change in birth weight, length or ponderal index. 95% confidence intervals are displayed in parentheses.
The birth weight analysis relates to 372 individuals of which 163 were male, 209 were female, 92 were monozygotic and 245 were dizygotic. The length and ponderal index analysis relates to 301 individuals of which 137 were male, 164 were female, 71 were monozygotic and 204 were dizygotic. Zygosity is unknown for twins from like-sexed pairs whose co-twin did not participate.

## 5.4 Overview of glucose tolerance findings

### 5.5.1 Representativeness of the study group

The response rate in the glucose tolerance study was low with 38% of the target population taking part. Analyses relating to complete twin pairs were based on 28% of the possible twin pairs in the target population. Comparison of participants with non-participants revealed that female twins were more likely to participate in the study, accounting for 56% of the study group. However, comparisons of birth size (weight and length), gestational age and socio-economic status at birth did not reveal any significant differences between the two groups suggesting that the study group was representative of twins in the target population in relation to birth characteristics. No comparisons were possible in relation to adult socio-economic status or adult lifestyle since only birth record data was available for non-participants.

### 5.5.2 Birth size and adult glucose tolerance in twin pairs

#### 5.5.2.1 Comparison between monozygotic and dizygotic twins

We had hypothesised that levels of disease in monozygotic twins may be higher than those in dizygotic twins due to the more adverse prenatal environment of monochorionic twins. However, comparison of size at birth and adult body mass index in the monozygotic, dizygotic like-sexed and dizygotic unlike-sexed twins revealed no significant differences in the three groups. Similarly, there were no significant differences between the three groups in relation to two-hour glucose or to two-hour insulin or fasting insulin and its precursors.

#### 5.5.2.2 Similarities within twin pairs

The prevalence of non-insulin dependent diabetes and impaired glucose tolerance in the study group was 1.6% and 6.3%. These rates are comparable with those reported in other European populations of this age group.[137] Probandwise concordance rates, which indicate the similarity in members of a twin pair in relation to disease status were 0.80 in monozygotic twins compared with 0.27 in dizygotic twins. These rates suggest greater similarity within monozygotic twin pairs compared with dizygotic twins pairs. However, calculation of these rates was based on a small number of cases of disease. Therefore we examined the correlation in levels of each of the outcome variables in monozygotic and dizygotic twins by plotting the level in one

twin against that of their co-twin and by calculating intra-class correlation coefficients. This analysis revealed that adult levels of glucose tolerance and the other outcome variables were more highly correlated in the monozygotic twins than dizyogtic twins. Within pair variance for all of the outcome variables was lower in monozygotic than dizygotic twins.

Intra-class correlation coefficients were used to make estimates of heritability for each of the outcome variables. These estimates suggested that genetic factors played an important role in determining adult levels of glucose tolerance and insulin resistance. However, this classical twin analysis takes no account of the fact that monozygotic twins have a more similar prenatal environment than dizygotic twins. We therefore carried out a multi-level modelling analysis in order to assess the relative contributions of prenatal environment (as determined by size at birth), adult body mass index and genetic factors in determining patterns of variance in the outcome variables.

Multi-level modelling analysis confirmed that within pair variance in two-hour glucose and insulin and fasting insulin and its precursors was significantly greater in dizygotic than monozygotic twin pairs. Neither length at birth or birth weight were predictive of these patterns of variance.

#### 5.5.2.3 Within pair differences in size at birth and glucose tolerance

A within pair regression analysis was carried out in order to test the hypothesis that within pair differences in adult glucose tolerance would be predicted by within pair differences in size at birth. Analyses were adjusted for adult body mass index and sex. As suggested by scatter plots of the data, the regression analysis revealed that differences in size at birth were not predictive of adult levels of glucose tolerance. However, there was an inverse association between difference in length at birth and differences in adult two-hour insulin and fasting split proinsulin in monozygotic pairs. Similarly within pair birth weight difference was inversely associated with difference in fasting split proinsulin in monozygotic pairs. These findings are suggestive of a link between birth size and adult insulin resistance that is independent of genetic factors since levels of fasting insulin and its precursors are highly correlated with levels of insulin resistance.[16] However, this trend was not consistent since there was

no association between birth size and fasting insulin or proinsulin which would be expected in insulin resistance.

Further exploration of within pair differences in length at birth revealed that in twin pairs whose length differed by an inch or more, the shorter twins had higher levels of two-hour glucose, two-hour insulin and 32-33 split proinsulin. The differences were larger the greater the discordance between the twins. These trends were seen in all twins and also when the analysis was restricted to monozygotic pairs. Similar analysis in relation to birth weight discordance suggested that levels of glucose tolerance and body mass index were greater in both members of the most discordant monozygotic pairs. Although these findings suggest an effect of birth size that is independent of genetic factors, they need to be interpreted with caution since in both the length and birth weight analysis the trends seen were largely dependent on a small number of very discordant pairs.

### 5.5.3 Birth size and adult glucose tolerance in twin individuals

Comparison of birth size, adult body mass index and glucose tolerance in monozygotic and dizygotic twins revealed that, although monozygotic twins were lighter and shorter at birth, these differences were not statistically significant. Similarly, there was no significant difference in mean adult body mass index or glucose tolerance. Comparison of men and women revealed that men had significantly higher birth weight and adult body mass index. Levels of two-hour insulin were significantly lower and levels of fasting split proinsulin significantly higher in the men but there were no significant differences in adult glucose tolerance.

Associations between birth size and adult glucose tolerance were explored using regression analysis. As for twin pairs, scatter plots did not suggest that birth size was predictive of adult levels of glucose tolerance. No association was found between birth size and adult glucose tolerance or insulin resistance in the regression analysis. These findings were not strengthened when the twins were subdivided according to sex or zygosity.

# 6 BLOOD PRESSURE

In this chapter, the findings of the blood pressure study are presented. The characteristics of participants and non-participants are compared in order to assess the representativeness of the study group. Similarities within twin pairs are examined in order to examine the influence of size at birth and genetic factors in determining observed similarities. Within pair differences in adult blood pressure are examined in order to assess whether they are explained by differences in size at birth. The association of birth size with blood pressure is also examined in twin individuals in order to make comparisons with other fetal origins studies of singleton populations.

## 6.1 Characteristics of the study group

### 6.1.1 Response rate

A total of 507 subjects (52% of the target population) agreed to have their blood pressure measured, comprising 197 complete and 113 incomplete twin pairs. The response rate for complete twin pairs was 41%.

### 6.1.2 Comparison of participants and non-participants

The birth weight, length at birth, gestational age and socio-economic status of participants in the study was compared with that of non-participants using data from the birth records (table 6.1)

**Table 6.1: Comparison of characteristics of twins participating in the blood pressure study and non-participants**

| | Target population non-participants (n=466) | Twin individuals for whose blood pressure was measured (n=507) | Mean difference (95% CI) unless otherwise stated |
|---|---|---|---|
| **Birth weight (lbs)** | 5.49 (1.08) | 5.50 (1.05) | 0.004 (-0.53, 0.54) |
| **Length at birth (inches)** | 18.64 (1.19) | 18.57 (1.10) | -0.06 (-0.22, 0.10) |
| **Gestation** | 37.7 (2.6) | 37.8 (2.5) | 0.1 (-0.22, 0.45) |
| **Sex:** | | | |
| **Male** | 56.7% | 44.2% | $\chi^2$=15.11 |
| **Female** | 43.3% | 55.8% | (p<0.001) |
| **Year of birth:** | | | |
| **1950** | 21.7% | 18.9% | $\chi^2$for trend=4.013 |
| **1952** | 18.2% | 24.3% | (p=0.045) |
| **1952** | 17.6% | 21.7% | |
| **1953** | 16.5% | 19.5% | |
| **1954** | 26.0% | 15.6% | |
| **Occupational status of father:** | | | |
| **Professional & executive;** | 3.6% | 5.7% | $\chi^2$ for trend=1.222 (p=0.269) |
| **Skilled worker;** | 59.9% | 60.9% | |
| **Unskilled worker;** | 12.2% | 12.0% | |
| **Unknown.** | 24.2% | 21.3% | |
| **Quality of Housing:** | | | |
| **Good** | 42.5% | 51.7% | $\chi^2$ =8.222 |
| **Poor** | 57.5% | 48.3% | (p=0.004) |

Mean birth weight and length at birth did not differ significantly in subjects whose blood pressure was measured and those who did not participate in the study. As in the glucose tolerance study, women were over-represented in the blood pressure study accounting for 55.8% of participants In addition participants tended to be older with subjects who were born in 1951 or 1952 being more likely than those born in 1954 to

participate. Socio-economic status at birth, as defined by paternal occupational status, did not differ significantly between the groups. However, the twins who participated were more likely to be living in a ward area of 'good' housing quality at birth than those who declined to take part.

### 6.1.3 Characteristics of twin pairs participating in the blood pressure study

Mean birth weight, length at birth, gestational age and adult body mass index in monozygotic, dizygotic like-sexed and dizygotic unlike-sexed twins were compared using analysis of variance (table 6.2).

There were no significant differences in birth weight, length at birth or adult body mass index between the three groups. Mean birth weight was similar in the monozygotic, dizyogtic like-sexed and dizygotic unlike-sexed twin pairs at 5.4, 5.5 and 5.6lb respectively. Similar mean length at birth was also seen in the three groups at 18.5, 18.5 and 18.6 inches respectively. Mean gestational age was significantly greater in the dizygotic unlike-sexed pairs at 38.4 weeks compared with 37.8 and 37.3 weeks in the monozygotic and like-sexed dizygotic pairs. Mean adult body mass index was lower in the monozygotic than dizyogtic twins (25.5kg/$m^2$ compared with 26.3 kg/$m^2$ in the like-sexed and 25.9 kg/$m^2$ in the unlike-sexed dizygotic twins) but these differences were not statistically significant. Mean values of systolic and diastolic blood pressure were also compared and similar levels were seen in monozygotic and dizygotic like-sexed and unlike-sexed twins with no statistically significant difference between the three groups.

Twenty-three subjects were receiving treatment for raised blood pressure. Their inclusion might potentially weaken any association between blood pressure and birth weight if treatment had led to a marked decrease in blood pressure. However calculation of mean blood pressure values revealed that blood pressure in the group on anti-hypertensive medication was higher than that of the rest of the study group. Mean values of systolic blood pressure and diastolic blood pressure were more than 10mmHg higher than the mean values for the study group overall at 146.4 and 86.8 mmHg respectively compared with mean levels of 131mmHg and 75.6mmHg in the study group. Those on treatment for hypertension were therefore included in the

study since to exclude then would have removed some of the most informative subjects from the analysis.

**Table 6.2: Characteristics of twin pairs participating in the blood pressure study**

| | All | Monozygotic twin pairs | Dizygotic like-sexed twin pairs | Dizygotic unlike-sexed twin pairs | P value |
|---|---|---|---|---|---|
| **Number of pairs (men/ women)** | 197 | 57 (25/32) | 80 (35/45) | 60 | |
| **Birth weight (lbs)** | 5.5 (1.0) | 5.4 (0.99) | 5.5 (1.1) | 5.6 (0.9) | 0.283 |
| **Length at birth (inches)** | 18.5 (1.1) | 18.5 (1.1) | 18.5 (1.1) | 18.6 (1.1) | 0.736 |
| **Ponderal index at birth ($kg/m^3$)** | 24.2 (3.4) | 23.9 (3.0) | 24.2 (3.4) | 24.4 (3.6) | 0.478 |
| **Body mass index ($kg/m^2$)** | 25.9 (4.3) | 25.5 (3.9) | 26.3 (4.7) | 25.9 (4.0) | 0.320 |
| **Gestational age (weeks)** | 37.8 (2.4) | 37.8 (2.4) | 37.3 (2.5) | 38.4 (2.0) | 0.004 |
| **Systolic blood pressure (mm Hg)** | 131.0 (15.7) | 130.5 (12.4) | 133.8 (15.0) | 130.0 (16.4) | 0.494 |
| **Diastolic blood pressure (mm Hg)** | 75.6 (12.4) | 75.0 (11.3) | 79.6 (11.2) | 76.0 (11.6) | 0.814 |
| **Age (years)** | 43.7 (1.4) | 43.7 (1.4) | 43.6 (1.5) | 43.7 (1.3) | 0.882 |

## 6.2 Similarities within twin pairs

Probandwise concordance rates and intra-class correlation coefficients were calculated in order to explore the role of genetic factors in determining adult blood pressure.

### 6.2.1 Probandwise concordance rates

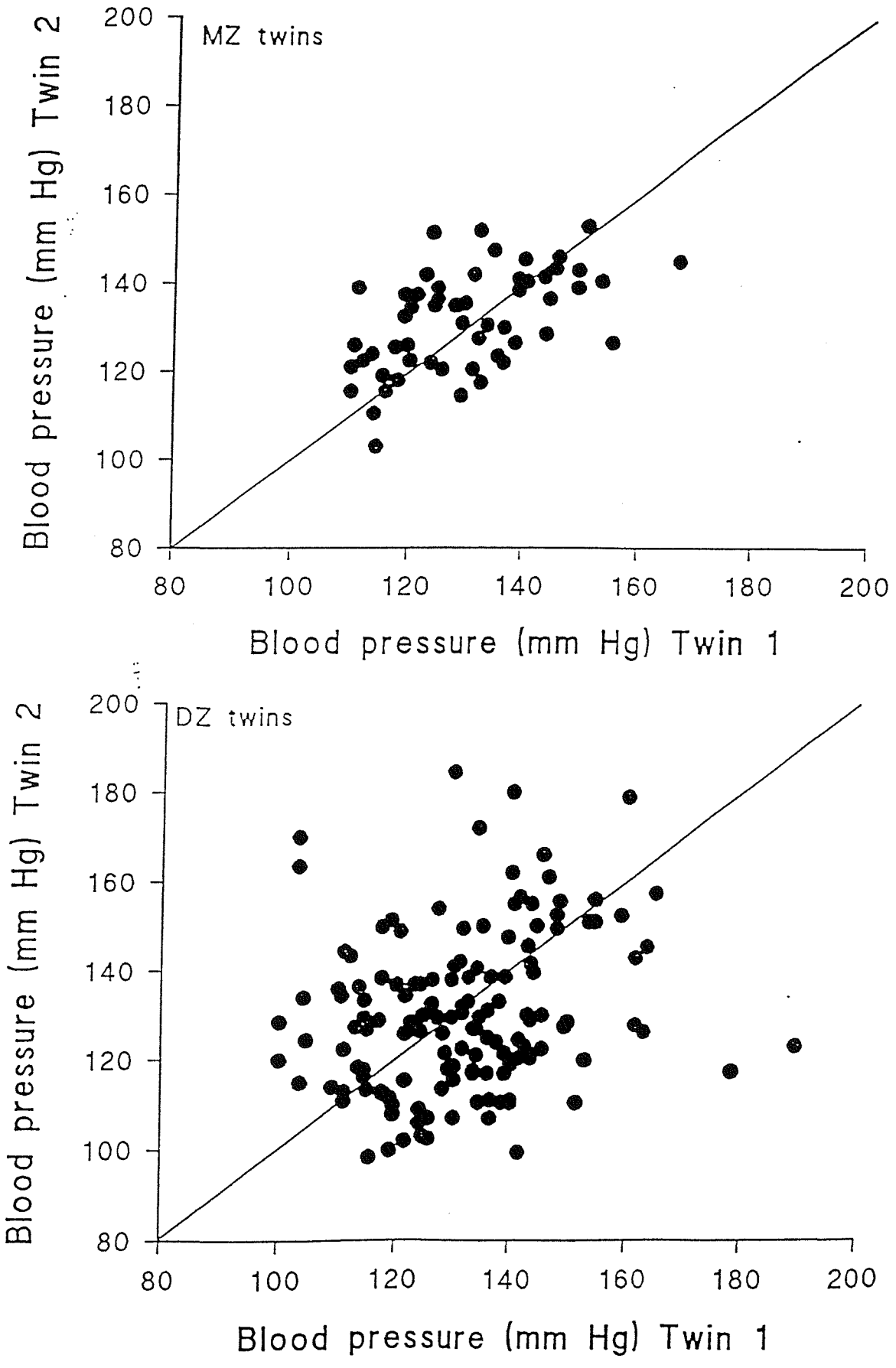
Blood pressure was measured in a total of 504 individuals. There were 197 complete twin pairs of which 57 were monozygotic and 140 dizygotic. Twenty-three individuals were receiving treatment for hypertension and a further 17 had systolic blood pressure levels greater than 160mm Hg and diastolic levels greater than 90mm Hg. Based on these numbers, the prevalence of hypertension was 7.9%.

Only three twin pairs (two monozygotic and one dizygotic) were concordant for hypertension and there were 34 discordant pairs who were all dizygotic. Concordance rates were not calculated since they would not have been meaningful when based on such a small number of concordant pairs.

### 6.2.2 Intra-class correlation coefficients

The similarities within twin pairs were explored by plotting systolic blood pressure in one twin against that of their co-twin (figure 6.1). Each point on the graph represents a twin pair. The line is the line of identity and points falling on this line represent twin pairs whose systolic blood pressure levels correlate exactly. Systolic blood pressure was more highly correlated in the monozygotic twins than in the dizygotic twins.

**Figure 6.1: Correlations between systolic blood pressure levels in pairs of monozygotic (MZ) and dizygotic (DZ) twins**



Intra-class correlation coefficients were calculated from the within and between pair variances in the outcome variables. The plots displayed in figure 6.1 may have been biased by the labelling of twin 1 and 2. Calculation of intra-class correlation coefficients allows for this in that it is the within pair difference that is examined rather than absolute levels of blood pressure. Table 6.3 shows the within and between pair variances in systolic blood pressure and diastolic blood pressure. The within pair variance in systolic blood pressure was lower in the monozygotic twins and this was reflected in the intra-class correlation of 0.52 compared with 0.23 in dizygotic twins. Similarly, coefficients for diastolic blood pressure in monozyogtic and dizygotic twins were 0.60 and 0.25 respectively.

**Table 6.3 Within pair ($\delta^2$) and between pair ($\delta b^2$) standard deviations and intraclass correlation coefficients (r) for blood pressure according to zygosity**

| | Systolic blood pressure (mm Hg) | | | | Diastolic blood pressure (mm Hg) | | | |
|---|---|---|---|---|---|---|---|---|
| | $\delta^2$ | $\delta b^2$ | r | h | $\delta^2$ | $\delta b^2$ | r | h |
| **Mono-zygotic twins** | 0.69 | 0.74 | 0.52 | 0.58 | 0.76 | 1.17 | 0.60 | 0.72 |
| **Dizygotic twins** | 1.70 | 0.52 | 0.23 | | 1.49 | 0.50 | 0.25 | |

Heritability estimates (h), derived from intra-class correlation coefficients, suggested a strong contribution of genetic factors to the variance in levels of adult diastolic blood pressure and, to a lesser extent, systolic blood pressure with estimates of 0.72 and 0.58 respectively. A non-parametric analysis was also used to derive heritability estimates in order to allow for the possible influence of outliers. The heritability estimates derived using non-parametric methods were identical to those derived using parametric methods.

## 6.3 Differences within and between twin pairs

### 6.3.1 Within pair difference in size at birth and blood pressure

Inspection of the relation between within pair difference in systolic blood pressure and within pair difference in birth weight, length and ponderal index at birth using scatter plots did not suggest that differences in size at birth were predictive of differences in adult systolic blood pressure (figure 6.2 to 6.4).

**Figure 6.2: Relation between within pair difference in birth weight and within pair difference in systolic blood pressure in monozygotic and dizygotic twins**

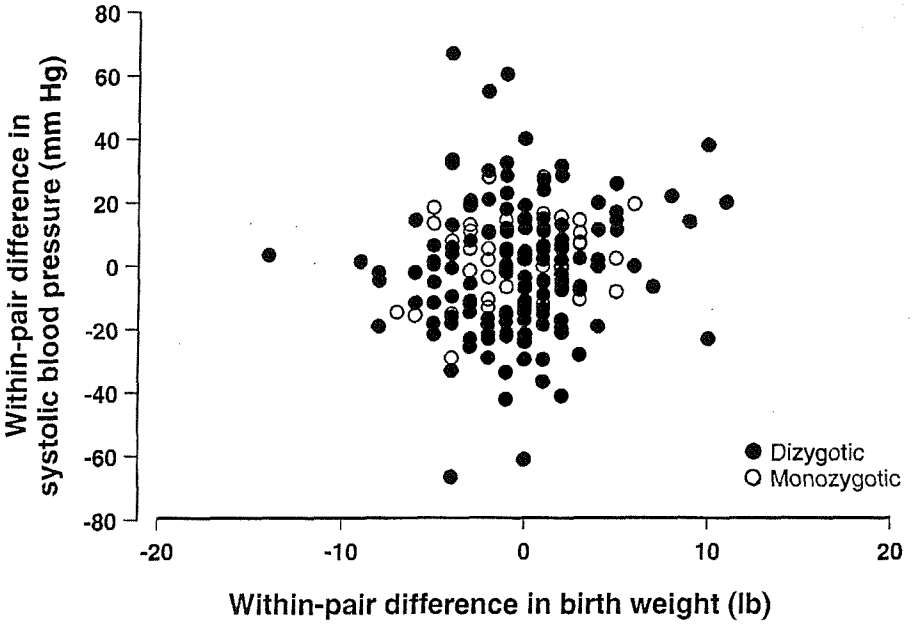


Figure 6.3: Relation between within pair difference in length at birth and within pair difference in systolic blood pressure in monozygotic and dizygotic twins

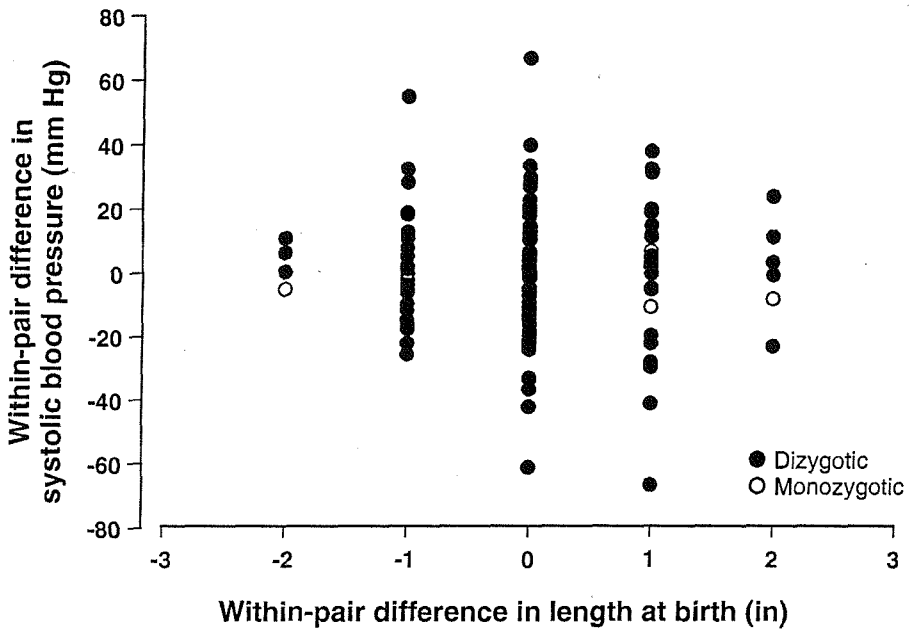
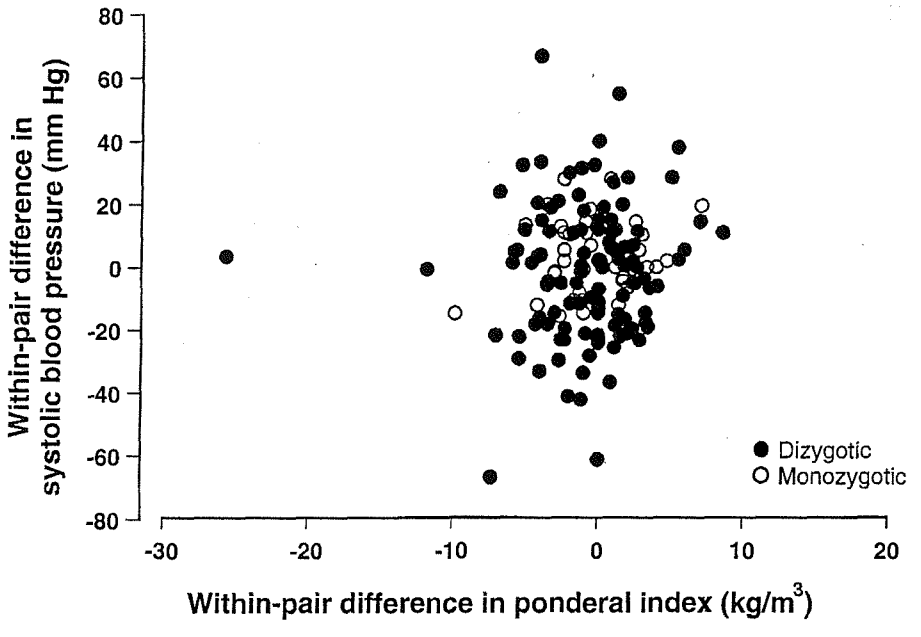



Figure 6.4: Relation between within pair difference in ponderal index at birth and within pair difference in systolic blood pressure in monozygotic and dizygotic twins



Multiple regression was used to explore the influence of within pair difference in levels of eight potential confounding factors on the within pair difference in systolic blood pressure (table 6.4). Only adult body mass index was predictive of adult blood pressure. All regression analyses in twin pairs were adjusted for adult body mass index and sex.

**Table 6.4: Simultaneous effect of within pair difference in eight potential confounding variables on within pair difference in systolic blood pressure**

| Change in systolic blood pressure (mm Hg) | | | |
|---|---|---|---|
| | **All twins** | **Monozygotic twins** | **Dizygotic twins** |
| **Body mass index ($kg/m^2$)** | 1.13 (0.54, 1.72) | 1.54 (0.03, 3.05) | 1.07 (0.38, 1.76) |
| **Sex** | -8.54 (-14.77, -2.31) | Not applicable | -9.22 (-17.12, -1.31) |
| **Current social class** | -0.80 (-2.77, 1.18) | -1.75 (-4.53, 1.03) | -0.69 (-3.34, 1.97) |
| **Current level of physical activity** | -1.43 (-3.03, 0.18) | 0.64 (-1.69, 2.97) | -2.06 (-4.15, 0.03) |
| **Level of physical activity (15-25 years)** | 0.01 (-2.70, 2.73) | -0.62 (-5.12, 3.87) | 0.18 9-3.21, 3.56) |
| **Level of physical activity (26-40 years)** | -1.29 (-4.11, 1.54) | -1.97 (-6.31, 2.37) | -1.04 (-4.66, 2.59) |
| **Smoking status** | 3.07 (0.14, 6.01) | 2.67 (-3.42, 8.76) | 3.16 (-3.34, 6.66) |
| **Alcohol consumption** | 0.33 (-1.71, 2.34) | 0.94 (-2.53, 4.42) | 0.04 (-2.50, 2.58) |

Figures represent the change in systolic blood pressure for every unit change in the relevant confounding variable. 95% confidence intervals are shown in parentheses. Definitions of confounding variables are given in section 2.9.5.

Multiple regression was used to examine the relation between within pair differences in the three measures of birth size and the within pair variation in systolic blood pressure (table 6.5). Analyses were adjusted for sex and body mass index. Differences in birth size within twin pairs did not predict differences in systolic blood pressure. The same was true when monozygotic and dizygotic twin pairs were examined separately. Similarly, differences in birth size did not predict differences in diastolic blood pressure. Diastolic blood pressure increased by 1.76mmHg for every 1lb increase in birth weight difference, (95% confidence intervals –0.4, 3.9). Similar trends were observed in relation to length at birth and ponderal index.

**Table 6.5: Within pair regression analyses of birth weight, length and ponderal index at birth on adult systolic blood pressure in monozygotic and dizygotic twins**

| | **Change in systolic blood pressure (mm Hg)** | | |
|---|---|---|---|
| | **all twins** | **monozygotic twins** | **dizygotic twins** |
| **birth weight** | 1.612 | 3.32 | 1.088 |
| **(lbs)** | (-1.264, 4.410) | (-0.832, 7.476) | (-2.52, 4.70) |
| **length at birth** | -0.569 | 0.355 | -0.871 |
| **(inches)** | (-4.260, 3.122) | (-4.694, 5.403) | (-5.539, 3.813) |
| **ponderal index** | 0.090 | 0.475 | 0.007 |
| **( $kg/m^3$)** | (-0.684, 0.865) | (-0.618, 1.169) | (-0.963, 0.977) |

Figures represent the change in systolic blood pressure for every unit change in birth weight, length or ponderal index. 95% confidence intervals are displayed in parentheses.
The birth weight analysis relates to 196 twin pairs of which 56 are monzygotic and 140 are dizygotic.
The length and ponderal index analyses relates to 155 twin pairs of which 45 are monozygotic and 110 are dizygotic.

### 6.3.2 Differences between heavier and lighter twins

We also examined mean levels of adult systolic blood pressure in the heavier twins and lighter twins. This analysis has been used to overcome any bias introduced by incorrect coding of twin 1 and twin 2 in other studies. As in the glucose tolerance study, this analysis was not informative: mean systolic blood pressure was higher in the heavier twins at 131.5mmHg compared with 130.9mmHg in the lighter twins and 130.2 in the twins of equal weight. As discussed in section 5.3.2, this analysis is weak because information is lost about the size of birth weight differences and because it breaks the pairing of twins. The regression analysis described above took account of potential errors in coding of twin 1 and twin 2 by constraining the regression line to pass through the origin.

### 6.3.3 Multi-level modelling analysis

Table 6.6 shows the relationship of birth weight and length at birth with within and between pair variances in systolic blood pressure for monozygotic and dizygotic twins. Within pair differences were significantly greater in dizygotic than in monozygotic twins. The between pair differences did not differ significantly in the monozygotic and dizygotic twins. Neither birth weight nor length at birth were predictive of these patterns of variance.

**Table 6.6: Multi-level modelling analyses: within and between pair variance in systolic blood pressure according to birth weight and length at birth**

| Number of complete pairs | Between pair (level 2) Variances | | | Level 1 (within pair) variances | | | Fixed predictor (with sex, bmi & zygosity) | B | SE (B) | p |
|---|---|---|---|---|---|---|---|---|---|---|
| | MZ | DZ | P for diff | MZ | DZ | P for diff | | | | |
| | 67.8 | 60.5 | NS | 64.4 | 138.3 | <0.0005 | none | _ | _ | _ |
| **155** | 66.5 | 59.7 | NS | 64.5 | 138.6 | <0.0005 | length | 0.490 | 0.834 | NS |
| | 65.6 | 59.3 | NS | 64.7 | 138.6 | <0.0005 | length x zygosity | 0.884 | 1.676 | NS |
| | 81.3 | 73.2 | NS | 71.0 | 134.2 | <0.0005 | none | _ | _ | _ |
| **196** | 81.9 | 73.7 | NS | 70.5 | 133.8 | <0.0005 | birth weight | 0.092 | 0.200 | NS |
| | 82.2 | 73.8 | NS | 70.4 | 133.8 | <0.0005 | birth weight x zygosity | 0.033 | 0.402 | NS |

## 6.4 Birth size and blood pressure in twin individuals

### 6.4.1 Characteristics of twin individuals participating in the blood pressure study

The birth size, adult body mass index and adult blood pressure of twin individuals was compared according to sex and zygosity (table 6.7).

**Table 6.7: Characteristics of twins participating in the blood pressure study according to sex and zygosity**

| | All n=507 | Males n=224 | Females n=283 | Mean difference (95% CI) | MZ n=115 | DZ n=335 | Mean difference (95% CI) |
|---|---|---|---|---|---|---|---|
| **Age** | 43.6 (1.4) | 43.6 (1.3) | 43.6 (1.5) | -0.006 (-0.3, 0.3) | 43.7 (1.4) | 43.7 (1.4) | 0.03 (-0.3, 0.4) |
| **Birth weight (lbs)** | 5.5 (1.1) | 5.8 (1.1) | 5.3 (1.0) | 0.5 (0.3, 0.7) p=<0.001 | 5.4 (1.0) | 5.5 (1.1) | -0.1 (-0.4, 0.1) |
| **Length at birth (inches)** | 18.6 (1.1) | 18.7 (1.2) | 18.5 (1.0) | 0.3 (0.1, 0.5) p=0.01 | 18.5 (1.1) | 18.6 (1.1) | -0.09 (-0.4, 0.2) |
| **Ponderal index at birth ($kg/m^3$)** | 24.0 (3.3) | 24.5 (3.5) | 23.6 (3.1) | 0.9 (-0.24, 1.53) | 23.9 (3.0) | 24.2 (3.5) | 0.3 |
| **Body mass index ($kg/m^2$)** | 26.1 (4.5) | 26.6 (3.8) | 25.7 (4.9) | 0.9 (0.1, 1.7) p=0.02 | 25.5 (3.9) | 26.1 (4.5) | 0.6 (-1.6, 0.3) |
| **Gestation (weeks)** | 37.8 (2.5) | 37.9 (2.5) | 37.7 (2.4) | 0.2 (-0.2, 0.7) | 37.8 (2.4) | 37.7 (2.4) | 0.1 (-0.5, 0.6) |
| **Systolic blood pressure (mm Hg)** | 131.0 (15.7) | 134.0 (14.5) | 128.6 (16.3) | 5.4 (2.7, 8.1) p=<0.001 | 130.5 (12.4) | 131.2 (16.8) | -0.7 (-4.0, 2.7) |
| **Diastolic blood pressure (mm Hg)** | 75.3 (12.1) | 79.2 (11.0) | 72.2 (12.1) | 7.0 (5.0, 9.1) p=<0.001 | 75.0 (11.2) | 75.7 (12.7) | -0.7 (-3.3, 2.0) |

Figures in parentheses are standard deviations unless otherwise stated.

Monozygotic twins were lighter than dizygotic twins with mean birth weight of 5.4lb compared to 5.5 lb in dizygotic twins. They were also shorter at birth with mean

length at birth of 18.5 inches compared with 18.6 inches in dizygotic twins. These differences in size at birth were not, however, statistically significant. Similarly, adult body mass index was also lower in the monozygotic twins but again differences were not statistically significant. Gestational age was similar in the two groups. Likewise, levels of systolic and diastolic blood pressure in monozygotic twins did not differ significantly from those in dizygotic twins.

Comparison between men and women revealed that men had significantly higher mean birth weight, length at birth and adult body mass index than women. The mean birth weight of men was 5.8lb compared with 5.3lb in the women. Corresponding values for length and body mass index were 18.7 and 18.5 inches and 26.6 and 25.7 $kg/m^2$. Men also had significantly higher levels of systolic and diastolic blood pressure at 134 and 79.2 mm Hg compared with 128.6 and 72.2 mm Hg in the women.

### 6.4.2 Associations between birth size and adult blood pressure

Inspection of scatter plots of birth weight, length at birth and ponderal index at birth against systolic blood pressure did not suggest that size at birth was predictive of adult blood pressure in this cohort of individuals (figure 6.5 to 6.7).

**Figure 6.5: Relation between birth weight and adult systolic blood pressure in monozygotic and dizygotic twin individuals**

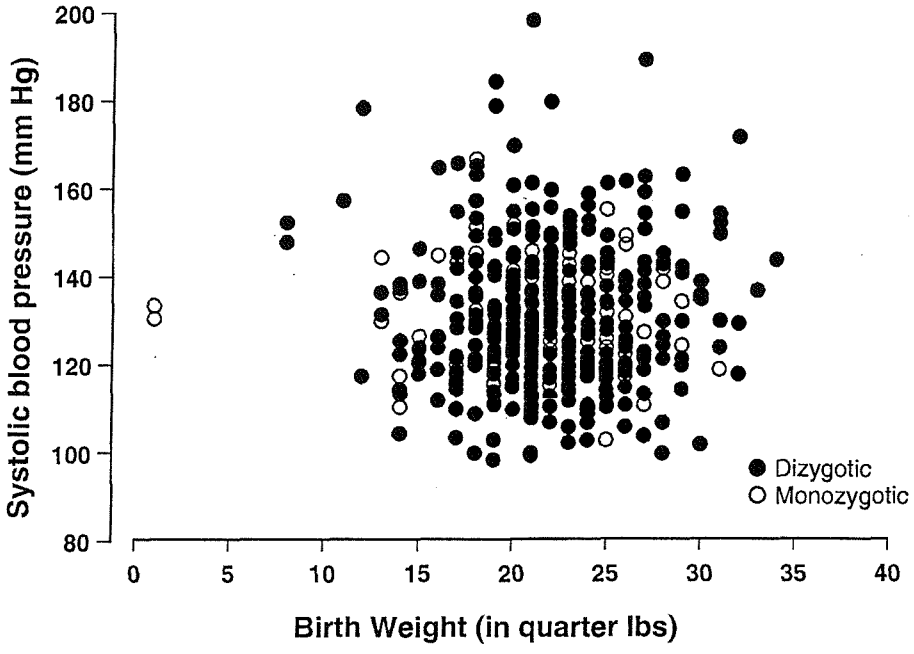


Figure 6.6: Relation between length at birth and adult systolic blood pressure in monozygotic and dizygotic twin individuals

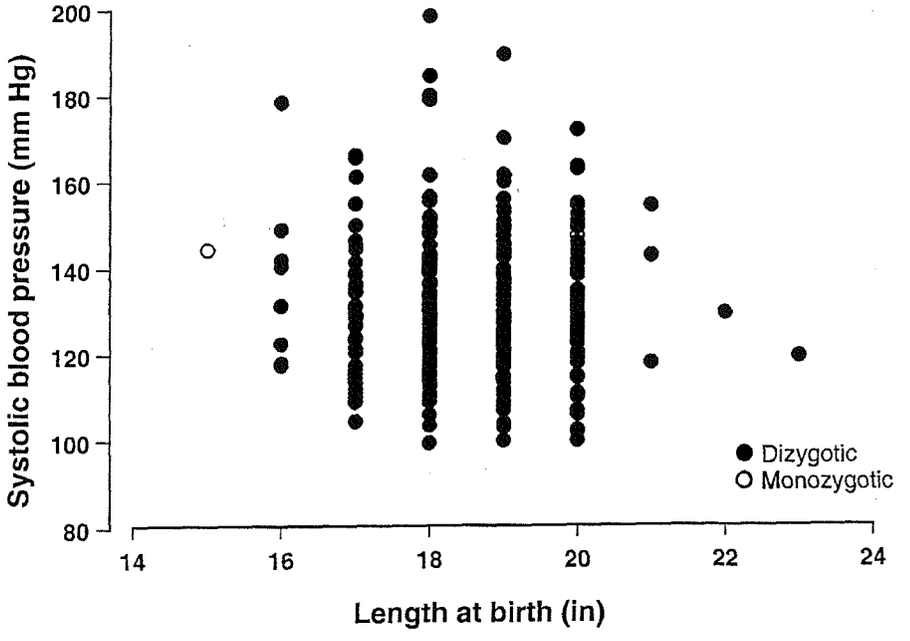
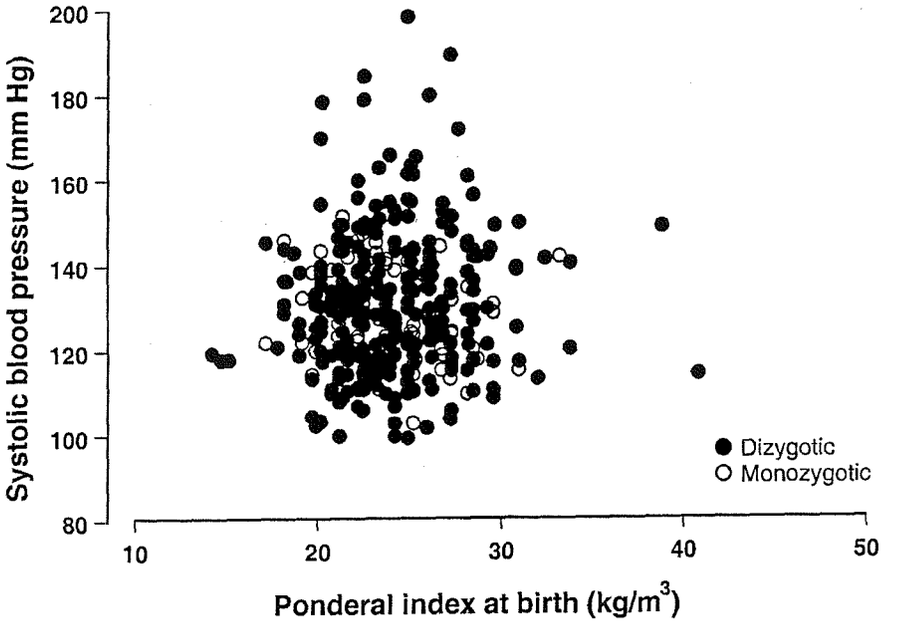



Figure 6.7: Relation between ponderal index at birth and adult systolic blood pressure in monozygotic and dizygotic individuals



The influence of potential confounding variables on adult systolic blood pressure levels was explored using multiple regression analysis (table 6.8). There was a positive association between adult body mass index and systolic blood pressure. All regression analyses of the association between birth size and adult blood pressure were adjusted for body mass index and sex.

**Table 6.8: Simultaneous effect of eight potential confounding variables on adult blood pressure in twin individuals**

| Change in systolic blood pressure (mm Hg) | | | |
|---|---|---|---|
| | **All twins** | **Monozygotic twins** | **Dizygotic twins** |
| **Body mass index ($kg/m^2$)** | 0.90 (0.57, 1.23) | 0.81 (0.06, 1.56) | 0.99 (0.56, 1.43) |
| **Sex** | -3.10 (-6.17, -0.02) | 0.47 (-5.51, 6.44) | -3.67 (-7.78, 0.45) |
| **Social class at birth** | -0.23 (-1.58, 1.13) | -0.34 (-2.96, 2.28) | -0.11 (-1.84, 1.62) |
| **Current social class** | 0.68 (-0.49, 1.84) | 0.02 (-2.04, 2.0) | 0.48 (-1.10, 2.08) |
| **Current level of physical activity** | -0.40 (-1.65, 0.85) | 0.57 (-1.80, 2.95) | -0.67 (-2.31, 0.97) |
| **Level of physical activity (15-25 years)** | 0.43 (-1.50, 2.37) | 0.91 (-2.71, 4.52) | 0.49 (-2.03, 3.0) |
| **Level of physical activity (26-45 years)** | -0.67 (-2.62, 1.28) | -1.34 (-4.86, 2.12) | -0.75 (-3.34, 1.85) |
| **Smoking status** | 1.40 (-0.27, 3.07) | 1.49 (-1.66, 4.63) | 1.94 (-0.26, 4.14) |
| **Alcohol consumption** | 0.05 (-0.01, 0.10) | -0.02 (-0.15, 0.11) | 0.07 (-0.01, 0.14) |

Figures represent the change in systolic blood pressure for every unit change in the relevant confounding variable. 95% confidence intervals are shown in parentheses. Definitions of confoudnign variables are given in section 2.9.5.

The relation between birth weight, length at birth and ponderal index and systolic blood pressure in 504 twin individuals was explored using multiple regression analysis (table 6.9). There was no association between birth weight and systolic blood pressure after adjustment for sex and body mass index. Analysis of males and females separately and of monozygotic and dizygotic individuals did not strengthen these findings. Similarly length at birth and ponderal index were not predictive of adult levels of systolic blood pressure.

Birth size was not predictive of diastolic blood pressure in the twins. Blood pressure increased by 0.96mmHg for every 1lb increase in birth weight (95% confidence intervals –0.04, 1.92) after adjustment for sex and body mass index. Similar non-significant trends were observed in relation to length at birth and ponderal index.

**Table 6.9: Relation between birth weight, length and ponderal index at birth and systolic blood pressure in 504 twin individuals**

| | Change in systolic blood pressure (mm Hg) | | | | |
|---|---|---|---|---|---|
| | **all twins** | **males** | **females** | **mono-zygotic individuals** | **dizygotic individuals** |
| **birth weight (lbs)** | -0.22 (-1.54, 1.08) | 0.68 (-1.08, 2.44) | -1.08 (-3.0, 0.84) | -0.086 (-2.24, 2.36) | 0.31 (-2.0, 1.4) |
| **length at birth (inches)** | -0.38 (-1.75, 0.99) | 0.76 (-1.05, 2.56) | -1.59 (-3.65, 0.46) | 1.57 (-0.69, 3.83) | -0.85 (-2.60, 0.90) |
| **ponderal index (kg/m3)** | -0.035 (-0.42, 0.49) | -0.0045 (-0.59, 0.59) | 0.08 (-0.61, 0.77) | -0.84 (-1.66, 0.03) | 0.17 (-0.39, 0.74) |

Figures represent the change in systolic blood pressure for every unit change in birth weight, length or ponderal index. 95% confidence intervals are displayed in parentheses.

The birth weight analysis relates to 504 individuals of which 224 are male, 280 are female, 115 are monozygotic (51 male and 64 female) and 335 are dizygotic (143 male and 192 female). The length and ponderal index analysis relates to 407 individuals of which 186 are male, 221 are female, 91 are monozygotic and 271 are dizygotic. Zygosity is unknown for twins from like-sexed pairs whose co-twins did not participate.

## 6.5 Overview of blood pressure findings

### 6.5.1 Representativeness of the study group

The response rate in the blood pressure study was 52%. Analyses relating to complete twin pairs were based on 41% of the possible twin pairs in the target population. Comparison of participants with non-participants revealed that female twins were more likely to participate in the study than male twins, accounting for 55.8% of the study group. Participants also tended to be older than non-participants. However, comparisons of birth size (weight and length), gestational age and socio-economic status at birth did not reveal any significant differences between the two groups suggesting that the study group were representative of twins in the target population in relation to birth characteristics. No comparisons were possible in relation to adult socio-economic status or adult lifestyle since only birth record data was available for non-participants.

### 6.5.2 Birth size and adult blood pressure in twin pairs

#### 6.5.2.1 Comparison between monozygotic and dizygotic twins

We had hypothesised that levels of disease in monozygotic twins may be higher than those in dizygotic twins due to the more adverse prenatal environment of monochorionic twins. However, comparison of size at birth and adult body mass index in the monozygotic, dizygotic like-sexed and dizygotic unlike-sexed twins revealed no significant differences in the three groups. Similarly, there were no significant differences between the three groups in relation to adult blood pressure.

#### 6.5.2.2 Similarities within twin pairs

The prevalence of hypertension in the study group was 7.9%. This is comparable with reported prevalence rates in the general population in the United Kingdom.[138] Probandwise concordance rates were not calculated since there were very few concordant pairs making calculation of rates potentially misleading. Therefore we examined the correlation in levels of blood pressure in monozygotic and dizygotic twins by plotting the level in one twin against that of their co-twin and by calculating intra-class correlation coefficients. This analysis revealed that adult levels of blood pressure were more highly correlated in the monozygotic twins than dizyogtic twins. Within pair variance was lower in monozygotic than dizygotic twins while between

pair variance was similar in the two groups. Intraclass correlation coefficients were used to make estimates of heritability. These estimates were 0.72 for systolic blood pressure and 0.58 for diastolic blood pressure. This suggests that genetic factors play an important role in determining adult levels of blood pressure particularly in relation to systolic blood pressure. However, this classical twin analysis takes no account of the fact that monozygotic twins have a more similar prenatal environment than dizygotic twins. We therefore carried out a multi-level modelling analysis in order to assess the relative contributions of prenatal environment (as determined by size at birth size), adult body mass index and genetic factors in determining patterns of variance in systolic blood pressure.

As in the glucose tolerance study, multi-level modelling analysis confirmed that within pair variance in systolic blood pressure was significantly greater in the dizygotic than the monozygotic twin pairs. Neither length at birth or birth weight were predictive of these patterns of variance.

#### 6.5.2.3 Within pair differences in size at birth and blood pressure

A within pair regression analysis was carried out in order to test the hypothesis that within pair differences in adult blood pressure would be predicted by within pair differences in size at birth. Analyses were adjusted for adult body mass index and sex. As suggested by scatter plots of the data, the regression analysis revealed no association between within pair differences in size at birth (birth weight, length and ponderal index) and adult blood pressure. Similar trends were observed in relation to diastolic blood pressure.

### 6.5.3 Birth size and adult blood pressure in twin individuals

Comparison of birth size, adult body mass index and blood pressure in monozygotic and dizygotic twins revealed that, although monozygotic twins were lighter and shorter at birth, these differences were not statistically significant. Similarly, there was no significant difference in mean adult body mass index or blood pressure. Comparison of men and women revealed that men had significantly higher birth weight and adult body mass index and significantly higher systolic and diastolic blood pressure as adults.

Associations between birth size and adult systolic blood pressure were explored using regression analysis. As for twin pairs, scatter plots did not suggest that birth size was predictive of adult systolic blood pressure. No association was found between birth size and adult blood pressure in the regression analysis. These findings were not strengthened when the twins were subdivided according to sex or zygosity.

# 7 DISCUSSION

## 7.1 Were our findings consistent with the fetal origins hypothesis?

### 7.1.1 Comparison of twins with singletons

Twins are growth retarded and have lower birth weight than singletons being on average 900g lighter at birth than singletons.[7] According to the fetal origins hypothesis, we therefore expected to find levels of glucose tolerance and blood pressure in twins greater than those observed in singletons. Our study did not include a singleton group for comparison. However, published studies based on adults of a similar age group provide suitable data for comparison. Levels of glucose intolerance and blood pressure in our study were comparable with published levels in the general population of a similar age distribution. Mean two-hour glucose was 5.4mmol/l (standard deviation 1.3) in our twins aged 40 to 45 years and this compares with mean levels of 5.6mmol/l (standard deviation 1.7) seen in adults aged 20 to 44 years in the NHANES 2 study, a large scale study of more than 3,000 adults in the United States.[119] Mean systolic blood pressure level in our study was 131mm Hg (standard deviation 15.7) with means of 134 and 129mm Hg in the male and female twins respectively. These levels are similar to mean levels in a comparable age group in the National Health Survey for England of 1994.[136] The National Health survey is a large scale survey of a representative sample of all private households in England. In the 35 to 44 age group mean levels of systolic blood pressure, which was measured during a home visit, in the men and women were 132mm Hg and 125mm Hg respectively.

One possible explanation for not finding higher levels of glucose intolerance and blood pressure in the twins than the general population would be if our findings were based on a sample of twins who were more healthy than twins in general. If this were the case we would have expected the mean birth weight and gestational age of the twins studied to be higher than those of other population-based samples of twins. Comparisons with other published data on twins born at around the same time suggests that our twins were a representative sample of twins and so selection bias is unlikely to explain our findings: the mean birth weight and gestational age of the twins are comparable with those recorded in other large population-based studies of twins born at around the same time. Mean twin birth weight in a Birmingham-based

study in 1947 was 5.3lb compared with 7.4lb in singletons and mean gestational age was 37 weeks compared with a mean of 40 weeks in singletons.[80] This study provides a useful comparison with our sample of twins since it was based in the same geographical area and, as in our study, included all births whether at hospital or at home. These findings are comparable with a mean birth weight of 5.5 lb and mean gestational age of 37.8 weeks in our cohort who were born between 1950 and 1954. Two further twin studies based in Italy and London, conducted in the 1950s, showed similar gestational age for twins of 36.5 weeks and 37 weeks respectively.[82] The mean birth weights of the twins in these studies were 5.5lb and 5.0 lb respectively. The London study was based on hospital births only, perhaps explaining why mean birth weight was lower than in the other studies since twin pregnancies considered at higher risk are likely to have occurred in hospital thus leading to over-representation of low birth weight twins.

Our findings of similar rates of disease in the twins and the general population are supported by a number of other twin studies. In a study based on the National Heart Lung Blood Institute (NHLBI) twin register, levels of systolic blood pressure and one-hour glucose tolerance in men aged 42 to 56 years were comparable with those of the general population.[96] In a further study based on the NHLBI twins the prevalence of non-insulin dependent diabetes at age 45 to 54 years was comparable with that of the general population.[92] Although these studies are based on a similar age group as the Birmingham twins, the NHLBI study is restricted to male army veterans. Another study based on men and women, however, also showed no difference in overall prevalence of disease between twins and singletons: Hong et al found that mean levels of each of the components of the insulin resistance syndrome were within the normal range for the general population in a study of middle-aged and elderly twins aged 52 to 86 years.[101] A more recent study comparing twins and singletons enrolled in the Dunedin study of Sudden Infant Death Syndrome, found that twins had significantly lower systolic blood pressure than singletons at ages 9 and 18 years.(107) Mean systolic blood pressure was 4.5mm Hg lower in the twins at nine years (95% confidence intervals 1.57 to 7.5). Although these findings suggest that rates of disease may be lower in twins, the observed trends were based on observations in only 22 twins who made up a small proportion (2.8%) of the sample studied.

### 7.1.2 Relation of birth size to adult disease in twin individuals

The inverse associations between birth size and adult glucose tolerance and blood pressure that underlie the fetal origins hypothesis are well documented.[39; 139] However, in an analysis of the twins in the Birmingham register who participated in the glucose tolerance and blood pressure studies, the relationships between birth size and adult blood pressure and glucose tolerance were not statistically significant. There was a weak inverse relationship between birth weight and adult glucose tolerance in the twins: two-hour glucose fell by 0.076mmol/l for every 1kg increase in birth weight (95% confidence intervals -0.33 to 0.16) after adjusting for sex and body mass index. Similarly, there was also a weak inverse relationship between birth weight and adult systolic blood pressure: systolic blood pressure fell by 0.48mmHg for every 1kg increase in birth weight (95% confidence intervals -3.3 to 2.4), after adjustment for sex and body mass index.

Comparison with other studies exploring the association between birth size and adult glucose tolerance suggest that the lack of an association between birth weight and glucose tolerance in the twins may be explained by weaknesses in the study. There is now strong evidence for the inverse association between birth weight and glucose tolerance: a recent overview of the evidence has shown that, of the 33 studies exploring the association, the majority (27) have reported an inverse association. Direct comparison with the findings of our study is difficult due to lack of consistency in the way such studies are reported. We did show an inverse relationship between birth weight and glucose tolerance but this was not statistically significant. The 95% confidence intervals around the regression coefficient for the birth weight/glucose tolerance association in the twins were relatively wide. This suggests that the failure to find a statistically significant association in the twins may be a reflection of the lack of statistical power to detect an effect.

An alternative interpretation of our lack of significant findings is that there are real differences in the associations between size at birth and adult glucose tolerance in twins compared with singletons. Comparisons with studies based on singletons of similar age group to the twins suggest that this may be the case. In a study of men and women aged 46 to 54 years (mean age 50 years) in Preston there was a strong

inverse association between two-hour plasma glucose concentration and birth weight. Two-hour plasma glucose concentrations fell with increasing birth weight (p =0.002 after adjusting for body mass index and sex).[17] In contrast, there was a weak negative relation between two-hour glucose and birth weight in the twins. According to the fetal origins hypothesis, we expected higher levels of glucose intolerance in the twins due to their lower mean birth weight. However, mean two-hour glucose levels were higher in the Preston subjects than in the Birmingham twins. Mean two-hour glucose in the Preston men was 5.5mmol/l and in the women 5.8mmol/l compared with 5.4mmol/l and 5.5mmol/l in the male and female twins respectively. Mean birth weight is not reported for the Preston study but only 16 men and 14 women, who made up 11% of the study population, had birth weights of 2.5kg (5.5lb) or less. In contrast, 50% of the sample of 372 twins who were tested for glucose tolerance had birth weights of 5.5lb or less. Similarly, studies based on adults of similar age group carried out in China and the United States also showed the inverse association between birth size and glucose tolerance.

A recent overview of studies investigating the association of birth size with adult blood pressure provides useful data for comparison with the blood pressure findings in the Birmingham twins. In the studies reporting regression coefficients a 1 kg increase in birth weight was associated with a decrease in systolic blood pressure ranging between 1 and 4 mmHg. The regression coefficient in the twins was lower with a 0.48mmHg decrease in systolic blood pressure per 1kg increase in birth weight. However, the 95% confidence intervals around the regression coefficient are wide and, with the lower limit -3.3mmHg, they include many of the values reported in other studies. This suggests that lack of statistical power due to low response rates in the study may provide an explanation for the lack of a significant association between birth weight and blood pressure in the twins.

As for the glucose tolerance study, an alternative explanation might be that impaired fetal growth has different consequences for twins than for singletons. Comparisons with a study of a singleton population of similar age group to the Birmingham twins would seem to provide some support for this theory. The study explored the association between birth weight and adult blood pressure in a group of adults aged 40 to 45 years in Aberdeen who were born at the same time as the twins in

Birmingham. There was a strong inverse relation between birth weight and blood pressure in the Aberdeen study: systolic blood pressure decreased by 4.4mm Hg for every 1kg increase in birth weight.[140] Mean levels of systolic blood pressure in men and women in the Aberdeen study where higher than in the twins even though mean birth weight was lower in the twins: mean systolic blood pressure levels in Aberdeen were 142 and 132mm Hg in men and women respectively compared with levels of 134 and 128mm Hg in the male and female twins. Mean birth weight in the Aberdeen study was 3.1kg (standard deviation 0.45kg) compared with 2.5kg (standard deviation 0.44kg) in the Birmingham twins. Therefore, there was almost a ten-fold difference in regression coefficients in the two studies with higher mean levels of systolic blood pressure in the Aberdeen men and women despite lower mean birth weight in the twins. One possible explanation for the differences between the Aberdeen cohort and the twins may relate to the geographical areas in which the studies were based. There are well documented geographical variations in the prevalence of cardiovascular disease in the United Kingdom. Prevalence of disease is highest in Scotland and this may explain why a strong birth weight/blood pressure association was detected in the Aberdeen cohort but not in the Birmingham twins.

Further support for the theory that impaired fetal growth has different significance in twins than in singletons comes from a number of recent studies which have failed to demonstrate differences in levels of mortality and morbidity in twins compared with the general population. In their study based on the Swedish twins register, Vagero and Leon found that levels of ischaemic heart disease mortality in the twins were similar to those observed in the general population.[110] Similarly, in a study based on the Danish twin register, Christiansen et al found mortality rates in a cohort of twins similar to those in the general population.[111]

### 7.1.3 Mortality in twins

Although we did not find higher rates of disease in the twins compared with the general population, mortality rates in the twins were raised. Infant and perinatal mortality rates were 4 and 3 times that of the general population respectively as described in chapter 4. Other large scale population-based studies of twins born in the United States and the United Kingdom at around the same time as the Birmingham twins have shown similar excesses.[85; 141; 142] The United Kingdom study

examined mortality in twin pregnancies in Dundee from 1956 to 1983.[142] The perinatal mortality rate in 1956 was 116 per 1000 total births which is comparable with the rate of 113 per 1000 in our study. In the American study, Kleinman et al used record linkage to compare 1960 and 1983 infant mortality statistics in twin and singleton births.[141] There was a four-fold difference in infant mortality in the white twins born in 1960 compared with the singletons with rates of 104.7 and 20.5 per 1000 total births respectively.

Overall mortality in the twins was high: the standardised mortality ratio was 259 (95% confidence intervals 221 to 300). Mortality was highest in the first year of life and, although it then declined progressively, it remained significantly higher than that of the general population until five years of age with standardised mortality ratios of 415 and 224 in the under 1-year age group and 1 to 4-year age group respectively. Our findings differ from those of two recent studies of mortality in twin cohorts, based on the Danish and Swedish twin registers, which revealed similar mortality rates to those of the general population.[110; 111] The Danish study examined mortality in a cohort of twins born before 1900 and found no difference in mortality compared with the general population.[111] The Swedish study of twins born from 1905 to 1925, who were still alive in 1971, likewise found no difference in overall mortality.[110] However, the picture from these studies is incomplete as they were based on historical cohorts born at a time when twin pregnancy was associated with a relatively high mortality. As the two studies only included twin pairs who had survived infancy or reached middle-age respectively, a significant number of twins who were of low birth weight and died in early life would have been excluded from the analyses. The extent of this underascertainment is particularly evident in the Danish study where the analysis was based on only a third of possible twin pairs. We were able to study all deaths from the perinatal period onwards in the Birmingham twins. Despite the relatively small number of deaths it is likely that complete ascertainment of deaths was achieved through use of the flagging system at the NHS central register. Our findings are further strengthened by the fact that the sample was population-based. In addition, as outlined above, perinatal and infant mortality rates in our study were typical of those described in other cohorts of twins suggesting that our sample was representative of twins in general.

We were also able to examine cause of death and factors associated with risk of death. More than half of the deaths in our cohort were due to conditions originating in the perinatal period. The most common causes of death were prematurity (International Classification of Disease (ICD) codes 7650 and 7651), atelectasis (ICD code 7705) and multiple pregnancy (ICD code 7615). There was also excess mortality in relation to respiratory and digestive disease and this was largely due to deaths that were infective in origin. Although we found excess mortality in the 1 to 4 year age group, no specific cause of death predominated in this age group. Survival analysis revealed that low birth weight, short gestation and male sex were significantly and independently associated with an increased risk of death.

Other studies of mortality in twin populations have also demonstrated that deaths due to prematurity account for much of the excess perinatal mortality seen in twins when compared with the general population. In a large scale prospective study of 60,000 pregnant women in the United States there were a total of 213 deaths which occurred up to the end of the neonatal period.[87] The most common causes of death were hyaline membrane disease and asphyxia due to birth trauma. These findings agree with ours in the Birmingham twins: both hyaline membrane disease and atelectasis are diseases of the lungs caused by immaturity. Other studies based in the United States and in Sweden all demonstrated that heaviest perinatal losses were seen in preterm, low birth weight twins.[141; 143; 144] The greater risks associated with prematurity are likely to reflect the high risk involved in premature delivery. These births occurred in the 1950s and 1960s before advances in obstetric and neonatal care which have dramatically reduced both the mortality associated with delivery of multiple pregnancies and the excess mortality in low birth weight premature infants. This explanation is backed up by the American study which compared mortality in twins in 1960 and 1983 and by the British study which examined mortality from 1953 to 1983.[141; 142] In the American study perinatal mortality in twins had reduced four-fold between 1963 and 1980 and this reduction was explained by increased survival of low birth weight infants. In the British study perinatal mortality had reduced from 116 per 1000 total births in 1956 to 16 per 1000 in 1983. This reduction in mortality was also attributable to reduced

mortality in premature infants. Other researchers have also found that male sex is associated with a greater risk of death: the American prospective study of 60,000 pregnant women also examined factors associated with mortality.[87] There was significantly higher mortality in the like-sexed twin pairs and this was due to excess deaths in the male twin pairs. As in our study these deaths occurred predominantly in the perinatal period. However, as in our cohort, the numbers of deaths on which these trends were based were small and it is difficult to draw any firm conclusions as to the underlying cause of the sex differences observed.

We have also shown that individuals whose co-twin had died were at greater risk of death themselves. A number of researchers have reported a similar within pair association for mortality.[145-147] Most notably, Hrubec et al studied Swedish twins born from 1886 to 1925 and examined factors associated with risk of death in a survival analysis similar to ours.[145] They also found that co-twin mortality had a significant, independent, positive relationship with the mortality risk of an individual. This association was observed in all twin pairs other than the oldest dyzygotic male twins. The researchers interpreted this as evidence of a familial effect on mortality. However, since twin pairs where both are low birth weight and one twin has died are likely to have experienced adverse conditions in utero, an alternative interpretation of these findings is that they reflect the influence of adverse prenatal environment extending beyond infancy.

### 7.1.4 Comparison of glucose tolerance and blood pressure in monozygotic and dizygotic twins

Two thirds of monozygotic twins are monochorionic and share a placenta. They have to compete for nutrients and studies show they are lighter at birth than dichorionic twins.[79; 81; 107; 108] We therefore expected the monozygotic twins in our study to have lower mean birth weight than the dizygotic twins. In addition, according to the fetal origins hypothesis, we expected to find higher levels of two-hour glucose and blood pressure in monozygotic than dizygotic twins. However, although the monozygotic twins were marginally lighter and shorter than the dizygotic twins, there were no statistically significant differences in mean birth weight or length at birth when the monozygotic and dizygotic twins we studied were compared. This was true for the

glucose tolerance study where mean birth weight in monozygotic twins was 5.3lb compared with 5.4lb and 5.6lb in the like-sexed and unlike-sexed dizygotic twins. Similarly, in the blood pressure study mean birth weight in the monozygotic twins was 5.3lb compared with 5.3lb and 5.5lb in the like-sexed and unlike-sexed dizygotic twins respectively. Further more, we did not find any statistically significant differences in mean levels of two-hour glucose or systolic blood pressure between monozygotic and dizygotic twins. Mean two-hour glucose in the monozyogtic twins was 5.4mmol/l compared with 5.5 and 5.6mmol/l in the like-sexed and unlike-sexed dizygotic twins. Mean systolic blood pressure was 130.5mm Hg in the monozygotic twins compared with 133.8 and 130.0mm Hg in the like-sexed and unlike-sexed dizygotic twins.

There are a number of possible reasons why we failed to find the expected differences between monozygotic and dizygotic twins. Firstly there was potential for selection bias in the design of the study. Twins pairs where one or both had died were excluded from the study. Studies have shown that this group will include twins of lower birth weight than surviving twins and monochorionic monozygotic twins are likely to be over-represented in this group.[81; 86; 87] This appears to be the case in this study since the mean birth weight of the Birmingham twins where one or both had died was 4.1lb compared with 5.5lb in the surviving twins. Although exclusion of this group provides a potential source of selection bias, they accounted for a relatively small proportion (11%) of the Birmingham cohort. Another potential source of selection bias relates to the fact that twin pairs where one or both had moved away from the Birmingham area were excluded from the study and it is possible that those who moved away differed from those who remained in the Birmingham area. It was possible to make some comparisons between the two groups using the birth register data. These showed that there were no significant differences relating to size at birth and gestational age. There were socio-economic differences between the two groups, however, in that the paternal occupation of those who were eligible to participate was less likely to be classified as 'Professional and Executive' than those who had moved away and they were more likely to have 'poor quality' housing. Nevertheless these socio-economic differences could only have accounted for the absence of a difference between monozygotic and dizygotic twins if one type of twin had been more likely to move away than the other and this seems unlikely. A further source of bias comes

from the low response rates in both the glucose tolerance and blood pressure studies. Although the group studied seem representative of the target population in terms of size at birth and gestation, it is possible that they may have differed from study participants in relation to levels of adult disease or risk factors for cardiovascular disease.

The chief weakness in comparing monozygotic and dizygotic twins in order to assess the influence of chorionicity is that only two thirds of the monozygotic group are monochorionic. A comparison of monochorionic and dichorionic twins would have been the only accurate method of determining whether there were differences in outcome caused by competition for nutrients.[95] This was not possible in our study since the birth register did not supply any data relating to placentation.

There is little published evidence to support the hypothesised differences in levels of blood pressure and glucose tolerance in monozygotic and dizygotic twins. No published studies have compared levels of blood pressure and glucose tolerance according to chorionicity of twins. The Danish study of like-sexed twin pairs aged 55 to 74 years examined glucose tolerance in the non-diabetic co-twins of monozygotic and dizygotic twins with diabetes or impaired glucose tolerance.[114] The monozygotic non-diabetic co-twins were significantly more likely to be insulin resistant than the dizygotic twins. Interpretation of these findings is difficult, however, since as in our study, the mean birth weights of the two groups were similar and the chorionicity of the twins was not known.

The higher concordance rates for diabetes and higher correlation in blood pressure in monozygotic twins compared with dizygotic twins are well documented as outlined in Chapter 1. We hypothesised that this greater similarity in monozygotic twins when compared with dizygotic twins could be explained by their more similar prenatal environment. Examination of concordance rates in our study was not informative due to the low prevalence of disease which was determined by the age group studied. We therefore examined levels of glucose tolerance and blood pressure levels in the twins.

Adult glucose tolerance and blood pressure were more highly correlated in the monozygotic than dizygotic twins. Classical twin analysis suggested high heritability

for adult glucose tolerance and blood pressure in the twins. However, since this type of analysis takes no account of prenatal environment, we carried out a multi-level modelling analysis which explored the influence of size at birth. This revealed that the greater correlation in monozygotic twins was not explained by the birth weight or length at birth of the twins.

These findings are difficult to interpret. The greater similarities within monozygotic twin pairs were not explained by their more similar birth size and this suggests that prenatal environment is not important in determining these similarities. However weight and length at birth are only proxy measures for factors operating in utero that are thought to underlie the birth size/adult disease association. They are likely to be relatively poor proxy measures for factors such as hormone or nutrient levels. Therefore our findings do not totally exclude a role for prenatal environment in determining similarities in disease susceptibility in monozygotic twins.

### 7.1.5 Within pair differences in glucose tolerance

According to the fetal origins hypothesis, we expected the smaller twin in birth size discordant pairs to have higher levels of glucose intolerance or blood pressure as an adult than their co-twin. When within pair differences in glucose tolerance were examined our findings were inconsistent. Differences in glucose tolerance were not predicted by differences in size at birth: two-hour glucose rose by 0.056mmol/l for every 1lb increase in birth weight (95% CI -0.044 to 0.16) in monozygotic twins. In contrast, an inverse relationship was observed in the dizygotic twins but it was not statistically significant: two-hour glucose fell by 0.046 mmol/l for every 1lb increase in birth weight. However, within pair differences in birth weight and length at birth were predictive of difference in fasting split proinsulin in monozygotic twins. Fasting split proinsulin fell by 0.232pmol/l for every 1lb increase in birth weight (95% confidence intervals -0.44 to -0.02) and by 0.403pmol/l for every 1inch increase in length at birth (95% confidence intervals -0.641 to -0.165). In addition difference in length at birth was predictive of differences in two-hour insulin in monozygotic twins. These findings are suggestive of a link between birth size and insulin resistance that is independent of genetic factors since levels of fasting insulin and its precursors and two-hour insulin are known to reflect insulin resistance as discussed in chapter 2.[131; 132] In addition it suggests that the association is independent of many factors thought to

confound the birth size/adult disease association because of the matching within twin pairs for factors such as maternal diet. However, further analysis revealed that the observed associations were largely dependent on a small number of twins pairs with large differences in birth weight or length at birth. Furthermore, estimation of levels of fasting split proinsulin and two-hour insulin are only a crude measure of insulin resistance and the absence of any associations between birth size and fasting insulin and proinsulin casts some doubt on any links between birth size and insulin resistance in the twins.

The inconsistency in our findings may be a reflection of the low response rates in the glucose tolerance study. Only 28% of twin pairs in the target population agreed to have their glucose tolerance measured. The 95% confidence intervals around the regression coefficient for the association between within pair differences in birth weight and glucose tolerance are relatively wide suggesting a lack of statistical power. In addition, although analysis of the birth register data suggested that study participants were representative of the cohort in terms of size at birth and gestational age, it was not possible to exclude the potential for selection bias in relation to levels of adult risk factors or disease.

There is little published evidence with which to compare our findings. Only one published study, based on the Danish twin register, has examined the association of birth weight and glucose tolerance within twin pairs. This may be partly due to publication bias. The potential for bias in relation to publication of non-significant findings is well documented.[148] There is evidence both that journals are less likely to publish non-significant findings and that researchers are less likely to submit such findings for publication.

The findings of the Danish study supported the fetal origins hypothesis: in twin pairs discordant for non-insulin dependent diabetes, the diabetic twins had significantly lower birth weights than their non-diabetic co-twin.[114] The mean birth weight of the monozygotic diabetic twins was 2634g compared with 2829g in their co-twins. The corresponding mean birth weights for the dizygotic discordant pairs were 2509g and 2854g. There are a number of possible reasons for the differences in the findings of the Danish study and our own. Most importantly the Danish study was based on

adults aged 55 to 74 years in comparison with our cohort aged 40 to 45 years. The prevalence of diabetes was higher in the Danish study and the sample size was greater at 303 identical twin pairs. This enabled analysis of disease discordant pairs. The prevalence of disease in our study was low (1.6% and 6.3% of the study group with diabetes and impaired glucose tolerance respectively) and so our analysis is based on continuous data rather than on the presence or absence of disease. The Danish study also had some potential for selection bias since only 303 pairs out of the 3074 targeted took part. It is possible that those who volunteered to take part were not typical of the group as a whole and may have been more likely to suffer from disease. Furthermore, the birth weight difference findings in the Danish study are based on a relatively small number of twin pairs (14 monozygotic and 14 dizygotic) since birth weight data was only available for 28 of the 45 disease discordant pairs.

### 7.1.6 Within pair differences in blood pressure

Our findings in relation to within pair differences in blood pressure were more consistent: differences in blood pressure were not predicted by birth weight, length or ponderal index at birth in the monozygotic or dizygotic twin pairs: within pair difference in systolic blood pressure rose by 3.32 for every 1lb increase in birth weight difference (95% CI –0.832 to 7.464) in monozygotic twins and the corresponding regression coefficient for dizygotic twins was 1.088 (95% -2.54 to 4.70). Similar trends were seen in relation to length at birth and ponderal index.

Our findings conflict with those of three published studies that have examined the association between within pair difference in birth weight and within pair difference in blood pressure in later life. All three have suggested that the lighter twin at birth does go on to have higher blood pressure as an adult.

The first study was a prospective cohort study of 166 pairs of American infant twins followed up at intervals until the age of 1 year. Differences in birth weight in 67 monozygotic twin pairs were negatively correlated with differences in blood pressure at 1 year of age ($r=0.37$, $P<0.01$).[115] In other words greater discordance in birth weight in monozygotic twins was associated with smaller within pair differences in blood pressure at 1 year. These findings provide evidence of the influence of size at

birth independent of genetic factors and, since two thirds of monozygotic twins are monochorionic, they also suggest that the influence of shared circulation in utero may lead to more similar blood pressure in infancy.

The second study of Australian twins aged eight years was based on a large cohort study set up to investigate the causes of Sudden Infant Death Syndrome (SIDS).[116] All multiple births were eligible for inclusion in the study although singletons were selected according to their level of risk of SIDS. For multiple births, blood pressure decreased by 7mm Hg for every 1 kg increase in birth weight (95% confidence intervals -10.1 to -3.9). Similar findings were observed in a within pair analysis although these findings were not statistically significant: blood pressure decreased by 5.3 mmHg for every 1kg increase in birth weight (95% confidence intervals -13.8 to 3.2). Similar regression coefficients were seen when the within pair analysis was confined to monozygotic and dizygotic pairs with regression coefficients of -6.5 and -4.9 respectively. This analysis was based on only 16 monozygotic and 39 dizygotic twin pairs, however, and these findings were not statistically significant.

The third study was based on 492 female twins with a mean age of 54 years who were recruited to the St Thomas's UK adult twin register.[117] Reported mean birth weights of lighter and heavier twins were 2.12kg (standard deviation 0.59) and 2.52kg (standard deviation 0.61) respectively. Difference in systolic blood pressure was examined across four strata of within pair difference in birth weight. The results showed that the greater the difference in birth weight the greater the difference in adult systolic blood pressure (test for trend: systolic p=0.05, diastolic p=0.09). Similar trends were seen when within pair differences in monozygotic and dizygotic twin pairs were examined although these were based on smaller numbers of pairs and were not statistically significant. Although the generalisability of the findings of this study is somewhat limited by its use of volunteer twins, the use of volunteers clearly had benefits in terms of recruiting large numbers of complete twin pairs: 492 twin pairs were studied in comparison with 192 pairs of twins in Birmingham. The trends reported in this study suggest a link between birth size and adult blood pressure that is independent of genetic factors.

Our findings also conflict with the Swedish twin mortality study where Vagero and Leon found that the shorter twin in height discordant pairs was more likely to die of ischaemic heart disease.[110] Although their findings relate to adult height rather than to birth measurements, there is some evidence to suggest that the smaller twin in birth size discordant pairs also goes on to be the shorter adult. Babson and Phillips followed up birth weight discordant monozygotic pairs until the age of 18 years and found that the smaller twin at birth also remained smaller at 18 years.[109] Wilson showed similar trends in birth weight discordant pairs followed up until the age of nine years.[7]

As in the analysis of within pair differences in glucose tolerance, it is possible that lack of statistical power is responsible for the inconsistency of findings in the Birmingham twins with those observed in other twin studies. Only 196 twin pairs took part in the blood pressure study representing 41% of the target population. This explanation is supported by the wide 95% confidence intervals around regression coefficients for the birth weight/blood pressure association in the monozygotic and dizygotic twins.

## 7.2 Possible explanations for lack of consistency with fetal origins hypothesis

### 7.2.1 Weaknesses in study methodology

Taken together our findings do not support the fetal origins hypothesis. However it is possible that limitations in the study may be partly responsible for the lack of significant associations between birth size and adult disease in the twins. Our study was based on a birth register in order to overcome difficulties of recruiting twins since the UK does not have a national twin register. Many studies of twins in this country have had to rely on ascertainment of twins on the basis of disease which has resulted in overestimation of concordance rates for disease.[78] We used the birth register as the sampling frame for the study in order to ensure our research was based on a population based sample of twins. There were a number of further advantages in this approach. Data relating to birth size was recorded in the birth register and so it was not necessary to rely on self-reported data. In addition we were able to obtain sufficient data from the birth register to trace the majority of twins by tagging records at the NHS central register.

However our approach also had a number of limitations. The study was restricted to a particular geographical area because, for practical purposes, it was not possible to follow up twins who had moved away from the Birmingham area. Although, there were no systematic differences between the twins in the target population and those who had moved away from the Birmingham area in relation to size at birth, it is possible that levels of adult risk factors or disease may have differed in the two groups, particularly given the geographical variations in prevalence of cardiovascular disease.

A further problem related to the difficulties in recruiting twins to take part in the study. Response rates were low particularly in relation to the numbers of complete twin pairs who participated. In the glucose tolerance study only 28% of complete pairs took part and in the blood pressure study the response rate was 41%. This was partly due to the difficulties of persuading subjects to attend a clinic to undergo a glucose tolerance test and also due to the frequency with which the subjects had changed address without notifying their general practitioner. In addition, a surprisingly high number of twins were unwilling to participate even though their co-twin had agreed to take part. The resultant lack of statistical power may explain the absence of any statistically significant associations between birth size and adult disease in the twins. Furthermore, the small number of twin pairs taking part makes it difficult to draw firm conclusions where findings are suggestive of links between birth size and adult disease. This was true in the glucose tolerance within pair analysis where the finding that the shorter twin in length discordant pairs appeared to more insulin resistant as an adult was based on only a small number of the most length discordant twin pairs. An alternative approach to using a birth register to trace recruit subjects would have been to use volunteers as in other recent twin studies in the UK. While there is potential for selection bias in such an approach since volunteers may differ from the rest of the population, there appear to be advantages in terms of recruiting larger samples of twins particularly in relation to complete twin pairs.[117]

Another potential source of selection bias in our study relates to the exclusion of all twin pairs where one or both had died. This group of twins had a lower mean birth weight and gestational age than the remainder of the cohort, as described in chapter 4.

They are likely to have experienced the most adverse conditions in utero and the findings of other studies suggest that they will have included a high proportion of monochorionic twins. They therefore represent a group in which we would expect to see higher levels of disease. However, they accounted for a relatively small proportion of the cohort (11%) and so are unlikely to explain the lack of association between birth size and adult disease. This is supported by the fact that other recent twin studies, which were also based on live born twins only, have shown associations between birth size and adult disease.[114; 116; 117]

The absence of data relating to placentation was a major weakness in exploring the influence of adverse early environment. Since two thirds of monozygotic twins are monochorionic, we hypothesised that monozygotic twins would have higher levels of disease than dizygotic twins. However, data relating to placentation was not available and so it was not possible to make any direct comparisons between twins according to chorionicity. This fact, coupled with the relatively low response rate for complete twin pairs, makes it difficult to draw any firm conclusions relating to the influence of chorionicity by extrapolating from comparisons between monozygotic and dizygotic pairs.

### 7.2.2 Fetal growth of twins differs from that of singletons

Despite the limitations of this study, there are a number of alternative explanations for the lack of consistency of our findings with the fetal origins hypothesis. One possible explanation is that low birth weight has different consequences for twins than for singletons. There are recognised differences in the fetal growth of twins and singletons that may account for the lack of an association between birth size and adult disease in this cohort of twins. Twins have a lower mean birth weight and shorter gestation than singletons as described in chapter 1.[82] The fetal origins hypothesis states that coronary heart disease and its associated conditions result from growth retardation in late pregnancy. Although a number of studies of humans have suggested that growth of twins is similar to that of singletons until the third trimester,[80; 86] there is some evidence that they down-regulate their growth from early gestation since studies based on serial ultrasound scan measurements show that twins may have lower growth rates than singletons from the first trimester onwards.[112] Evidence from animal studies suggests that the setting of a low growth trajectory

during early pregnancy protects the fetus from the effects of later undernutrition.[149] This is supported by observations in lamb twins which show that lambs who down-regulated their growth did not change their growth trajectory even after increase in nutrients.[84]

There is also some evidence to suggest that the metabolic changes associated with growth retardation in singletons are not observed in twins. One study showed that hypoinsulinaemia seen in growth retarded singletons was not observed in twins.[150] This was a small scale study, however, being based on only 20 human fetuses and the findings will need to be replicated in other populations before any firm conclusions can be drawn as to their significance.

Based on this evidence in animals and humans, it is possible that twins may be protected from the effects of undernutrition in late gestation by the early down-regulation of growth. The lack of any association between birth size and adult glucose tolerance and blood pressure in the twin individuals in our study lends support to the theory that fetal undernutrition has different consequences for twins than for singletons.

### 7.2.3 Role of genetic factors

A second possibility is that genetic factors are important determinants of adult glucose tolerance and blood pressure. Classical twin analysis revealed that levels of glucose tolerance and blood pressure were more highly correlated in monozygotic and dizygotic twins and corresponding heritability estimates suggested a strong influence for genetic factors. Although these findings were consistent with earlier twin studies this type of analysis is dependent on the assumption that environmental variance is equal for monozygotic and dizygotic twins. A number of authors have argued that the equal environments assumption may result in over-estimation of the importance of genetic factors since, if monozygotic twins share an environmental factor that influences the outcome of interest more than dizygotic twins, the proportion of variance due to genetic factors will be artificially elevated.[4; 151; 152] As outlined in chapter 1, monozygotic twins have a more similar prenatal environment than dizygotic twins and so the influence of genetic factors will be over-estimated in an analysis which takes no account of prenatal environment.

In order to overcome the limitations of a classical twin design, we performed a multi-level modelling analysis. This allowed estimation of the relative contributions of environmental and genetic factors in explaining observed patterns of variance in the outcome variables. It allowed us to examine the influence of size at birth in determining variance in adult glucose tolerance and blood pressure in the twins. This analysis showed that glucose tolerance and blood pressure were significantly more correlated in the monozygotic than the dizygotic twins. The greater correlation in monozygotic twins was not explained by the birth weight or length at birth of the twins. These findings suggest that genetic factors are important in determining adult levels of glucose tolerance and blood pressure in the twins.

## 7.3 Conclusions

### 7.3.1 The role of prenatal environment in twins needs further exploration

Examination of within pair differences in glucose tolerance in the twins revealed a significant association between birth size and insulin resistance in the monozygotic twin pairs. Since monozygotic twins share the same genes, our findings suggest an effect of birth size that is independent of genetic factors. Furthermore they suggest an association that is independent of many factors that are thought to confound the birth size/adult disease association since twins are perfectly matched for many factors such as maternal size and diet. However, these findings were based on a relatively small number of twin pairs and so will need to be replicated in further studies before any firm conclusions can be drawn as to their significance.

Overall our findings were not consistent with the fetal origins hypothesis. However this may be explained by limitations in our study particularly in relation to low response rates as discussed in section 7.2.1. This would seem to be confirmed by comparison with other twin studies exploring the fetal origins hypothesis which have suggested that the association of birth size with adult glucose tolerance and blood pressure can be demonstrated in twins.

It is also possible that we failed to show an association between prenatal environment and adult disease because small size at birth was not an accurate predictor of adverse environment in utero. Weight and length at birth are likely to be poor proxy measures

for many factors operating in utero such as hormone and nutrient levels and, as outlined above, low birth weight may have a different significance for twins than for singletons. Recent studies to explore the underlying mechanisms for the birth size/adult disease association have suggested that programming of endocrine axes is an important mechanism linking early environment and the development of cardiovascular disease in adult life. It has been suggested that programming of the hypothalamic-pituitary-adrenal axis may be responsible for linking fetal undernutrition with adult disease since raised levels of corticosteroids are known to affect carbohydrate metabolism and cause raised blood pressure. Preliminary evidence in both adults and children provides support for this.[63; 64]

The vascular anastomoses between the circulations of monochorionic twins provide a mechanism by which hormonal and other humoral factors may diffuse between the circulations of twins. This is not a new idea and was proposed by Price in 1950 as a potential explanation for the greater similarity in monozygotic twins.[106] Recent findings in human twins also suggest that the shared circulation in monochorionic twins leads to similar susceptibility to later disease. A subset of 90 twin pairs from the NHLBI cohort of male army veterans was studied to explore the hypothesis that type of placentation was responsible for the greater similarity in high density lipoprotein (HDL), HDL-Cholesterol and apolipoprotein A-1 observed in monozygotic twins when compared with dizygotic twins. Dermatoglyphics were used to make a retrospective assessment of chorionicity in the twins by assigning an index to each twin pair. The findings revealed significantly greater within pair variation in all three outcome variables in the dichorionic twins than the monochorionic twins.

Although based on only one study, this evidence suggests that the similar disease susceptibility of pairs of monochorionic twins could be due to interactions between the twins due to their shared circulation. Further studies to explore the link between prenatal environment and adult disease in twins are needed to explore this theory. Accurate diagnosis of chorionicity would be an essential prerequisite for such studies.

### 7.3.2 Impaired fetal growth has different consequences for twins

The lack of an association between birth size and adult glucose tolerance and blood pressure in twin individuals seen in this study suggests that low birth weight may

have different consequences for twins than for singletons. This is supported by the findings of a number of recent studies which have found similar mortality and morbidity from adult disease in twins and singletons despite the lower mean birth weight of twins.[110; 111; 113] Further more, experiments in animals and observations in human pregnancies suggest that twins down-regulate their growth from early in pregnancy as a protective mechanism against undernutrition in utero. These differences between twins and singletons limit the generalisability of findings in twins and draw into question the value of further studies comparing twins and singletons as a means of exploring the fetal origins hypothesis.

### 7.3.3 Implications for the Fetal Origins Hypothesis

Few published studies have explored the birth size/adult disease association in twins. A recurring theme throughout this thesis is the lack of published evidence with which to compare our findings in twins and this may be a reflection of reluctance of journals and authors to publish non-significant findings. To date, the evidence from published studies suggests that associations between birth size and adult disease can be demonstrated in twins. A number of studies have suggested that differences in adult disease are determined by differences in prenatal environment, which are independent of genetic factors. There is some support for a birth size/adult disease association in the findings of this study of twins born in Birmingham where differences in birth size were predictive of differences in adult glucose tolerance in monozygotic twins. However, our findings and those of other twin studies exploring the fetal origins hypothesis have been based on relatively small samples of twins and will need to be confirmed in larger scale studies.

Further studies of twins are needed to examine the influence of prenatal environment on risk of adult disease in twins. The study of within pair differences in twins provides a particularly powerful model for such studies since it enables control for many factors that are thought to confound the birth size/adult disease association. In particular, as recently argued, they are matched for maternal nutritional status prior to and during pregnancy and therefore provide a means of assessing the importance of the placenta and blood supply to the fetus in utero.[153]

# 8 Appendices

## Appendix A: Questionnaire used in Birmingham twin study

# BIRMINGHAM - QUESTIONNAIRE

Name: _____

Serial Number: | | | | | | |

Address:_____

_____

_____

Tel No:_____

GP: _____

Interviewer:_____ |  |

Date of interview:_____/_____/_____

Time interview commenced: _____:_____

Pregnant 1. Yes / 0. No.

---

Diabetes 1. Yes/ 0. No

Clinic: Accepted / Declined

Clinic Date:_____/_____/_____ Time:_____: _____

1. What is your date of birth? _____/_____/_____

2. Where were you born? _____

3. Are you

   1. Single?
   2. Married?
   3. Divorced/Separated?
   4. Widowed?
   5. Co-habiting?

4. Where was your mother born? _____

5. In what year was your mother born? _____

6. Is your mother still alive? 1. Yes GO TO Q7

   0. No ASK Q6a & Q6b

   6a. What did she die of? _____

   6b. How old was she when she died? _____

7. Is your father still alive? 1. Yes GO TO Q8

   0. No ASK Q7a & 7b

   7a. What did he die of? _____

   7b. How old was he when he died? _____

8. What was your father's job when you were born?
(If unemployed, last full time job)

_____

(Probe if necessary) What industry was that in?

_____

9. Did your mother have any twin pregnancies apart from yours?

1. Yes ASK Q10

0. No GO TO Q11

2. Don't know GO TO Q11

10. If yes, how many other twin pregnancies did she have?

_____

11. How many babies did your mother have in total, including you.
(including stillborn children)?

_____

12. Who was born first, you or your twin?

1. Me

2. My twin

3. Don't know

13. And overall, how many of your brothers and sisters were born before you?

_____

14. Has anyone in your family been diagnosed as a diabetic?
(First degree relations only: mother, father, brothers, sisters or children).

1. Yes PLEASE SPECIFY

0. No GO TO Q15

| Member of family | Age of onset of diabetes<br><br>Record DK if don't know | Do they have insulin injections<br><br>Yes/No/Don't know |
|---|---|---|
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |

15. Has anyone in your family been diagnosed as having high blood pressure? (Include first degree relatives only: mother, father, brothers, sisters or children)

1. Yes IF YES are they receiving treatment.

0. No GO TO Q16

2. Don't know GO TO Q16

| Member of family | Receiving treatment Yes/No/Don't know |
|---|---|
| | |
| | |
| | |
| | |
| | |
| | |
| | |

16. Is your twin the same sex as you?

1. Yes ASK Q17-19

0. No GO TO Q21

17. During childhood, were you and your twin described as :

1. Alike as two peas in a pod
2. Of ordinary family likeness
3. Quite different
4. Don't Know

18. Were you and your twin mistaken for each other as children?

1. Yes, very often
2. Now and then
3. Never
4. Can't remember

19. Who mixed you up?

1. Parents
2. Other relatives
3. Teachers
4. Strangers
5. Others
6. Nobody

20. I'm now going to ask how alike you and your twin are now, in relation to your physical appearance. Can you tell me is your:

| | Very Similar | Somewhat Similar | Different | Don't Know |
|---|---|---|---|---|
| Eye colour | 1 | 2 | 3 | 4 |
| Natural hair colour | 1 | 2 | 3 | 4 |
| Height | 1 | 2 | 3 | 4 |
| Weight | 1 | 2 | 3 | 4 |
| Facial appearance | 1 | 2 | 3 | 4 |
| Complexion | 1 | 2 | 3 | 4 |

21. Until what age did you and your twin live together (with parents or sharing a home)?

_____ years.

22. What is your current or most recent full-time job?

_____

(Probe if necessary) What industry is/was that in?

_____

23. (If married woman) What is/was your husband's current or most recent full time job?

_____

(Probe if necessary) What industry is/was that in?

_____

24a. Have you ever been treated by a doctor for high blood pressure?

1. Yes — IF YES AND SUBJECT IS A MAN ASK Q25. IF A WOMAN ALSO ASK Q24b.

0. No — GO TO Q26

24b. Was this only while you were pregnant?

1. Yes

0. No

25. Are you taking any tablets or medicines to lower your blood pressure?

1. Yes

0. No

26. Have you ever been told <u>by a doctor</u> that you have diabetes?

1. Yes — ASK Q26a

0. No — If a woman, go to Q 28
If a man, go to Q30

26a. At what age was it diagnosed? _____

26b. What treatment do you have for it?

1. Insulin injections

2. Tablets

3. Diet only

**<u>For Women</u>:** Questions 27-28

**<u>For Men</u>:** go to Question 30.

27. Have you ever been treated by a doctor for diabetes during pregnancy?

1. Yes — What treatment did you receive
   1. Insulin Injections
   2. Tablets
   3. Diet Only

0. No — GO TO Q28

28. I would now like to ask you a few questions about your pregnancies, that is if you have ever been pregnant.

How many children do you have? _____

Have you had any other pregnancies? _____

Record details of live births and stillbirths (ie over 28 weeks) only.

| **Pregnancy No.** | Liveborn L<br>Stillborn S | If Liveborn | | |
|---|---|---|---|---|
| | | Male M<br>Female F | Birthweight<br>Lbs | <br>ozs |
| 1. | | | | |
| 2. | | | | |
| 3. | | | | |
| 4. | | | | |
| 5. | | | | |
| 6. | | | | |
| 7. | | | | |
| 8. | | | | |

29a. At what age did your periods start? _____

29b. Have you stopped your periods yet?
1. Yes 0. No

If Yes, at what age _____

30. What **regular** medicines/pills/tablets are you taking?

PLEASE USE BLOCK CAPITALS.
COPY NAMES DIRECTLY OFF BOTTLES IF POSSIBLE.

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____
7. _____
8. _____
9. _____
10. _____

31. I'm now going to ask you a few questions about what you drink - that is ,if you do drink.
Do you ever drink alcohol nowadays, incuding drinks you brew or make at home ?

1. Yes GO TO Q33 0. No ASK Q32

32. Could I just check, does that mean you never have an alcoholic drink nowadays, or do you have an alcoholic drink very occasionally, perhaps for medicinal purposes or on special occasions like Christmas or New Year ?

1. Very occasionally ASK Q33 0. Never GO TO Q35

**SHOW CARD A**

33. I'd like to ask you whether you have drunk different types of alcoholic drinks in the last 12 months. I do not need to know about non-alcoholic or low alcoholic drinks. How often have you had a drink of ..... in the last 12 months?
Ring the appropriate number

| | Almost every day | 5 or 6 days a week | 3 or 4 days a week | once or twice a week | once or twice a month | once every couple of months | once or twice a year | Not at all in last 12 months |
|---|---|---|---|---|---|---|---|---|
| Shandy (exclude bottle/cans) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Beer, Lager, Stout, Cider | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Spirits or Liqueurs (e.g gin, whisky, rum, brandy,vodka, advocaat) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Sherry or Martini (including port, vermouth cinzano, dubonnet) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Wine (including Babycham, Champagne) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Any other alcoholic drinks?<br>1. Yes<br>2. No<br>If YES,<br>specify name of drink | | | | | | | | |
| _____ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| _____ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |

34. Ask for each group of alcoholic drinks coded 1 to 7 (drunk in the last 12 months)

How much ..... have you usually drunk on any one day ?

Enter the amount. Leave blank for the groups of drinks the informant has not drunk at all in the last 12 months.

| | Amount drunk on any one day during the last 12 months | | |
|---|---|---|---|
| Shandy (exclude bottles/cans) | | | Half pints |
| **Beer, Lager, Stout, Cider** | | | Half pints OR |
| | | | Large cans OR |
| | | | small cans |
| Spirits or Liqueurs (e.g Gin, Whisky, Rum, Brandy, Vodka, Advocaat) | | | Singles (count doubles as 2 singles) |
| Sherry or Martini (including Port, Vermouth, Cinzano, Dubonnet) | | | Glasses |
| Wine (inc Babycham, Champagne) | | | Glasses |

Any other alcoholic drinks ?

Specify name of drink and enter the amount usually drunk on any one day

_____ _____

_____ _____

35. Have you ever smoked regularly ? (i.e. at least once a day for a year or more)

1. Yes ASK Q35a

0. No GO TO Q38

35a. How old were you when you first smoked regularly ? _____

35b. What is the most you have ever smoked regularly ?

1. Cigarettes _____ / day

2. Roll-ups _____ / day

3. Tobacco _____ / ozs/week

4. Cigars _____ / day

35c. Do you still smoke regularly ?

1. Yes ASK Q36

0. No GO TO Q37

36. And about how many cigarettes a day do you usually smoke ?

1. Less than 1

2. No. smoked a day _____

37. How old were you when you last smoked regularly ? _____

38. I'd like to ask you about some of the things you have done at work or in your free time that involve physical activity **in the past 4 weeks** that is from ..... up to yesterday.

**Activity at work and around the house**

(Can I just check) were you in paid employment or self employed in the past 4 weeks?

1. Yes ASK Q39

0. No GO TO Q40

39. Thinking about your job in general would you say that you are .....

1. Very physically active
2. Fairly physically active
3. Not very physically active
4. Or not at all physically active in your job?

PREAMBLE FOR INFORMANTS WHO WERE IN WORK OR SELF-EMPLOYED:

I'd like you to think about the physical activities you have done when you were **not** doing your paid job.

**QUESTION TO ALL SUBJECTS**

40. Have you done any housework in the past 4 weeks?

1. Yes ASK Q40a
0. No GO TO Q41

a) Some kinds of housework are heavier than others. This card gives examples of heavy housework but it does not include everything. These are just examples. Was any of the housework you did in the past 4 weeks this kind of heavy housework?

**SHOW CARD B**

1. Yes ASK Q40b
0. No GO TO Q41

b) During the past 4 weeks on how many days have you done that kind of heavy housework?

No. of days _____

41. Have you done any gardening, DIY or building work in the past 4 weeks ?

1. Yes ASK Q41a

0. No GO TO Q42

a) Could you have a good look at this card which gives examples of heavy manual gardening and DIY work. Was the gardening or DIY you did in the past 4 weeks of the **heavy manual** kind?

**SHOW CARD C**

1. Yes ASK Q41b

0. No GO TO Q42

b) During the past 4 weeks, on how many days in total did you do this kind of heavy manual gardening or DIY?

No. of Days _____

42. Have you done any walks of a quarter of a mile or more in the past 4 weeks? That would usually be **continuous** walking lasting 5 to 10 minutes.

1. Yes ASK Q43

0. No GO TO Q45

2. Can't walk at all - GO TO Q45

43. I'd like you to think about **all** the walking you have done in the past 4 weeks either locally or away from here. Please include any country walks, walking in the course of your work, walking to and from work and any other walks that you have done.

Did you do any walks of 1 mile or more in the past 4 weeks? That would usually be **continuous** walking for at least 20 minutes.

1. Yes ASK Q43a

0. No GO TO Q45

a) During the past 4 weeks, how many times did you do any walks of 1 mile or more?

No of times _____

44. Which of the following best describes your **usual** walking pace .....

1. a slow pace
2. a steady pace
3. a fairly brisk pace
4. or a fast pace - at least 4 mph?

45. Can you tell me if you have done any of the activities on this card during the last 4 weeks?

**SHOW CARD D**

1. Yes ASK Q45a

0. No GO TO Q47

a) **RECORD THE SPORTS AND EXERCISE DONE IN COLUMN 1 OF THE TABLE ON THE NEXT PAGE.**

46. **Ask for each activity done in the past 4 weeks.**

a) Can you tell me on how many separate occasions did you (ACTIVITY) during the past 4 weeks?

b) How much time did you usually spend (ACTIVITY) on each occasion?

c) During the past 4 weeks was the effort of (ACTIVITY) usually enough to make you out of breath or sweaty?

| | Column 1 | (a) | (b) | | (c) | |
|---|---|---|---|---|---|---|
| | Activity done | No. of occasions. | Time spent per occasion. | | Effort | |
| | | | hrs | min | Yes | No |
| Cycling/Exercise bike | 01 | | | | 1 | 0 |
| Exercises (press ups, sit ups etc) | 02 | | | | 1 | 0 |
| Aerobics/keep-fit gymnastics/dance for fitness | 03 | | | | 1 | 0 |
| Other types of dancing | 04 | | | | 1 | 0 |
| Weight training | 05 | | | | 1 | 0 |
| Swimming | 06 | | | | 1 | 0 |
| Running/jogging | 07 | | | | 1 | 0 |
| Football/rugby | 08 | | | | 1 | 0 |
| Badminton/tennis | 09 | | | | 1 | 0 |
| Squash | 10 | | | | 1 | 0 |
| Other sports or exercise (specify) | OFFICE USE | | | | | |
| | | | | | 1 | 0 |
| | | | | | 1 | 0 |
| | | | | | 1 | 0 |
| | | | | | 1 | 0 |
| | | | | | 1 | 0 |

47. I am interested in the amount of physical activity you undertook, both at work and at home during the different stages of your adult life. Broadly indicate the most strenuous level of activity carried out **daily** during each of the following periods of your life. (see glossary)

AGE GROUP

| 15-25 | 26-40 |
|---|---|
| | |

LEVEL OF ACTIVITY

light (1)
moderate (2)
heavy (3)
very heavy (4)

48. Did you ever practice sports or physical exercise sufficient to produce sweating or shortness of breath?

AGE GROUP

| 15-25 | 26-40 |
|---|---|
| | |

never (1)
occasionally (2)
< 1 hour per week (3)
1-2 hours per week (4)
> 2 hours per week (5)

49. How old were you when you left school? _____

50. Did you go to college or university after leaving school?

1. Yes
0. No

51. Do you have any formal qualifications/exams?

1. None
2. CSE/ School cert/ GSCE grade D or lower
3. O Levels/ Matric/ GCSE grade A,B,C/ RSA secretarial
4. A Levels/ City and Guilds/ SEN/ ONC/ NNEB/ BTech (day release)
5. HND/ SRN/ Teaching Cert
6. Degree
7. Other

## Appendix B: Publications arising from this work

Baird J, Osmond O, Bowes I, Phillips DIW Mortality from birth to adult life: a longitudinal study of twins *Early Hum Devel* 1998; 53: 73-79.

Baird J, Osmond,O, MacGregor A, Sneider H, Hales CN, Phillips DIW Testing the fetal origins hypothesis in twins: the Birmingham twin study *Diabetologia* 2001;44:33-39.

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